13th SafePork 2019

ONE HEALTH – Tear down interdisciplinary walls

BERLIN | GERMANY

26–29 AUGUST 2019

Proceedings

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INFORMATION A–Z

About Berlin
The city is Germany's biggest and most popular meeting destination. It is far more than just a meeting place for leaders of science, business and politics. The German capital ranks as one of the most visited metropolis in Europe. Located at the heart of Europe it represents a connection between East and West. Berlin offers its visitors a uniquely varied cultural programme, one-of-a-kind historical landmarks and all sorts of entertainment. The city has a long tradition as a theatre metropolis and is justly proud to present a diverse range of productions. It is also known as a city of museums. Germany's largest cultural investment project - the Berlin Museum Island was completed in 2010. It hosts five museums which are part of the World Cultural Heritage List. From a quick snack to dinner at a gourmet restaurant, international cuisine, theme restaurants, beer gardens and regional cooking – Berlin's 7,000 restaurants and eateries cater for every taste, any occasion, any time of the day or night. Berlin's vibrant nightlife offers something for everyone.

Act of God
It is mutually agreed that in the event of total or partial cancellation of the congress due to fire, strike, natural disaster (either threatened or actual), government regulations or incidents not caused by the organizer, which would prevent its scheduled opening or continuance, the congress may be partially postponed or terminated. In this case, participants are not entitled to claim refunds on no account.

Certificate of Attendance
All registered attendees, whether registered in advance or on site, will receive a certificate of attendance issued on-site on Thursday, 29 August 2019.

Congress Language
The official language of the congress will be English. Simultaneous translation will not be provided.

Conference Venue
Langenbeck-Virchow-Haus
Luisenstraße 58/59
10117 Berlin
Germany

Please note that the Langenbeck-Virchow-Haus does not have any parking facilities.

Getting to the Congress Venue
Berlin has an excellent public transport system which is very effective and inexpensive. Tickets are available from machines at underground stations (Maestro debit cards accepted), at newspaper stands, or at Berlin Transport Authority's ticket offices. Tickets must be punched using the ticket validation machine on the tram or bus, or on the platforms before boarding the train.

Currency
The official currency in Germany is Euro (€). You can change money at banks and currency exchanges in airports or train stations. Typically accepted credit cards are American Express, MasterCard and Visa; Diners Club is rarely used, but accepted by major hotels, petrol stations or large shops. Otherwise cash should be be accepted.

Electricity
In Germany electricity is supplied at 220 V, 50 Hz. For some devices from abroad converters may be needed.

Helpful Phone Numbers
Police: 110
Fire: 112
Ambulance: 112
Taxi Berlin: +49 30 202020

Insurance
The congress organizers do not accept any liability for damages and/or loss of any kind which may be incurred by the congress, including delegates or by any persons accompanying them.
Delegates participate in all events at their own risk. Delegates are advised to take out insurances against loss, accidents or damage that could occur during the congress. Verbal agreements will not be binding unless they are confirmed in writing.

Internet Access
Free wireless internet access is available in the venue.

INFORMATION A–Z

Liability Disclaimer
The organisers cannot be held liable for any hindrance or disruption of congress proceedings arising from political, social or economic events or any other unforeseen incidents beyond their control. The organisers will not accept liability for any personal injuries sustained or for loss or damage to property belonging to congress delegates, either during or as a result of the congress or during all events. Registration of a delegate entails acceptance of these conditions.

Lost & Found
A Lost & Found box will be placed at the registration desk.

Name Badge
The name badge will be the official identification document of the meeting and must be worn at all times and should be worn at all times in order to gain entry to the meeting rooms and the exhibition halls. Admission to the conference will not be allowed without badge identification. In case of lost or forgotten badges, an administration fee of €10 will be charged.

Photography, Audio, Video and Mobile Phone Policy
Audio, photo and video recording by any device (e.g. cameras, laptops, PDAs, mobile phones, watches) is strictly prohibited during all oral and poster sessions, unless prior permission is obtained from the congress organizer. Use of mobile phones is strictly prohibited during scientific sessions. Mobile phones must be switched off while attending sessions.

Programme Changes
The organiser reserves the right to make changes if necessary. No full or partial refunds are made to the attendees in the event of cancellations or other changes in the programme. Please note that changes will be posted on screens at the entrance of the session halls. Delegates will be informed about changes.

Public Transportation
Arriving by airplane
From Tegel Airport (TXL) take the TXL bus (Direction: S+U Alexanderplatz via Hauptbahnhof) directly to the Langenbeck-Virchow-Haus (Stop: Haus Charité - Campus Mitte).
From Schönefeld Airport (SXF) take the train RB7 (Direction: Dessau, Hauptbahnhof) or RB14 (Direction: Nauen, Bahnhof) and get off at the stop "Alexander Platz". Take the TXL bus (Direction: Flughafen Tegel Airport) directly to the Langenbeck-Virchow-Haus (Stop: Haus Charité - Campus Mitte).

Arriving by train
From "Hauptbahnhof" (main station) travel by bus line 147 (direction Puschkinallee). Get off the bus at the stop "Luisenstraße/Charité".
Transfer times by Taxi
Tegel (TXL): approximately 20 minutes
Schönefeld (SXF): approximately 50–60 minutes

Registration
Registration is valid only if the complete fee and charges for other services have been paid in full. Registration on-site is possible during the entire congress within the opening hours of the registration desk. Only credit cards and cash payment will be accepted for on-site registration. Valid proof of status must be presented on-site when registering at lower rates. However, waiting can be eased, if participants register online in advance.

Registration Desk
The registration desk is situated in the foyer on the ground floor.

Opening Hours

Monday, 26 August	2:30PM–6:00PM
Tuesday, 27 August	8:00AM–6:00PM
Wednesday, 28 August	8:30AM–6:00PM
Thursday, 29 August	8:30AM–1:30PM

Social Programme
Get-Together
The Get-Toghter takes place on Monday, 26 August from 6:30 PM–8:30 PM at the TAT (Tieranatomisches Theater, Philippstraße 13, Campus Nord, Haus 3, 10115 Berlin) next to the conference venue.
Conference Dinner
The Networking Dinner takes place on Tuesday, 27 August at 7:00 PM. To attend the dinner, a ticket is required. This ticket can be bought at the registration counter on-site.
Pick-up: Pier Friedrichstraße/Reichstagufer, Stern und Kreisschiffahrt GmbH

Time Zone
Berlin belongs to the Central European Time Zone (GMT+1).

Tipping
For services in areas such as restaurants, taxis, at hair salons or any service stations, tipping is traditionally expected. Usually, the tip amounts to about 5 to 10%, according to the degree of your satisfaction with the service rendered.

NOTES

MONDAY, 26 AUGUST

10:00AM–2:30PM	Workshop 1 RIBMINS Cost Action Network Meeting – Workshop about risk-based meat inspection and integrated meat safety assurance
	Break
3:00PM–5:00PM	Workshop 2 African Swine Fever
5:15PM–6:15PM	Zoetis Symposium Ending piglet castration – requirements for a reliable detection of boar taint
6:30PM–8:30PM	Get-Together at Tieranatomisches Theater

TUESDAY, 27 AUGUST

9:00AM–09:15AM	**Opening Ceremony**
9:15AM–09:45AM	Keynote 1 The multiple dimensions of One Health
9:45AM–11:15AM	Round Table Discussion “One Health”
	Break
11:45AM–12:45PM	Abstract Session One Health – One World
	Break
1:45PM–2:30PM	Keynote 2 Antimicrobial resistance and antimicrobial stewardship in food producing animals
2:30PM–3:30PM	Abstract Session One Health – Antimicrobial Resistance
	Break
4:00PM–5:00PM	Abstract Session One Health – Antimicrobial Resistance
7:00PM	Conference Dinner and Conference Party

WEDNESDAY, 28 AUGUST

9:00AM–10:10AM	Keynote 3 EU Pig welfare priorities: castration, tail docking and beyond Keynote 4 Animal welfare at transport and slaughter
10:10AM–10:25AM	Abstract Session Animal Welfare
	Break
11:00AM–11:45AM	Keynote 5 Meat inspection and interventions to control biological hazards in pig abbatoirs in the European Union
11:45AM–1:15PM	Abstract Session Meat inspection & slaughter solutions
	Break
2:15PM–2:30PM	**Presentation of SafePork 2021 and 2023**
2:30PM–3:15PM	Keynote 6 Zoonotic pathogens in the pork supply chain – what should be the responsibilities of the preharvest sector?
3:15PM–4:00PM	Abstract Session Zoonotic pathogens I
	Break
4:30PM–5:30PM	Abstract Session Zoonotic pathogens I

THURSDAY, 29 AUGUST

9:00AM–10:00AM	Abstract Session Zoonotic pathogens II
	Break
10:30AM–11:15AM	KeyNote 7 Risk-based surveillance in the pork chain - requirements and challenges
11:15AM–1:00PM	Abstract Session Monitoring & surveillance systems
1:00PM–1:15PM	**Closing Ceremony & Best Poster Price**

MONDAY, 26 AUGUST 2019

WORKSHOPS

10:00AM–2:30PM **Workshop 1: RIBMINS Cost Action Network Meeting – Workshop about risk-based meat inspection and integrated meat safety assurance**
Chairs: Lis Alban (Denmark) & Bojan Blagojevic (Serbia)

2:30PM–3:00PM COFFEE BREAK

3:00PM–5:00PM **Workshop 2: African Swine Fever**
Chairs: Franz Conraths (Germany) & José-Manuel Sanchez-Vizcaino (Spain)

5:15PM–6:15PM **Zoetis Symposium: Ending piglet castration – requirements for a reliable detection of boar taint**
Dr. Johanna Mörlein (Germany)

6:30PM–8:30PM **Get Together**
Tieranatomisches Theater, Philippstraße 13, Campus Nord, Haus 3, 10115 Berlin

TUESDAY, 27 AUGUST 2019

ONE HEALTH – ONE WORLD

9:00AM–9:15AM **Opening Ceremony**

9:15AM–9:45AM **KeyNote: The multiple dimensions of One Health**
Jerry Shurson (USA)

9:45AM–11:15AM **Round Table Discussion “One Health”**
Moderator: Prof. Dr. Thomas Blaha (Germany)
with Prof. Dr. Lothar Wieler (Germany)
Dr. Katinka de Balogh (Thailand)
Prof. Dr. John Deen, Minnesota (USA)
Prof. Dr. Jaap Wagenaar (The Netherlands)
Prof. Dr. Jerry Shurson (USA)
Hung Nguyen-Viet, MSc, PhD (Vietnam)

11:15AM–11:45AM COFFEE BREAK

11:45AM–12:00PM **Review of biological and chemical health risks associated with pork consumption in Vietnam: major pathogens and hazards**
Megan Cook (Vietnam)

12:00PM–12:15PM **Evaluation of the implementation of one health in Kenya: a case study of the zoonotic disease unit**
Kelvin Momanyi (Kenya)

12:15PM–12:30PM **Serological prevalence of human Trichinellosis and Cysticercosis in Hoa Binh province of Northwest Vietnam**
Nguyen Thanh Luong (Vietnam)

12:30PM–12:45PM **Pork consumption habits and occurrence of Trichinellosis and Cysticercosis in communities of Southern Laos**
Vannaphone Putthana (Vietnam)

12:45PM–1:45PM LUNCH BREAK

ONE HEALTH – ANTIMICROBIAL RESISTANCE

1:45PM–2:30PM **KeyNote: Antimicrobial resistance and antimicrobial stewardship in food producing animals**
Jaap Wagenaar (The Netherlands)

2:30PM–2:45PM **Reduction of antimicrobial use in the pork food chain – did it reduce antimicrobial resistance?**
Bernd-Alois Tenhagen (Germany)

2:45PM–3:00PM **Evaluating average MIC over time using a Baysian latent class mixture model: examples from a Salmonella enterica serovar Typhimurium and S. enterica serovar 4,[5],12:i:-**
Annette O‘Connor (USA)

3:00PM–3:15PM **Characterization of a multidrug-resistant (MDR) Salmonella enterica serovar I 4,[5],12:i:- isolate associated with a 2015 foodborne outbreak from pork**
Bradley Bearson (USA)

3:15PM–3:30PM **Resistance to colistin and production of extended-spectrum-lactamases and/or AmpC enzymes in Salmonella isolates collected from pigs in NW Spain between 2008 and 2009**
Raul C. Mainar-Jaime (Spain)

3:30PM–4:00PM COFFEE BREAK

4:00PM–4:15PM **Quantitative investigation of ESBL resistance in the Danish pork meat chain with estimation of the full burden of ESBL resistance carried in other bacteria than E. coli.**
Soren Aabo (Denmark)

4:15PM– 4:30PM **Antibiotic resistance in E. coli from pigs is associated with their antibiotic treatments and with resistance in E. coli from their dams**
Elke Burow (Germany)

4:30PM–4:45PM **Patterns of antimicrobial use in heavy pig production**
Federico Scali (Italy)

4:45PM–5:00PM **Handling of cases where a pig producer calls in regarding delivery of slaughter animals prior to the end of the withdrawal period**
Lis Alban (Denmark)

7:00PM **Conference Dinner & Conference Party**
Pick-up: Pier Friedrichstraße/Reichstagufer, Stern und Kreisschiffahrt GmbH

WEDNESDAY, 28 AUGUST 2019

ANIMAL WELFARE

9:00AM–10:10AM	**KeyNote: EU Pig welfare priorities: castration, tail docking and beyond** Nancy De Briyne (The Netherlands)
	KeyNote: Animal welfare at transport and slaughter Rebecca Holmes (Germany)
10:10AM–10:25AM	**Ethical implications of the alternatives to surgical piglet castration** Thomas Blaha (Germany)
10:25AM–11:00AM	COFFEE BREAK

MEAT INSPECTION & SLAUGHTER SOLUTIONS

11:00AM–11:45AM	**KeyNote: Meat inspection and interventions to control biological hazards in pig abattoirs in the European Union** Sava Buncic (Serbia)
11:45AM–12:00PM	**Pork safety assessment and first results from pilot interventions targeting slaughter and retail in selected provinces of Northern Vietnam** Fred Unger (Vietnam)
12:00PM–12:15PM	**Handling of lesions indicative of prior septicemia in sows** Jesper Valentin Petersen (Denmark)
12:15PM–12:30PM	**Statistics of meat inspection: How to standardise the assessment of ante-mortem and post-mortem inspection of pigs nationwide? – Development of an educational concept for Germany** Lüppo Ellerbroek (Germany)
12:30PM–12:45PM	**Interactive meat inspection – do we all decide in the same way?** Nina Langkabel (Germany)
12:45PM–1:00PM	**Assessing the food safety risk associated with federally regulated pork establishments in Canada using the Canadian food inspection agency's establishment-based risk assessment model** Sylvain Quessy (Germany)
1:00PM–1:15PM	**Safe pork or safer pork? What has been changed and is to be changed in the EU hygiene legislation?** Edwin Ernst (Germany)
1:15PM–2:15PM	LUNCH BREAK
2:15PM–2:30PM	**Presentation of SafePork 2021 and 2023**

ZOONOTIC PATHOGENS I

2:30PM–3:15PM	**KeyNote: Zoonotic pathogens in the pork supply chain – what should be the responsibilities of the preharvest sector?** Peter Davies (USA)
3:15PM–3:30PM	**The successful control of Salmonella in pigs in Norway** Truls Nesbakken (Norway)

3:30PM–3:45PM	**Applying Salmonella vaccination at the top of a UK pig production pyramid** Judy M. Bettridge (United Kingdom)
3:45PM–4:00PM	**Effect of group vaccination of sows and gilts against Salmonella Typhimurium on Salmonella serology and excretion in sows and their offspring** Linda Peeters (Belgium)
4:00PM–4:30PM	COFFEE BREAK
4:30PM–4:45PM	**Assessment of the relative role of meat of domestic pigs, sheep, cattle, wild boars and moose for the exposure of humans to Toxoplasma gondii** Abbey Olsen (Denmark)
4:45PM–5:00PM	**Risk factors for the occurrence of antibodies against Toxoplasma gondii in organic pig fattening farms in Austria and prospect for their control** Tatjana Sattler (Germany)
5:00PM–5:15PM	**Occurrence of Trichinellosis in indigenous pigs of ethnic minorities in Hoa Binh Province, Vietnam** Hung Nguyen-Viet (Vietnam)
5:15PM–5:30PM	**Reduction of sporulating and non-sporulating pathogens during anaerobic digestion of livestock manure in biogas plants** Martine Denis (France)

THURSDAY, 29 AUGUST 2019

ZOONOTIC PATHOGENS II

9:00AM–9:15AM	**Hepatitis E – analyzing the occurrence in slaughter pigs for a risk assessment of raw meat products** Janine Dzierzon (Germany)
9:15AM–9:30AM	**Hepatitis E virus: an investigation of within-herd transmission and factors affecting risk of infection in slaughter age pigs** Susan Withenshaw (United Kingdom)
9:30AM–9:45AM	**The entry of Listeria monocytogenes into the food chain via slaughter pigs** Verena Oswaldi (Germany)
9:45AM–10:00AM	**Population genetic structure of Listeria monocytogenes strains isolated from the pig and pork meat production chain in France** Benjamin Félix (France)
10:00AM–10:30AM	COFFEE BREAK

MONITORING & SURVEILLANCE SYSTEMS

10:30AM–11:15AM	**KeyNote: Risk-based surveillance in the pork chain – requirements and challenges** Lis Alban (Denmark)
11:15AM–11:30AM	**Salmonella in pigs from weaning to slaughter** Vahab Farzan (Canada)
11:30AM–11:45AM	**Detection of Salmonella antibodies in oral fluid samples from pigs: A tool for easier monitoring of fattening herds?** Juergen Harlizius (Germany)
11:45AM–12:00PM	**A biomolecular DIVA-strategy for Salmonella spp. – diagnostics in Swine** Henning Lindhaus (Germany)
12:00PM–12:15PM	**The smartphone based PCR lab in a bag** Carsten Schroeder (Germany)
12:15PM–12:30PM	**Establishing a serum bank of confirmed cysticercosis positive and negative samples** Maurice Murungi (Kenya)
12:30PM–12:45PM	**Change of livestock trade networks during epidemic outbreaks** Hartmut H. K. Lentz (Germany)
12:45PM–1:00PM	**Decomposition of wild boar carcasses** Franz Conraths (Germany)
1:00PM–1:15PM	**Closing Ceremony & Best Poster Price**

INVITED SPEAKER

Dr. Lis Alban
holds a DVM and a Ph.D. in veterinary epidemiology from University of Copenhagen in Denmark. She is affiliated as a Chief Scientist with the Danish Agriculture & Food Council (DAFC). DAFC is an organization that represents the entire agricultural business of Denmark – from the farmers to the processing industry. At DAFC, she undertakes epidemiological investigations and is responsible for the conduct of risk assessments primarily within food safety including antimicrobial resistance. Her main interest is surveillance and control of pig-borne hazards such as Salmonella, Trichinella, Toxoplasma, and residues of antimicrobials in meat. Modernization of meat inspection is also an area of active research, where she combines the different parts of her work into a new framework for control of meat. She is also an Adjunct Professor at the University of Copenhagen. Her involvement in both academia and industry allows her to focus on identifying intelligent solutions to the challenges in current meat production. She prefers working using the Danish Model, which involves collaboration between stakeholders, academic partners and the veterinary authorities. She is a diplomate of the European College of Veterinary Public Health and is on the Editorial Board of the journal Preventive Veterinary Medicine.

Prof. Dr. Thomas Blaha, Ph.D., Dipl. ECPHM and ECVPH
graduated in veterinary medicine from the University of Leipzig, Germany, in 1971. At the Institute of Applied Animal Hygiene (Eberswalde, Germany) he got the academic title "Dr. med. vet." in 1973. After some years in veterinary practice he joined the Institute of Bacterial Animal Diseases (Jena, Germany), where he achieved his Ph.D. in 1983 – there he was first junior, and later until 1991 senior scientist in the area of infectious intestinal and respiratory pig diseases. From 1991 to 1996, and from 2001 to 2015, Thomas was Professor of Applied Epidemiology and Preventive Veterinary Medicine at the University of Veterinary Medicine, Foundation, of Hannover, Germany. From 1996 to 2001 he held as Full Professor the renowned Endowed "Al Leman Chair" in Swine Health and Epidemiology at the College of Veterinary Medicine of the University of Minnesota, USA. During his years as university professor both in Germany and in the USA, he was several times consulting the WHO, the FAO and the European Union in various countries on preventive veterinary medicine, zoonoses and especially Salmonella control, reduction of antimicrobial use, and animal welfare in food animals.

Prof. Sava Buncic
obtained his veterinary (DVM), MPhil and PhD degrees at the University of Belgrade (Serbia). He held positions of Senior Researcher at the Meat Research Institute in Hamilton, New Zealand; Senior Lecturer at the School of Veterinary Sciences, University of Bristol (United Kingdom); and Full Professor at Department for Veterinary Medicine, University of Novi Sad (Serbia). He has worked as Scientific Consultant in Food Safety, including for Ministries of Science of several European countries, a range of scientific bodies in European Union (such as EFSA and RIA) and within United Nations (such as FAO/WHO and Codex Alimentarius). Prof. Buncic is Diplomate of the European College of Veterinary Public Health (ECVPH) and served as the Chair of its Education Committee. He was member of Editorial Boards of several leading scientific journals on food safety (e.g. Journal of Food Protection; Foodborne Pathogens & Disease). His main areas of expertise are Microbial Food Safety Risk Assessment, Meat Inspection and Safety Assurance, and Veterinary Public Health.

Dr. Peter Davies
College of Veterinary Medicine, University of Minnesota, received his veterinary degree from the University of Melbourne, Australia, in 1975 and his PhD from the University of Sydney, Australia, in 1983. Peter is a veterinary epidemiologist specializing in infectious diseases of food animals, particularly swine, and has been a Professor at the College of Veterinary Medicine, University of Minnesota, USA, since 2003. His professional experience includes 6 years of clinical veterinary practice in Australia, New Zealand and the United Kingdom; 2 years as a livestock advisor on a rural development project in Pernambuco, Brazil; 4 years as a swine specialist with the Department of Agriculture in Adelaide, South Australia; and 25 years as an academic researcher. He has held endowed chair appointments as the MAF Professor of Food Safety and Public Health, Massey University, New Zealand (2002-2003), and Leman Chair of Swine Health and Production, University of Minnesota (2003-2009). In addition to swine health research, Dr. Davies research has focused on the epidemiology of zoonotic and foodborne pathogens, including antimicrobial resistance, at farm level, to understand the relationships between attributes of the farm environment and management that influence the occurrence of infectious agents, including assessment and mitigation of the associated risks to animals and people. He is an author of over 100 peer-reviewed manuscripts and more the 250 conference papers. Current projects include a longitudinal study of infectious disease risks (MRSA, Influenza A virus, Hepatitis E virus) at the swine-human interface, and understanding antibiotic use practices in the US swine industry. From 2000 – 2007, he was a member of the Scientific Advisory Committee for the International Research Center for Veterinary Epidemiology in Denmark. He served as a member of the Presidential Advisory Committee on Combatting Antibiotic Resistant Bacteria in the USA from 2015 to 2018, and in 2019 received the Howard Dunne Memorial Award for service to the US swine industry and the American Association of Swine Veterinarians.

Dr. Katinka de Balogh
is a veterinarian graduated from the Ludwig Maximilian University in Germany, holding a doctorate in tropical parasitology and specialization tropical diseases and veterinary public health. She has held positions at the World Health Organization and worked as lecturer at the veterinary faculties in Zambia, Mozambique and the Netherlands. Over the last 16 years she has been working for the Food and Agriculture Organization at its Headquarters in Rome, Italy and presently holds the position of Senior Animal Health and Production Officer at the FAO Regional Office for Asia and the Pacific based in Bangkok, Thailand where she is the lead technical officer for projects ranging from pig production in Democratic People's Republic of Korea, controlling foot and mouth disease (FMD) and peste des peitits ruiminants (PPR) in Afghanistan to livestock breeding in Tonga. She is also the focal point for the FAO/OIE/WHO Tripartite regional collaboration as well as for antimicrobial resistance and rabies.

Prof. John Deen, DVM, M.Sc., Ph.D., Dipl. ACAW and ABVP
graduated in veterinary medicine from the University of Guelph, Canada, in 1984. He started his professional carrier as associate in a veterinary practice and laboratory for swine and poultry in Shakespeare, Ontario, 1984-1986 before he became a veterinary practitioner in swine health management from 1986-1990. From 1990 to 1991 he became a part-time faculty member in the Department of Population Medicine at the Ontario Veterinary College, Canada. From 1992 to 1999, John was Assistant and Associate Professor in the Department of Food Animal and Equine Medicine, North Carolina State University, before he became Full Professor in the Department of Veterinary Population Medicine at the College of Veterinary Medicine, University of Minnesota. He has been since several years Distinguished Global Professor for One Health, consulting and teaching all over the world. His research is focusing on public awareness, education, and collaboration on One Health.

Nancy de Briyne
studied veterinary medicine in Ghent (Belgium), graduating in 1996. After working as a veterinary practitioner in Belgium and the UK, she works since 2000 for the Federation of Veterinarians of Europe (FVE). In 2015, she became diplomate of the European College of Animal Welfare and Behavioural medicine, subspecialty Animal Welfare Science, Ethics and Law.
Within FVE she is specifically responsible for dossiers in field of animal welfare, veterinary medicines, education and communication. Presently, she is Deputy Executive Director of the FVE.
She has worked extensively in the field of increasing veterinary education in animal welfare and veterinary medicinal products, publishing in 2009 an overview of animal welfare teaching in veterinary undergraduate education in Europe and working on Day 1 Competences in the field of animal welfare for veterinarians. She also published several publications on welfare issues in relation to pigs and on antibiotic use by veterinarians. Her aspiration is to create the right conditions for veterinarians to be and continually strive to be, the leading advocates for a good welfare of animals in a continually evolving society.

Dr. Rebecca Holmes
Born in Cambridge /GB
School in Heidelberg/Germany
1988 – 1995 Studies of Veterinary Medicine at the "Freie Universität" Berlin
2002 PhD at the Ludwig-Maximilian-University, Munich
1996 – 2002 Junior Veterinary Surgeon in an animal hospital in Northern Bavaria
2002 to date Official Veterinarian on various levels of veterinary administration in Bavaria and Baden-Württemberg/Germany
2017 to date Head of the working group "Animal Protection during stunning and slaughter" "Tierärztliche Vereinigung für Tierschutz" (TVT)
2017 to date head of an inspection group for the Bavarian Inspection Authority for Food Safety and Veterinary Affairs. Responsible for veterinary inspections in slaughterhouses, on cutting plants and in poultry husbandries over 40.000 animals.

Prof. Gerald (Jerry) Shurson
received his B.S. degree in Animal Science and Agricultural Economics at the University of Minnesota, and his M.S. and Ph.D. degrees in swine nutrition at Michigan State University. He is currently Professor in the Department of Animal Science at the University of Minnesota, with responsibilities for research, on-campus teaching, and extension. He serves on numerous graduate student committees and has advised 45 Ph.D and M.S. students. Jerry is best known for his research contributions on determining the nutritional value of corn co-products produced by the fuel ethanol industry, but his diverse research program also involves numerous studies to better understand fiber, lipid, amino acid, and trace mineral nutrition in swine. He also serves as the Coordinator of the University of Minnesota Integrated Animal Systems Biology Team, which has numerous industry research partnerships to evaluate mechanisms of growth and health responses from feed additives and other nutritional interventions. His research program has resulted in 115 refereed publications, 187 abstracts, 26 book chapters and white papers, 4 DDGS Handbooks, 94 conference proceedings, and has generated over $18 million in research funding. He has presented his research findings to audiences in over 25 countries, and has unique global perspectives and experiences of the role of animal nutrition in food security, environmental sustainability, and pre-harvest food safety. He also has served in several professional leadership roles including Director and President of the Midwest Section of the American Society of Animal Science, and Director of the University of Minnesota Swine Center. In recognition of his outstanding contributions to corn co-product research, technical advising, and international market development, Shurson received the 2012 Award of Excellence, which is the highest award given by the U.S. ethanol industry. In 2014, Shurson was also selected as the recipient of the American Feed Industry Association Nonruminant Nutrition Research award for his outstanding accomplishments in swine nutrition research.

Prof. Dr. Jaap Wagenaar
was trained as veterinarian and completed his PhD study at Utrecht University and the USDA-National Animal Diseases Center, Ames, IA, US. In 1996 he started his research group at the Central Veterinary Institute (now: Wageningen Bioveterinary Research) in Lelystad, the Netherlands, on food safety and in particular on Campylobacter. From 2004-2006 he worked with WHO (Headquarters, Geneva, Switzerland, and for the Tsunami-relief operations with WHO Indonesia), the Centers for Disease Control and Prevention (Atlanta, US) and the USDA Western Regional Research Center (Albany, Ca, US).
In 2006 he was appointed as chair in Clinical Infectious Diseases at the Faculty of Veterinary Medicine, Utrecht University. His research group is focussing on Campylobacter and antimicrobial resistance. He is currently coordinator of a large EU-project on antimicrobial resistance (EFFORT). He is member of the WHO-AGISAR-group (Advisory Group on Integrated Surveillance of Antimicrobial Resistance) and WHO-Global Foodborne Infections Network, a global capacity building network. He is member of the scientific panel of the Netherlands Veterinary Medicines Institute (SDa) and involved in the major reduction of antimicrobial use in livestock. He is director of the WHO Collaborating Center for Campylobacter and of the OIE-reference laboratory for Campylobacteriosis, and is acting frequently as expert for WHO, FAO and OIE.

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The multiple dimensions of One Health

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The Ultimate Challenge

We live in a complicated, interconnected world that is changing rapidly. In fact, in Thomas Friedman's recent book called "Thank You for Being Late – An Optimists Guide to Thriving in the Age of Accelerations", he explains how our lives are being transformed on many levels all at once by changes in technology, globalization, climate change, and biodiversity (Friedman, 2016). He further suggests that although these changes are occurring faster than our human ability to adapt, if we slow down and use our time to reimagine work, politics, and community, we can overcome these stresses.

As the global human population continues to increase, it is accelerating the stress placed on finite natural resources such as land, water, and air, which are the foundations of life on earth. The recent climate change report released by the United Nations in October of 2018 was an urgent call to action. Climate scientists indicate that if the rise in the earth's temperature exceeds 1.5°C, we will experience the most devastating effects of climate change including destruction of ecosystems and unpredictable weather patterns. Therefore, our ultimate challenge is to find ways to feed a growing population of people without destroying the planet. Fortunately, the United Nations adopted 17 sustainable development goals that can serve as guidelines to implement universal, integrated, and transdisciplinary approaches for transforming the world by 2030 (United Nations, 2015). Producing and consuming nutrients are the common thread that sustains life, and not surprisingly, food is the common link among these 17 sustainable development goals. Using these guidelines, The Economist (2018) developed a global food security index to provide a common framework for understanding the fundamental causes of food insecurity in countries and geographical regions around the world. Some of the key findings in this report were:

- Climate change will affect food production for marine and terrestrial systems as environmental conditions change
- Fertile land, fresh water, and oceans are essential resources that provide the foundation for food security
- Political stability is essential for agricultural production and relief efforts
- Financial risks threaten food affordability, especially for low-income households
- Global trade contributes to food security, but importing countries are vulnerable to increasing protectionism

Similarly, the Barilla Institute for Food and Nutrition (Parma, Italy) developed a Food Sustainability Index to quantitatively and qualitatively characterize the sustainability of national food systems based on food loss and waste, sustainable agriculture (water use, land use, biodiversity, human capital, greenhouse gas emissions), and nutritional challenges (life quality, life expectance, dietary patterns). The most recent report published in 2018 showed that countries that tend to have high incomes, high levels of human development, smaller populations, and slower rates of urbanization, made more progress in improving sustainability of food production than other countries. The countries with the highest ranking in the Food Sustainability Index were France, Japan, Germany, Spain, and Sweden. However, more progress is needed in these top ranking countries, and we need to accelerate the rate of food sustainability improvement in lower ranking countries if we are going to have a significant global impact on food security and sustainability.

One Health

Leonardo da Vinci, who is regarded as one of the most diversely talented geniuses who ever lived, once said "realize that everything connects to everything else". This simple statement completely describes the interconnectivity of the challenges we are facing in feeding the world sustainably and the concept of One Health. The many technological advances and economic benefits that previously provided global health improvements, have now led to an enormous environmental and ecological footprint that is having adverse effects on human health (McMichael and Butler, 2011). One Health has been defined as the collaboration of multiple disciplines and sectors working locally, regionally, nationally, and globally to achieve optimal health by recognizing the interconnections between people, animals, plants and their shared environment. The Centers for Disease Control and Prevention identified 3 key factors that are changing the interactions between humans, animals, and the environment, which have led to the emergence and re-emergence of several diseases (https://www.cdc.gov/onehealth/basics/index.html):

1. Human populations are increasing and expanding in new geographic areas, resulting in more people living in close contact with wild and domestic animals, which increases the likelihood for transmission of diseases from animals to humans.
2. Climate change, deforestation, and intensive agricultural production practices have altered land use. These changes in environmental conditions and habitats create new opportunities for disease transmission to animals.
3. Increased international trade and travel increase the likelihood and rate that diseases can spread around the world.

Although the original concept of One Health included the interactions between humans, animals, and the environment, the environmental component is often neglected (Essack, 2018).Furthermore, the effects of climate change cannot solely focus on human health (Watts, 2018), and must include the interactive effects with animal health and the environment (Zinsstag et al., 2018). **Therefore, achieving One Health must involve transcending and interconnecting all of the components of the global food system** including:

- ecosystem resilience and biodiversity
- sustainable land and soil resources
- abundant and clean water
- climate change
- human, animal, and plant health
- innovative technology for food production, storage, and transport
- equitable food access, production, and distribution
- demographic changes
- culture and lifestyle
- government policy

If all of these components can be optimized, we will have healthier nations that have broad access to abundant, safe, affordable, and nutritious foods produced by thriving farms that are efficient, resilient, sustainable, and profitable. However, if we are going to accomplish this, we need a new paradigm that is transdisciplinary and places more emphasis on the interconnections of ecosystems, soil, water, plant, and microbiome resources with animal and human health. Furthermore, Adeel (2017) indicated that achieving all water-related UN sustainable development goals and interconnections is crucial for achieving universal health, food security, gender equality, sustainable consumption, resilient urbanization, and conservation of marine and terrestrial ecosystems.

"Breaking Down Walls"

The first step in achieving One Health and sustainable food production is to "break down walls" between individual disciplines and interconnect and transcend all disciplines and organizations that are involved in the global food system. This will require a major paradigm shift because most of us became knowledgeable experts in our respective disciplines by becoming reductionist scientists in a narrowly defined field of study. While this level of scientific discovery and translation into practice is still essential, our challenge is to collaborate and integrate this knowledge across disciplines and countries using a systems-based approach to achieve greater impact in solving these complex problems. For example, Nakamura et al. (2019) summarized the scientific literature related to research involving the UN's sustainable development goals and observed that European nations dominated in sustainability related research, with the greatest levels of bilateral and multilateral international collaboration than other nations or regions. North America and the Asia-Pacific regions contributed less to global sustainable development goal research and international collaboration than Europe, with Africa, Arab countries, and Latin America contributing the least, despite major concerns in these regions. These results indicate that there is tremendous need to foster international research collaborations if we are going to be successful in achieving sustainable development and One Health goals. We also need to be conscious of the need to integrate social sciences with the biological sciences to develop meaningful strategies for feeding the world sustainably. For example, Sörqvist and Langeborg (2019) indicated that human heuristic behaviors related to environmental sustainability can actually be more harmful than doing nothing at all. In fact, it is rare to find thoughtful, science-based strategies combined with effective execution of actions in our changing world geopolitical leadership. In other words, we need action, not just rhetoric to solve these problems. Geopolitics often becomes an obstacle that prevents collaborative, transdisciplinary science that can lead to real change in overcoming these challenges in our global food system. Smyth et al. (2017) suggested that achieving improved food security has been limited due to the lack of acknowledgement and rejection of science-based evidence by non-governmental environmental organizations, which has resulted in food security becoming a political issue. Brown et al. (2019) indicated that achieving the Paris Agreement goal of limiting the average global temperature increase to 1.5 °C will unlikely be achieved, because plans in individual countries to address this problem remain vague and insufficient. It is very disconcerting to observe that most of the many government policy decisions and regulations are made based on limited consideration of scientific knowledge. We must let science guide our decisions if we are going to be successful in addressing these global challenges.

One Health – Earth

The health of earth is rapidly deteriorating. To gain an appreciation for the interconnectedness and multiple dimensions of One Health and implications for the global pork industry, it is essential to begin with a brief description of the many challenges that are being created by global climate change. Climate scientists predict that the increase in average global temperature will exceed 1.5°C increase by 2030 if greenhouse gas emissions that cause global warming, continue to increase at the current rate. The stability of earth depends on sea ice in the Arctic and Antarctica because it reflects solar radiation to prevent global warming. Unfortunately, the amount of sea ice has been declining at a rate of about 13% per decade since 1980. If this rate continues, the earth will be ice-free by the year 2040. Therefore, if we don't prevent this increase in global temperature, there will be devastating consequences on ecosystems and unpredictable weather patterns that will exacerbate our challenges of achieving global One Health. The stability of ecosystems depends on the interconnections of diverse habitats, where humans, animals, plants, insects, oceans, and microbiomes are co-dependent and flourish. If rainfall is more predictable and certain, then ecosystems can survive more richly and with variety. However, the outcomes of climate change include changes or loss of habitat for wildlife; species extinction; changes in animal location and migration patterns; as well as more severe and frequent droughts, floods, and wildfires. Furthermore, increased length of growing seasons, extended periods of extreme heat, and changes in precipitation patterns will lead to lost crops. Hurricanes will become stronger and more intense, sea levels will continue to rise, and more frequent flooding will occur, resulting in less and lower quality fresh water leading to drinking water shortages. There will also be increases in some species of plant and animal pests that will alter the health of ecosystems. All of these changes will have negative consequences for the health of microbiomes, soil, water, plants, animals, and humans and availability of resources to produce and deliver food.

One Health – Agriculture

Changes in atmospheric temperatures and carbon dioxide, along with an increased frequency and intensity of extreme weather events will likely reduce crop yields. This will not only affect food security for the growing human population but will also have dramatic effects on reducing the availability of grains and by-products for use in animal feeds. Furthermore, although increased temperatures during the growing season will likely accelerate plant growth, it will alter the nutritional composition of grains and forages through reductions in protein and mineral content. Changes in climate conditions will likely promote more fungi and mold growth in feed grains, leading to increased production of mycotoxins, which have detrimental effects on animal health and productivity. In addition, increased frequency and duration of extreme heat will lead to more heat stress that decreases animal fertility and growth, as well as increases susceptibility to disease. Warmer temperatures, wet climates, and increased carbon dioxide will alter the composition of weeds, insects, and fungi in ecosystems, and enable some species to thrive while others will not. For example, populations of mosquitoes are expected to increase dramatically under warm, wet conditions. Because mosquitoes are vectors for transmission of numerous human and animal diseases, achieving One Health will become more challenging. The increased prevalence of parasites and insects will likely increase pesticide use and change the approaches and practices used by veterinarians for preventing and treating diseases. Ultimately, all of these changes could lead to decreased food availability and reduce access to food by interrupting food delivery, increasing food spoilage and food prices, and decreasing nutritional quality of food. These disruptions are already occurring our global food system, but if they continue to increase, they will lead to more humanitarian crises and cause national security concerns.

One Health – Animal Agriculture

Climate change is expected to have profound negative direct and indirect effects on the health and well-being of food producing animals (Lacetera, 2019). Direct effects include an increase in extreme weather events that can affect transport of feed and feed ingredients from manufacturing to farms; flooding can reduce crop and pasture production; and extreme cold and snowstorms can cause health problems and death of cattle in open ranges. Nardone et al. (2010) also noted that the carrying and buffering capacity of agro-pastoral systems may also be reduced. Equally important will be the increased frequency and duration of extreme heat that will lead to prolonged heat stress, which causes disruptions in metabolism, increased oxidative stress, immune suppression, and death of animals in extreme cases (Lacetera, 2019). The indirect effects include potential reductions in the quantity and quality of feedstuffs and drinking water, low adaptability of genotypes to heat stress, along with potential for increased survival and distribution of pathogens and vectors (Nardone et al., 2010). All of these factors will create even greater challenges for achieving nutrient utilization efficiency and sustainably feed a growing world population that is consuming a greater proportion of animal-derived food products in their daily diet. The global livestock industry accounts for 70% of all agricultural land use, 30% of total land surface use, 8% of water use, and is also responsible for 18% of greenhouse gas emissions. Therefore, changes in animal production practices, especially focused on the sustainability and environmental impacts of feed ingredients, will be essential for reducing the negative environmental impacts of food animal production on global climate change.

Future Perspectives for One Health in Pork Production

Pork is the most widely consumed animal-derived food in the world. In fact, pork production is expected to continue to increase during the next 30 years (Alexandratos and Bruinsma, 2012) due to continued increases in human population and dietary trends toward more animal protein consumption per capita (Lassaletta et al., 2014; Bai et al., 2018). Therefore, the global pork industry will continue to be an important contributor to feeding the world sustainably. We need to move toward a new paradigm that involves designing and implementing holistic, systems approaches to deal with the current and emerging challenges in pork production to achieve One Health. Producing safe and wholesome pork is much more than being free of drug and chemical residues, and food borne pathogens. We need to become more focused on prevention (process controls) rather than focusing on treating disease. Certification schemes need to be harmonized and implemented uniformly among countries rather than relying on carcass inspections and sample testing for identifying unsafe physical, chemical, and microbiological components before they enter the food chain. One health in pork production involves developing new strategies for early detection and surveillance to prevent the spread of pathogens during increasing global mobility of people, animals, feed ingredients, and food. Furthermore, pork production has contributed the unintended consequences of antimicrobial resistance and its effects on soil, water, plant, animal, and human microbiomes. We need to develop and implement strategies to mitigate these effects. One Health in pork production involves balancing the needs for high quality protein for the growing human population while preserving and optimizing the use of finite resources. It involves recycling and re-purposing of food waste nutrients into swine feed to reduce carbon footprint of pork production. One Health also involves coping with effects of climate change such as heat stress, grain shortages, natural disasters, and changes in ecosystems that can all influence swine health and productivity. Achieving One Health in pork production in the future will involve using genomics techniques to develop commercial swine genotypes that are resistant to specific pathogens. Veterinary practices will need to evolve into a new paradigm to ensure that pork production farms implement practices that improve environmental sustainability, feed and food safety, higher standards for biosecurity, and cope with the many consequences of global climate change.

Prophylaxis vs. treatment

We need to implement more effective disease prevention and food safety approaches rather than continuing to rely on treatment of sick pigs, and inspections and testing of carcasses before entering the food chain. Numerous feed and food quality control schemes have been developed and implemented to varying degrees in the global food chain. These include but are not limited to ISO, HAACP, GMP+ certifications. However, different countries have different standards and expectations of quality management, which creates a tremendous problem for harmonization of global trade of feed and food products. Process controls (HARPC) for sanitary feed and food manufacturing, packaging, transport, and storage must be further developed and implemented to reduce the risk of pathogen transmission in complex global supply chains. Block chain technology applications have tremendous possibilities, and the eventual implementation of this technology in agriculture and food production will greatly improve transparency in feed and food safety. However, implementation of block chain technology will depend on our ability to digitize products for traceability throughout the chain. In fact, the recent outbreaks of African Swine Fever and Classical Swine Fever in Asia, as well as the Porcine Epidemic Diarrhea virus in North American created an urgency to implement new process controls and create heightened biosecurity for feed mills and transport. These heightened measures are an important step toward minimizing the risk of transmission of these viruses and other devastating foreign animal diseases from endemic countries to those that are free of these viruses. However, despite the emerging opportunities to further develop and implement quality control and sanitary measures in all aspects related to One Health in pork production, we still need to increase implementation of well-established practices such as vaccinations, animal hygiene, and on-farm biosecurity measures. There is also tremendous potential to determine host effector mechanisms of disease resistance that may lead to the development of new biotherapeutics for disease control and growth optimization in pigs. Molecular genetic tests have been developed and are being used

to select pigs for improved traits. Genetic markers associated with immunity and disease resistance have been identified, and studies have shown that different vaccine responses can be attributed to different genetic lines. Research results have also shown that inheritance is associated with E. coli F18 infections (Fryendahl et al., 2003), which has led to breeding companies providing E. coli F18 resistant breeding stock. Recent studies have also shown that porcine reproductive and respiratory syndrome virus can be controlled through genetic improvements in disease resistance and tolerance (Rowland et al., 2012; Burkhard et al., 2018). These types of research discoveries led Topigs Norsvin (swine breeding company) to identify the major genetic marker associated with natural resistance to PRRS, and to incorporate it into their genetic selection program.). However, although disease resistance can be quantified, it is more difficult to measure disease tolerance, which is poorly understood in pigs (Nakov et al., 2019). Other studies have shown that using CRISPR editing can provide resistance to cornonavirus infection in pigs (Whitworth et al., 2019). Therefore, the development of new gene editing approaches offer promising opportunities for developing commercial genotypes that are resistant to many of the common pathogens that are threats to One Health in global pork production.

Disease surveillance and early detection

Information technology, global markets, and climate are changing faster than our human ability to adapt (Friedman, 2016). We have very sophisticated and complex analytical technology that allows us to detect very low concentrations of substances that may be hazardous to health, but although miniscule amounts of various compounds can be detected, it does not necessarily mean that they pose any health concern. The use of “big data” has enabled practitioners to achieve precision public health by conducting more widespread and specific research trials using segmented populations at risk for various health problems, surveillance and signal detection, predicting future risk, targeting interventions, and understanding diseases (Dolley, 2018). Data-driven business models (Brownlow et al., 2015) have been used to develop similar models for precision livestock farming that can improve animal health and welfare and transparency of production processes (Smith et al., 2015). Various types of sensors are available and are being evaluated for applications in pig production systems to identify behavior changes that can lead to early detection of reduced health and welfare (Matthews et al., 2016). Sensors can be used for animal identification, automatic weight detection, water intake monitoring, and pig coughing (Vranken and Berckmans, 2017). Neural networks linked with sensors to collect environmental data can be used for early detection of respiratory disease in pigs (Cowton et al., 2018), and using sound data and audio surveillance systems can be used for detection of pig wasting diseases (Chung et al., 2013). Technology is also under development to use bio-sensing and photonics technologies for early and rapid field detection of swine viruses by non-specialized personnel (Montagnese et al., 2019).

Global trade and human mobility

Advances in transportation and global infrastructure have provided almost unlimited distribution of feed, food, and other consumer goods around the world. However, we still have enormous inefficiencies and inequities in global food distribution. In fact, about one-third of the food produced globally is lost or wasted before and after it reaches consumers (FAO, 2011). This has led to abundant amounts of food not reaching vulnerable populations of people, along with wasting valuable land, water and energy resources, and contributing to increased greenhouse gas emissions through disposal of food waste in landfills. The most effective options for reducing food waste is to implement practices to reduce waste, followed by feeding hungry people, and recycling these nutrients into animal feed, rather than composting, using anaerobic digestion for energy consumption, or disposing in landfills (Papargyropoulou et al., 2014). Several studies have been conducted showing that recycling food waste into swine feed can recapture lost economic value, serve as excellent energy and nutrient sources (Fung et al, 2018; Jinno et al., 2018; Fung et al, 2019), and can have a dramatic impact on reducing environmental footprint (Salemdeeb et al., 2017). However, concerns about proper thermal treatment to destroy pathogens has limited some governments from approving legislation for this purpose. International travel by humans is another major risk factor for transmission of human and animal diseases (Tatem et al., 2006; Lindahl and Grace, 2015), with nearly 940 million international trips taken by people in 2010 (WHO, 2012). Global increases in economic activity, tourism, and human migrations are causing a dramatic increase in movement of disease vectors and the pathogens they carry (Tatem et al., 2006). Tonnes of live animal and unprocessed animal products are shipped internationally around the world every day, which provide many opportunities for rapid transmission of zoonotic pathogens and foreign animal diseases (Marano et al., 2006). Smuggling of wild animals into countries has always been a high risk factor for human health, and controlling illegal imports is a constant problem. Furthermore, import restrictions do not apply to all species that may be

a health threat because it is not always known which animals carry disease. Much more attention is needed to screen passengers and their belongings at country ports of entry to prevent the unwanted introduction of zoonotic and foreign animal diseases. Foreign animal diseases are major global trade and market disrupters that affect feed ingredient demand and prices, ability to export and import meat to and from countries, and affect food prices and food security for consumers. Global trade has dramatically increased the risk of transmission of pathogens from endemic countries to other countries, which not only can have devastating effects on domestic pork production but also creates trade barriers. The awareness of the significance of global trade on the potential risk of transmission of foreign animal diseases has increased as a result of recent outbreaks of Porcine Epidemic Diarrhea Virus and African Swine Fever Virus in pig populations around the world. The ability of viruses to survive in feed ingredients for extended periods of time was evaluated recently by Dee et al. (2018). These researchers determined the survival (PCR, virus isolation, and/or bioassay) of 11 viruses of global significance to the livestock industry, using Trans-Pacific or Trans-Atlantic transboundary models of representative feed ingredients, transport times, and environmental conditions. Senecavirus A (surrogate for Foot and Mouth Disease Virus), Feline Calicivirus (surrogate for Vesicular Exanthema of Swine Virus), Bovine Herpes Virus Type-1 (surrogate for Pseudorabies Virus), Porcine Reproduction and Respiratory Syndrome Virus, Porcine Sapelovirus (surrogate for Swine Vesicular Disease Virus), African Swine Fever Virus, and Porcine Circovirus Type-2 maintained infectivity during several weeks of transport. More of these viruses survived in conventional soybean meal, lysine HCl, choline chloride, and vitamin D than in organic soybean meal, soy oil cake, distillers dried grains with solubles, and complete feed. These results showing that feed ingredients can serve as vectors for virus transmission has led to a heightened level of biosecurity in some global feed ingredient supply chains. Research is underway to conduct risk assessments and implement sanitary process controls in feed ingredient supply chains to reduce the risk of introducing foreign animal pathogens though feed ingredients imported from countries that are undergoing outbreaks of African Swine Fever. Feed ingredient selection and sourcing not only affects the potential risk of pathogen transmission, but it can also affect environmental sustainability of pork production. Many by-products, such as rendered animal by-products, have been used as economical nutrient sources in swine diets for many years, while also contributing to improved environmental sustainability. However, if inadequate thermal treatment is used, these ingredients can potentially serve as vectors for transmission of undesirable pathogens to pigs. The first case of Porcine Epidemic Diarrhea Virus in North America was attributed initially to a source of spray dried porcine plasma that was fed to pigs. Although a direct cause and effect link was not been definitively confirmed, it led many veterinarians in North America to recommend using only grain-based ingredients in swine diets. However, as several studies subsequently showed, soybean meal and corn can be greater risk factors for transmitting corona viruses than spray dried porcine plasma and other rendered animal by-products (Trudeau et al., 2017). Therefore, feeding strictly grain-based diets does not reduce the risk of virus transmission to pigs, and in doing so, it actually increases gut health problems, and reduces feed efficiency and growth rate. Trade barriers among countries also exist based on different standards and perceptions about the relative food safety risks. More than 70% of genetically engineered crops and biomass is fed to food-producing animals. Regulatory and peer-reviewed studies have shown that genetically engineered crops are safe for feeding to livestock, where more than 100 regulatory submissions have shown equivalent composition and safety between genetically engineered vs. conventional crops, and no rDNA fragments have ever been detected in meat, milk, and eggs (Van Eenennaam, 2013). Government regulations have disproportionately focused on potential risks, rather than the benefits, which has slowed the adoption of genetically engineered crop use in small and poor developing countries. Although metabolic growth enhancers (e.g. ractopamine) have enabled to pork industry to improve the efficiency and sustainability of pork production, government policies in various countries around the world differ in their assessment of safety and acceptance of using these technologies, which has led to trade barriers (Davis and Belk, 2018). Furthermore, various countries use different standards for maximum residue limits of antibiotics in meat and organ tissues, which further impacts market accessibility in global trade. These are only a few more examples of why we need to let science guide regulatory decisions when attempting to feed the world sustainably. There continues to be a need for global harmonization of reasonable feed and food safety standards to overcome food insecurity in many countries.

Contributions to and impacts of climate change on pig production

Climate change plays a dual role in achieving One Health in pork production systems. First, we need to implement technologies that reduce negative environmental impacts of pork production systems. Secondly, we need to develop strategies to try to

mitigate the consequences of climate change on pig health, welfare, and productivity. During the past 80 years, the U.S. pork industry has achieved a 76% reduction in land use, 25% reduction in water use, 8% reduction in global warming potential, and a 7% reduction in energy use (National Pork Board, 2019). Although progress has been made, more concerted and dramatic efforts are needed to achieve further reductions. Lassaletta et al. (2019) developed a model of pig production systems in 26 geographic regions to characterize the shared socioeconomic pathways and identify key factors that will determine their future sustainability. These factors include using improved genotypes with greater productivity and efficiency, use of alternative feed sources that do not compete with human food, reduce crude protein content in swine diets, optimize use of swine manure as fertilizer for crop production, and moderation of human consumption of pork. Nutrition is the primary means to minimize the negative environmental impacts of pork production. Many life cycle assessment studies have been conducted to characterize environmental impacts of food animal production in various countries (de Vries et al., 2010; Tan and Yin, 2017; Weiss and Leip, 2012). Sustainability indicators for nitrogen (Groenestein et al., 2019), phosphorus (Li et al., 2019), and nutrient use (Uwizeye et al., 2016) have been described for livestock production systems. Dourmad et al. (2013) reviewed the impact of pig nutrition on nitrogen, phosphorus, copper, and zinc in pig manure, and emissions of ammonia, greenhouse gases and odor. Several studies have been conducted to assess the environmental footprint (e.g. acidification potential, eutrophication potential, renewable and non-renewable resource use) in classifying feed ingredients used in swine diets (Eriksson et al. 2005; Kebreab et al., 2016; Mackenzie et al., 2016; Wilfart et al., 2016). This approach is useful for developing supply chain management programs for sourcing grain and other feed ingredients that minimize the carbon footprint of pork production systems. In fact, several multi-national feed companies, large swine integrators, as well as governmental and industry organizations have developed environmental sustainability programs with the goal of producing a “zero carbon” pig. Furthermore, new feed ingredients, such as insect meal, microalgae by-products, and bacteria meal, are emerging into the feed ingredient market that are not only more environmentally sustainable, but also appear to have unique chemical compounds that may play a significant role in enhancing pig health and performance. Although several studies have been conducted to determine environmental impacts and sustainability of feed resources, implementation of meaningful practices are only beginning. Climate change will increase the frequency and duration of excessive heat exposure and stress on pigs. Oxidative stress is a major challenge for optimizing pig health and performance. Although there are many commercial antioxidants used to preserve vitamin potency and minimize oxidation of lipids in animal feeds, the use of antioxidant compounds to minimize systemic oxidative stress in pigs has not been adequately evaluated. Furthermore, although some immunity enhancing feed ingredients and additives exist, more attention is needed on developing products that improve innate immunity because new strains of pathogenic viruses and bacteria continue to emerge.

Ecosystem resilience and biodiversity

The biodiversity of ecosystems is extremely important role in achieving One Health of pork production, but is rarely considered. One of our greatest challenges is to continue to use global agricultural land for animal feed, biomass, and human food production while simultaneously maintaining natural ecosystems and reducing climatic and environmental impacts. Intensification of agriculture, which includes the use of fossil fuels, has reduced biodiversity and negatively affected many of the ecosystem services that food production relies upon (Tsiafouli et al., 2017). Several human interventions have led to loss of habitat, biodiversity, and destruction of ecosystems. Use of pesticides have drastically diminished bee populations, which are essential for crop pollination. The need to provide more environmentally friendly alternatives to burning fossil fuels has led to the diversion of grains and oilseeds to biofuels production and provided economic incentives for using monoculture crop production systems, which have created new challenges for weed and pest control, and negatively affected ecosystem biodiversity. Conversion of non-aerable land to aerable land reduces the ability of trees and plants to sequester carbon dioxide. Therefore, new frameworks need to be developed that integrate knowledge from diverse ecosystem components across multiple scales and time to preserve and enhance ecosystem services provided to agricultural systems (Tsiafouli et al., 2017). Soybeans and soybean meal are the main protein sources used in swine diets in many countries around the world. The expansion of soybean production in countries like Brazil, has led to deforestation of thousands of hectares of land. Deforestation is a major ecosystem concern because of the loss of biodiversity and forests that utilize carbon dioxide. As a result, multiple organizations, such as the Consumer Goods Forum have developed global sustainable soy sourcing guidelines. Similarly, the United Soybean Board, American Soybean Association, and the U.S. Soybean Export Council have also established goals for the U.S. soybean industry to reduce land use by 10%, reduce soil erosion by 25%, increase energy use efficiency by 10%, and reduce greenhouse gas emissions by 10% by the year 2025.There is tremendous interest in defining and understanding the microbiome of ecological systems on many levels. Complex, diverse microbial communities are found everywhere in the environment and have a major influence on the health of soil, plants, forests, oceans, animals, and humans. We have known for a long time that humans and animals have microorganisms both internally and externally, but we are only just beginning to understand the role of the microbiome in the health and well-being of the host (Miller et al., 2018). The Earth Microbiome Project began in 2010 with the goal of developing a global catalog of the uncultured microbial diversity on earth. One of the initial findings of this ambitious research effort has shown that major shifts in microbial composition of prairie soils in the Midwestern U.S. has occurred due to agricultural use, which has changed the relative abundance of Verrucomicrobia and its influence on carbon dynamics (Gilbert et al., 2014). The science of microbiome communities is only beginning, but promises to be a key component for developing meaningful strategies to improve One Health on many dimensions. Phosphorus is an essential nutrient for living organisms and is a critical resource for the bioeconomy and food security. However, phosphate rock is a finite resource and global reserves are decreasing (Mogollón et al., 2018). If inorganic phosphorus fertilizer and manure are not managed properly, phosphorus can have an ecologically damaging effect on freshwater eutrophication. Global trade of phosphorus has changed the global phosphorus cycle resulting in critical nutrient imbalances among countries and ecosystems (Nesme et al., 2018). The over-abundance of phosphorus that has reduced water quality, and the eventual global depletion of phosphorus reserves for future agricultural production, has led to a convergence of phosphorus security and water quality initiatives (Leinweber et al, 2018). In fact, the European Sustainable Phosphorus Platform is one example of a collaborative effect involving over 150 organizations to improve phosphorus utilization efficiency in agriculture and food production while developing strategies to reuse, recover, and recycle phosphorus in a circular economy. In addition, a recent report by the United Nations identified 5 major environmental challenges including 1) synthetic biology and biotechnology, 2) ecological connectivity, 3) melting permafrost (carbon dioxide losses), 4) maladaptation to climate change, and 5) disruption of the global nitrogen cycle (UNEP, 2019). The increase in livestock and agricultural production, along with transportation, energy and industry, have led increased emissions of nitrate in water, and ammonia and nitrous oxides in air, which has significant negative effects on climate change, air quality, and the ozone layer. The European Nitrogen Assessment estimated that 80% of the nitrogen used in food production is wasted, with an associated global cost of 300-400 billion US$ per year. These results continue to emphasize the need for precision nutrition when formulating and feeding swine diets to minimize nitrogen excretion in manure and reduce ammonia and nitrous oxide emissions in order to contribute toward improving global One Health. Finally, Aleksandrowicz et al. (2016) conducted a review of impacts of changing human diets on greenhouse gas emissions, land and water use, and health, and concluded that environmental and health benefits are possible by shifting current Western diets to a variety of more sustainable diets. In fact, Rose et al. (2019) argued that environmental sustainability should be included as a key component when educating individuals and groups about dietary choices, and in setting national dietary guidance recommendations. Consumer purchasing decisions contribute substantially to environmental degradation, resource depletion, and social problems (Gandenberger et al., 2011, Gardner et al., 2004). This concern has led to many public and private initiatives to communicate sustainability information about food to consumers Grunert et al., 2014). In fact, ecolabelindex.com has identified 463 ecolabels (e.g. Rainforest Alliance, World Wildlife Federation, Ocean Conservancy) in 199 countries and 25 industry sectors. However, although consumers have medium to high levels of concern about sustainability issues, they have lower levels of concern when making food choices (Grunert et al., 2014). Therefore, the future use of eco-labeling of food products will be dependent on actual behavioral changes of consumers when making food purchasing decisions.

Antimicrobial paradigm shift

The development of antimicrobial resistance in the global microbiome is one of the greatest threats to One Health. We have developed and used many chemicals such as antimicrobials, herbicides, and pesticides that have provided many benefits in global health, and contributed immensely toward feeding the world by preventing and treating diseases. However, these technologies have led to unintended consequences that have caused the development of antimicrobial resistance and food safety concerns (Barton, 2014). Studies have shown the antibiotic resistant genes

spread from the pig production environment to meat throughout the pork production chain, including the feed supply (Liu et al., 2019). Österberg et al. (2016) reported that although antimicrobial resistance to *E. coli* was less common in pigs produced in organic systems compared to conventional systems, there were large differences in resistance between countries within each type of production system. Scoppetta et al. (2017) evaluated antibiotic use and development of resistance on 14 farms in the Umbra region of Italy and reported that farms varied in their level of antibiotic resistance. In addition to the development of bacterial resistance, inappropriate use and unintended carry over from feed to food of antibiotic residues continues to be a threat to One Health. Although the World Health Organization has designated antimicrobial resistance as serious threat to global public health, the U.S. has lagged behind the E.U. in restricting or banning the use of antibiotic in animal agriculture. All global governments and society must take action to address this problem, but efforts by federal government policy makers and regulators have been insufficient (Martin et al., 2015). Interesting new scientific discoveries are beginning to reveal that advances in biotechnology may result in restoring the efficacy of antibiotics by using antibiotic-peptide conjugates (Marquardt and Li, 2018). Additional approaches for treating and controlling disease caused by microorganisms include CRISPR/Cas9 gene editing technology, genetically modified bacteriophages, peptides, and nanoantibiotics, along with improved vaccines, immunoglobulins, and eubiotics (Marquardt and Li, 2018). The discontinuation of using antibiotics for growth promotion purposes in many countries has led to the consideration and use of numerous "alternatives" to antibiotics, which vary in their efficacy. These feed additives must be evaluated based on direction, magnitude, and consistency of growth responses, while also determining if we adequately understand their mechanisms of action, if they are synergistic, antagonistic, or additive in combinations with other additives, and if they provide a predictable return on investment when used. Unfortunately, the mode of action of most of these feed additives is not well understood, which prevents their strategic use in optimizing swine health in the absence of antibiotics. In addition to health and food safety concerns from antibiotic use, many segments of consumers have developed strong preferences and make food choices based on how their food is produced. This has led to many restaurants, food service providers, and supermarkets to provide animal-derived food products produced with various types of food claims ranging from organic, "no antibiotics ever", "no medically important antibiotics", "no growth-promoting antibiotics", to "judicious use of antibiotics". The use of these label claims has provided incentives for many pork producers to adopt production practices that greatly reduce or eliminate antibiotic use in their production systems to meet these market demands. However, in doing so, more management pressure is required to achieve greater biosecurity and hygiene standards in these production systems, which are at greater risk for increased mortality and reduced productivity and efficiency. Another less known contribution to antimicrobial resistance is a result of extensive use of herbicides and pesticides in crop production. Increasing pesticide use in agriculture has resulted in the selection and emergence of multiple antibiotic resistance in pathogenic strains (Curutiu et al., 2017; Jørgensen et al., 2018). Furthermore, studies have shown that herbicides also contribute to the development of antimicrobial resistance (Jiang et al., 2018; Kurenbach et al., 2015). This is an increasing concern because climate change is expected to alter the survival of selected weeds and pests, which may lead to further increases in pesticide and herbicide use in the future. Furthermore, the consequences of extensive and long-term use of antibiotics, herbicides, and pesticides on altering microbiomes of soil, water, and ecosystems is not well defined but has major implications for our ability to achieve One Health.

The science paradox

In some ways, we live in a "science illiterate" and "sound bite" society where limited, or lack of scientific knowledge and context leads to an inability for people to distinguish scientific facts from fiction, leading to the wide dissemination of inaccurate information through various social media venues. This results in many people making uninformed decisions about the need to optimize the balance between the environment, human, animal, and plant health for future sustainability. In other ways, people with greater science literacy and education have more polarized beliefs on controversial science topics based on religious and political beliefs (Drummond and Fischhoff, 2017). People who consider themselves to be political conservatives and supporters of free-market capitalism are less likely to believe in climate change and have concerns about its impacts (McCright et al., 2016; Bohr, 2014; Hamilton, 2011; Lewandowsky et al., 2013; McCright and Dunlap, 2011). However, there appears to be little association between religious or political polarization on acceptance of nanotechnology and genetically modified foods (Drummond and Fischoff, 2017). The relationship between scientific literacy and sources of information affect overall consumer knowledge and perception of genetically modified organisms and foods (Wunderlich and Gatto, 2015). In contrast to the findings by Drummond and Fischoff (2017) of the effect of education level on acceptance of climate change science, people who are familiar with genetic engineering tend to be more resistant to the use of bioengineering than those who have greater scientific knowledge of the technology (Wunderlich and Gatto, 2015). In the U.S., there has been a resurgence of infectious human diseases resulting from lack of comprehensive vaccinations, which has created increasing public health concerns. Studies have shown that people who have greater trust in health care professionals are more knowledgeable about the risks and benefits of vaccines, with individuals who are older, more affluent, and educated, being more likely to choose vaccination for themselves and their families (Song, 2014).

Healthy food

Meat consumption has been a core component of human survival for centuries, but its role in a healthy diet has been greatly debated for several decades (McNeill et al., 2017). Pork is the most widely consumed animal protein in the world, and research evidence suggests that the consumption of lean pork results in similar changes in human body composition compared with lean beef and chicken (Murphy et al., 2014). Pork not only contains all of the essential amino acids required by humans, but it is also a rich source of minerals (phosphorus, selenium, zinc, and iron) and vitamins (thiamin, B_{12}, B_6, and niacin). Achieving adequate daily consumption of these essential nutrients is difficult to achieve for meeting daily requirements of people consuming vegan or vegetarian-based diets. However, the amount of fat in pork products can vary from 10 to 16% depending on the amount of trimming, and consumption of saturated fatty acids has been shown to be associated with increased risk of cardiovascular disease (Jakobsen et al., 2009; Skeaff and Miller, 2009; Micha and Mozaffarian, 2010; Mozaffarian et al., 2010). Compared to beef and lamb, pork has less fat, greater concentrations of polyunsaturated fatty acids, and lower trans fatty acid content, which makes it a healthier meat choice for humans because substitution of saturated and trans fatty acids for polyunsaturated fatty acids in the diet reduces the risk of cardiovascular disease (Scollan et al., 2017). In fact, the concentrations of long-chain *n*-3 fatty acid content of intramuscular fat of pork, can be increased by feeding diets containing linseed and linseed oil (Nuernberg et al., 2005; Haak et al., 2008; Guillevic et al., 2009), flaxseed (Turner et al., 2014), rapeseed oil (Bertol et al., 2013; Gjerlaug-Enger et al., 2015), fish oil (Haak et al., 2008), and microalgae (Meadus et al., 2010) to growing-finishing pigs. While the use of fish oil in swine diets is not sustainable within the broad scope of global One Health, the use of microalgae is certainly is more sustainable, and initial studies have shown it has nutritional benefits in swine diets (Lei, 2018). However, we also need to be cognizant of the potential for harmful mycotoxins, such as aflatoxins, which are known to be carcinogenic, to be deposited in pork meat when pigs are fed mycotoxin-contaminated diets (Völkel, et al., 2011). A recent study conducted by Lee et al. (2017) showed that about 54% of 1,920 urine samples collected from pig slaughter facilities in Vietnam contained an average of 0.63 µg/kg of aflatoxin M_1. With the increased likelihood of global climate change increasing the prevalence and concentrations of mycotoxins in feed grains and grain by-products, more attention needs to be devoted to understanding the potential adverse effects on human health from consuming pork containing mycotoxins and their metabolites.

Conclusions

We live in a complex, globally interconnected and diverse world where numerous changes are occurring rapidly at an accelerating pace that are affecting our ability to feed the world sustainably and achieve One Health. We must "break down walls" between narrowly focused disciplines, accelerate our collaborations on many One Health dimensions, and begin using more holistic systems approaches for discovering and applying scientific knowledge if we are going to have a meaningful impact of solving the many complex problems in food and pork production systems. Scientific discoveries and human interventions have created enormous improvements in food security, food safety, and overall well-being for many segments of the global population. However, many of these interventions have led to many unintended consequences on many levels and dimensions that have created serious challenges including climate change, the development of antimicrobial resistance, and the future sustainability of the planet. We must let science guide our decisions to overcome these challenges by working collaboratively across scientific disciplines, government agencies, industries, academia, countries, and regions to not only discuss science based strategies but to also act on them. There are many dimensions of One Health that must be considered in future to protect precious resources on earth and ensure well-being and health for all.

References

Adeel, Z. 2017. A renewed focus on water security within the 2030 agenda for sustainable development. Sustainability Science 12:891-894.

Aleksandrowicz, L., R. Green, E.J.M. Joy, P. Smith, and A. Haines. 2016. The impacts of dietary change on greenhouse gas emissions, land use, water use and health: A systematic review. PLoS ONE 11:e0165797. doi:10.1371/journal.pone.0165797.

Alexandratos, L., and J. Bruinsma. 2012. World agriculture towards 2030/2050, the 2012 revision. In: FAO (Ed.), ESA Working Paper no. 12-13. Food and Agriculture Organization of the United Nations, Rome, Italy.

Bai, Z., W. Ma, L. Ma, G.L. Velthof, Z. Wei, P. Havlik, O. Oenema, M.R.F. Lee, and F. Zhang. 2018. China's livestock transition: driving forces, impacts and consequences. Sci. Adv. 4. https://doi.org/10.1126/sciadv.aar8534.

Barton, M.D. 2014. Impact of antibiotic use in the swine industry. Current Opinion in Microbiology 19:9-15.

Bertol, T.M., R.M.L. de Campos, J.V. Ludke, N.N. Terra, E.A. de Figueiredo, A. Coldebella, J.I. dos Santos Filho, V.L. Kawski, and N.M. Lehr. 2013. Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. Meat Sci. 93:507-516.

Bohr, J. 2014. Public views on the dangers and importance of climate change: predicting climate change beliefs in the United States through income moderated by party identification. Clim. Change 126:217-227.

Brown, C., P. Alexander, A. Arneth, I. Holman and M. Rounsevell. 2019. Achievement of Paris climate goals unlikely due to time lags in the land system. Nature Climate Change https://doi.org/10.1038/s41558-019-0400-5

Brownlow, J., M. Zaki, A. Neely, A., and F. Urmetzer. 2015. Data and Analytics - Data-Driven Business Models: A Blueprint for Innovation. http://www.cambridgeservicealliance.org/uploads/downloadfiles/2015%20March%20Paper%20-%20The%20DDBM-Innovation%20Blueprint.pdf

Burkard, C., T. Opriessnig, A.J. Mileham, T. Stadejek, T. Ait-Ali, S.G. Lillico, C.B.A. Whitelaw, and A.L. Archibald. 2018. Pigs lacking the scavenger receptor cysteine-rich domain 5 CD163 are resistant to porcine reproductive and respiratory syndrome virus 1 infection. J. Virology 92:e00415-18. https://doi.org/10.1128/JVI.00415-18

Chung, Y., S. Oh, J. Lee, D. Park, H-H. Chang and S. Kim. 2013. Automatic detection and recognition of pig wasting diseases using sound data in audio surveillance systems. Sensors 13:12929-12942.

Cowton, J., I. Kyriazakis, T. Plötz and J. Bacardit. 2018. A combined deep learning GRU-autoencoder for early detection of respiratory disease in pigs using multiple environmental sensors. Sensors 18:2521. Doi: 10.3390/s19082521.

Curutiu, C., V. Lazar, and M.C. Chifiriuc. 2017. Pesticides and antimicrobial resistance: from environmental compartments to animal and human infections. New Pesticides and Soil Sensors. http://dx.doi.org/10.1016/B978-0-12-804299-1.00011-4.

Davis, H.E., K.E. Belk. 2018. Managing meat exports considering production technology challenges. Anim. Frontiers 8:23-29.

Dee, S.A., F.V. Bauermann, M.C. Niederwerder, A. Singrey, T. Clement, M. de Lima, C. Long, G. Patterson, M.A. Sheahan, A.M.M. Stolan, V. Petrovan, C.K. Jones, J. De Jong, J. Ji, G.D. Spronk, L. Minion, J. Christopher-Hennings, J.H. Zimmerman, R.R.R. Rowland, E. Nelson, P. Sundberg, and D.G. Diel. 2018. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. PLoS ONE 13(3):e0194509. https://doi.org/10.1371/journal.pone.0194509

de Vries, M. and I.J.M. de Boer. 2010. Comparing environmental impacts for livestock products: A review of life cycle assessments. Livestock Science 128:1-11.

Dolley, S. 2018. Big data's role in precision public health. Front. Public Health 6:68 Doi: 10.3389/fpubh.2018.00068.

Dourmad, J.-Y., F. Garcia-Launay, and A. Narcy. 2013. Pig nutrition: impact on nitrogen, phosphorus, Cu and Zn in pig manure and on emissions of ammonia, greenhouse gas and odours. Bat-farm European Workshop Reconciling Livestock Management to the Environment, March 2013, Rennes, France.

Drummond, C., and B. Fischoff. 2017. Individuals with greater science literacy and education have more polarized beliefs on controversial science topics. PNAS 114:9587-9592.

Eriksson, I.S., H. Elquist, S. Stern and T. Nybrandt. 2005. Environmental systems analysis of pig production – The impact of feed choice. Int. J. LCA 10:143-154.

Essack, S.Y. 2018. Environment: The neglected component of the One Health triad. The Lancet 2:e238-e239.

FAO. 2011. Global food losses and food waster – Extent, causes and prevention. Rome, Italy, http://www.fao.org/3/mb060e/mb060e00.pdf

Friedman, T.L. 2016. Thank You for Being Late – An Optimist's Guide to Thriving in the Age of Accelerations. Farrar, Straus, and Grioux publishers. ISBN: 9780241301449

Frydendahl, K., T.K. Jensen, J.S. Anderson, M. Fredholm, G. Evans. 2003. Association between the porcine Escherichia coli F18 receptor genotype and phenotype and susceptibility to colonization and postweaning diarrhea caused by E. col 0138:F18. Vet. Microbiol. 93:39-51.

Fung, L., P.E. Urriola and G.C. Shurson. 2019. Energy, amino acid, and phosphorus digestibility and energy prediction of thermally processed food waste sources for swine. Transl. Anim. Sci. Doi: 10.1093/tas/txz028.

Fung, L., P.E. Urriola, L. Baker, and G.C. Shurson. 2018. Estimated energy and nutrient composition of different sources of food waste and their potential for use in sustainable swine feeding programs. Transl. Anim. Sci. 3;143-152.

Gandenberger, C., H. Garrelts, and D. Wehlau. 2011. Assessing the effects of certification networks on sustainable production and consumption: the cases of FLO and FSC. J. Consum. Policy 34:107-126.

Gardner, G., E. Assadourian, and R. Sarin. 2004. The State of Consumption Today. State of the World. http://erikassadourian.com/wp-content/uploads/2013/07/SOW-04-Chap-1.pdf

Gjerlaug-Enger, E., A. Haug, M. Gaarder, K. Ljøkjel, R.S. Stenseth, K. Sigfridson, B. Egelandsdal, K. Saarem, and P. Berg. 2015. Pig feeds rich in rapeseed products and organic selenium increased omega-3 fatty acids and selenium in pork meat and backfat. Food Sci. Nutr. 3:120-128.

Gilbert, J.A., J.K. Jansson, and R. Knight. 2014. The Earth Microbiome project: successes and aspirations. BMC Biol. 12:69.

Groenestein, C.M., N.J. Hutchings, H.D. Haenel, B. Amon, H. Menzi, M.H. mikkelsen, T.H. Misselbrook, C. van Bruggen, T. Kupper, and J. Webb. 2019. Comparison of ammonia emissions related to nitrogen use efficiency of livestock production in Europe. J. Cleaner Prod. 211:1162-1170.

Grunert, K.G., S. Hieke, and J. Wills. 2014. Sustainability labels on food products: Consumer motication, understanding and use. Food Policy 44:177-189.

Guillevic, M., M. Kouba, and J. Mourot. 2009. Effect of a linseed diet on lipid composition, lipid oxidation and consumer evaluation of french fresh and cooked pork meats. Meat Sci. 81:612-618.

Haak, L., S. De Smet, D. Fremaut, K. Van Walleghem, and K. Raes. 2008. Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. J. Anim. Sci. 86:1418-1425.

Hamilton, L.C. 2011. Education, politics and opinions about climate change evidence for interaction effects. Clim. Change 104:231-242.

Jakobsen, M.U., E.J. O'Reilly, B.L. Heitmann, M.A. Pereira, K. Bälterm G.E. Fraser, U. Goldbourt, G. Hallmans, P. Knekt, S. Liu, P. Pietinen, D. Spiegelman, J. Stevens, J. Vitamo, W.C. Willett and A. Ascherio. 2009. Major types of dietary fat and risk of cornonary heart disease: a pooled analysis of 11 cohort studies. Am. J. Clin. Nutr. 89:1425-1432.

Jiang, W. J. Gao, Z. Cheng, P. Wang, Z. Zhou and D. Liu. 2018. The effect of antibiotics on the persistence of herbicides in soil under the combined pollution. Chemosphere 204:303-309.

Jinno, C., Y. He, D. Morash, E. McNamara, S. Zicari, A. King, H.H. Stein and Y. Liu. 2018. Enzymatic digestion turns food waste into feed for growing pigs. Anim. Feed Sci. Technol. 242:48-58.

Jørgensen, P.S., A. Aktipis, Z. Brown, Y. Carriére, S. Downes, R.R. Dunn, G. Epstein, G.B. Frisvold, D. Hawthorne, Y. T. Gröhn, G.T. Gujar, D. Jasovskỳ, E.Y. Klein, F. Klein, G. Lhermie, D. Mota-Sanchez, C. Omoto, M. Schlüter, H. M. Scott, D. Wernli, and S.P. Carroll. 2018. Antibiotic and pesticide susceptibility and the Anthropocene operating space. Nature Sustainability 1:632-641.

Kebreab, E., A. Liedke, D. Caro, S. Deinling, M. Binder, and M. Finkbeiner. 2016. Environmental impact of using specialty feed ingredients in swine and poultry production: A life cycle assessment. J. Anim. Sci. 94:2664-2681.

Kurenbach, B., D. Marjoshi, C.F. Amábile-Cuevas, G.C. Ferguson, W. Godsoe, P. Gibson and J.A. Heinemann. 2015. Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. mBio 6:e00009-15. Doi:10.1128/mBio.00009-15.

Lacetera, N. 2019. Impact of climate change on animal health and welfare. Animal Frontiers 9:26-31.

Lassaletta, L., F. Estellés, A.H.W. Beusen, L. Bouwman, S. Calvet, H.J.M. van Grinsven, J.C. Doelman, E. Stehfest, A. Uwizeye and H. Westhoek. 2019. Future global pig production systems according to the Shared Socioeconomic Pathways. Sci. Total Environ. 665:739-751.

Lassaletta, L., G. Billen, E. Romero, J. Garnier and E. Aguilera. 2014. How changes in diet and trade patterns have shaped the N cycle at the national scale: Spain (1961-2009). Reg. Environ. Change 14:785-797.

Lee, H.S., J. Lindahl, H. Nguyen-Viet, N.V. Khong, V.B. Nghia, H.N. Xuan and D. Grace. 2017. An investigation into aflatoxin M_1 in slaughtered fattening pigs and awareness of aflatoxins in Vietnam. BMC Vet. Res. 13:363.

Lei, X.G. 2018. Sustaining the future of animal feed protein. Indust. Biotechnol. 14:74-76. Doi: 10.1089/ind.2018.29120.xgl.

Leinweber, P., U. Bathman, U. Buczko, C. Douhaire, B. Eichler-Löbermaann, E. Frossard, F. Ekardt, H. Jarvie, I. Krämer, C. Kabbe, B. Lennartz, P.-E. Mellander, G. Nausch, H. Ohtake and J. Tränckner. 2018. Handling the phosphorus paradox in agriculture and natural ecosystems: Scarcity, necessity, and burden of P. Ambio. 47(Suppl. 1):S3-S19.
Lewandowsky, S. G.E. Gignac, and k. Oberauer. 2013. The role of conspiracist ideation and worldviews in predicting rejection of science. PLoS ONE 8:e75637.
Li, M., T. Wiedmann and M. Hadjikakou. 2019. Towards meaningful consumption-based planetary boundary indicators: The phosphorus exceedance footprint. Global Environmental Change 54:227-238.
Lindahl, J.F. and D. Grace. 2015. The consequence of human actions on risks for infectious diseases: a review. Infection Ecol. Epidemiol. 5:30048. http://dx.doi.org/10.3402/iee.v5.30048
Liu, Z., U. Klümper, L. Shi and M. Li. 2019. From pig breeding environment to subsequently produced pork: Comparative analysis of antibiotic resistance genes and bacterial community composition. Front. Microbiol. 10:43. Doi: 10.3389/fmicb.2019.00043.
Mackenzie, S.G., I. Leinonen, N. Ferguson, I. Kyriazakis. 2016. Can the environmental impact of pig production systems be reduced by utilizing co-products as feed? J. Cleaner Prod. 115:172-181.
Marano, N., P. Arguin, M. Pappaioanou, B. Chomel, E. Schelling, V. Martin, J.C. Butler, C.B. Beard and L. King. 2006. International attention for zoonotic infections. Emerg. Infect. Dis. 12:1813-1815.
Martin, M.J., S.E. Thottathil, and T.B. Newman. 2015. Antibiotics overuse in animal agriculture: a call to action for health care providers. Amer. J. Public health 105:2409-2410.
Marquardt, R.R., and S. Li. 2018. Antimicrobial resistance in livestock: Advances and alternatives to antibiotics. Anim. Front. 8:30-37.
Mathews, S.G., A.L. Miller, J. Clapp, T. Plötz, I. Kyriazakis. 2016. Early detection of health and welfare compromises through automated detection of behavioural changes in pigs. The Vet J. 217:43-51.
McCright, A.M., S.T. Marquart-Pyatt, R.L. Shwom, S.R. Brechin and s. Allen. 2016. Ideology, capitalisms, and climate: explaining public views aboutn climate change in the United States. Energy Res. Soc. Sci. 21:180-189.
McCright, A. and R.E. Dunlap. 2011. The politicization of climate change and polarization in the American public's views of global warming, 2001-2010. Sociol. Q. 52:155-194.
McMichael, A.J. and C.D. Butler. 2011. Promoting global population health while constraining the environmental footprint. Annu. Rev. Public Health 32:179-197.
McNeill, S.H., K.E. Belk, W.W. Campbell and C.L. Gifford. 2017. Coming to terms: meat's role in a healthful diet. Anim. Front. 7:34-42.
Meadus, W.J., P. Duff, B. Uttaro, J.L. Aalhus, D.C. Rolland, L.L. Gibson and M.E. Dugan. 2010. Production of docosahexaenoic acid (DHA) enriched bacon. J. Agric. Food Chem. 58:465-472.
Micha, R., and D. Mozaffarian. 2010. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids 45:893-905.
Miller, E.T., R. Svanbäck and B.J.M. Bohannan. 2018. Microbiomes as metacommunities: Understandin host-associated microbes through metacommunity ecology. Trends Ecol. Evol. 33:926-935.
Mogollón, J.M., A.H.W. Beusen, H.J.M. van Grinsven, H. Westhoek and A.F. Bouwman. 2018. Future agricultural phosphorus demand accornding to the shared socioeconomic pathways. Global Environ. Change 50:149-163.
Montagnese, C., P. Barattini, A. Giusti, G. Balka, U. Bruno, I. Bossis, A. Gelasakis, M. Bonasso, P. Philmis, L. Dénes, S. Peransi, M. Rodrigo, S. Simón, A. Griol, G. Wozniakowski, K. Podgorska, C. Pugliese, L. Mozaffarian, D., R. Micha and S. Wallace. 2010. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med 7:e1000252.
Mozaffarian, D., R. Micha and S. Wallace. 2010. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med 7:e1000252.
Murphy, K.J., B. Parker, K.A. Dyer, C.R. Davis, A.M. Coates, J.D. Buckley and P.R.C. Howe. 2014. A comparison of regular consumption of fresh lean pork, beef and chicken on body composition: A randomized cross-over trial. Nutrients 6:682-696.
Nakamura, M., D. Pendlebury, J. Schnell and M. Szomszor. 2019. Navigating the structure of research on sustainable development goals. Institute for Scientific Information. Webofsciencegroup.com
Nakov, D. S. Hristov, B. stankovic, F. Pol, I. Dimitrov, V. ilieski, P. Mormede, J. Hervé, E. Terenina, B. lieubeau, D.K. Papanastasiou, T. Bartzanas, T. Norton, D. Piette, E. Tullo and I.D.E. van Dixhoorn. 2019. Methodologies for assessing disease tolerance in pigs. Front. Vet. Sci. 5:329. doi: 10.3389/fvets.20a8.00329
Nardone, A., B. Ronchi, N. Lacetera, M.S. Ranieri, and U. Bernabucci. 2010. Effects of climate change on animal production and sustainability of livestock systems. Livestock Sci. 130:57-69.

National Pork Board. 2019. U.S. Pork… Sustainable for People, Pigs, and the Planet. https://www.pork.org/news/new-study-shows-u-s-porks-long-term-sustainability-progress/
Nesme, T., G.S. Metson and E.M. Bennet. 2018. Global phosphorus flows through agricultural trade. Global Environ. Change 50:133-141.
Nuernberg, K., K. Fisher, G. Nuernberg, U. Kuechenmeister, D. Klosowska, G. Eliminowska-Wenda, I. Fiedler and K. Ender. 2005. Effects of dietary olive and linseed oil on lipid composition, meat quality, sensory characteristics and muscle structure in pigs. Meat Sci. 70:63-74.
Österberg, J., A. Wingstrand, A.N. Jensen, A. Kerouanton, V. Cibin, L. Barco, M. Denis, S. Aabo and B. Bengtsson. 2016. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four European countries. PLoS ONE 11(6):e0157049.
Papargyropoulou, E., R. Lozano, J.K. Steinberger, N. Wright and Z. bin Ujang. 2014. The food waste hierarchy as a framework for the management of food surplus and food waste. J. Cleaner Prod. 76:106-115.
Rose, D., M.C. Heller, and C.A. Roberto. 2019. Position of the society for nutrition education and behavior: The importance of including environmental sustainability in dietary guidance. J. Nutr. Educ. Behav. 51:3-15.
Rowland, R.R.R., J. Lunney and J. Dekkers. 2012. Control of porcine reproductive and respiratory syndrome (PRRS) through genetic improvements in disease resistance and tolerance. Frontiers in Genetics 3:1-6. Doi: 10.3389/fgene.2012.00260.
Salemdeeb, R., E.K.H.J. zu Ermgassen, M.H. Kim, A. Balmford and A. Al-Tabbaa. 2017. Environmental and health impacts of using food waste as animal feed: a comparative analysis of food waste management options. J. Cleaner Prod. 140:871-880.
Scollan, N.D., E.M. Price, S.A. Morgan, S.A. Huws and K.J. Shingfield. 2017. Can we improve the nutritional quality of meat? Proc. Nutrition Soc. 76:603-618.
Scoppetta, F., M. Sensi, M.P. Franciosini and M. Capuccella. 2017. Evaluation of antibiotic usage in swine reproduction farms in Umbria region based on the quantitative analysis of antimicrobial consumption. Italian J. Food Safety 6:6886.
Skeaff, C.M. and J. Miller. 2009. Dietary fat and coronary heart disease: summary of evidence from prospective cohort and randomized controlled trials. Ann. Nutr. Metab. 55:173-201.
Smith, D., S. Lyle, A. Berry, N. Manning, M. Zaki and A. Neely. 2015. Internet of animal health things (IoAHT) – Opportunities and challenges. Cambridge Service Alliance www.cambridgeservicealliance.org
Smyth, S.J., P.W.B. Phillips and D. Castle. 2017. (Mis) information and the politicization of food security. Anim. Front. 7:33-38. Doi: 10.2527/af.2017.0116.
Song, G. 2014. Understanding public perceptions of benefits and risks on childhood vaccinations in the United States. Risk Anal. 34:541-555.
Sörqvist, P. and L. Langeborg. 2019. Why people harm the environment although they try to treat it well: An evolutionary-cognitive perspective on climate compensation. Front. Psychol. 10;348. Doi: 10.3389/fpsyg.2019.00348.
Tan, B. and Y. Yin. 2017. Environmental sustainability analysis and nutritional strategies of animal production in China. Annu. Rev. Anim. Biosci. 5:171-184.
Tatem, A.J., D.J. Rogers and S.I. Hay. 2006. Global transport networks and infectious disease spread. Adv. Parisitol. 62:293-343.
The Economist. 2018. Global Food Security Index 2018 – Building resilience in the face of rising food-security risks. https://foodsecurityindex.eiu.com/
Trudeau, M.P., H. Verma, F. Sampedro, P.E. Urriola, G. C. Shurson and S.M. Goyal. 2017. Environmental persistence of porcine coronaviruses in feed and feed ingredients. PLoS ONE 12:e0178094. https://doi.org/10.1371/journal.pone.0178094.
Tsiafouli, m.A., E.G. Drakou, A. Orgiazzi, K. Hedlund and K. Ritz. 2017. Editorial: Optimizing the delivery of multiple ecosystem goods and services in agricultural systems. Front. Ecol. Evol. 5:97 Doi: 10.3389/fevo.2017.00097.
Turner, T.D., C. Mapiye, J.L. Aalhus, A.D. Beaulieu, J.F. Patience, R.T. Zijlstra and M.E. Dugan. 2014. Flaxseed fed pork: n-3 fatty acid enrichment and contribution to dietary recommendations. Meat Sci. 96:541-547.
UNEP. 2019. Frontiers 2018/19 emerging issue of environmental concern. United Nations Environment Programme, Nairobi. https://wedocs.unep.org/bitstream/handle/20.500.11822/27538/Frontiers1819.pdf?sequence=1&isAllowed=y
United Nations. 2015. Transforming our world: the 2030 agenda for sustainable development. http://www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang=E
Uwizeye, A., P.J. Gerber, R.P.O. Schulte and I.J.M. de Boer. 2016. A comprehensive framework to assess the sustainability of nutrient use in global livestock supply chains. J. Cleaner Prod. 129:647-658.
Van Eenennaam, A.L. 2013. GMOs in animal agriculture: time to consider both costs and benefits in regulatory evaluations. J. Anim. Sci. Biotech. 4:37.
Völkel, I., E. Schröer-Merker and C.-P. Czerny. 2011. The carry-over of mycotoxins in products of animal origin with special regard to its implications for the European food safety legislation. Food Nutr. Sci. 2:852-867.

Vranken, E. and D. Berckmans. 2017. Precision livestock farming for pigs. Animal Frontiers 7:32-37.
Watts, N., W.N. Adger, S. Ayeb-Karlsson, Y. bai, P. Byass, D. Campbell-Lendrum, T. Colburn, P. Cox, M. Davies, M. Depledge, A. Depoux, P. Dominguz-Salas, P. Drummond, P. Elkins, A. Flahult, D. Grace, H. Graham, A. Haines, I. Hamilton, A. Johnson, I. Kelman, S. Kovats, L. Liang, M. Lott, R. Lowe, Y. Luo, G. Mace, M. Maslin, K. Morrissey, K. Murray, T. Neville, M. Nilsson, T. Oreszczyn, C. Parthemore, D. Pencheon, E. Robinson, S. Schütte, J. Shumake-Guillemot, P. Vineis, P. Wilkinson, N. Wheeler, B. Xu, J. Yang, Y. Yin, C. Yu, P. Gong, H. Montgomery and A. Costello. 2017. The Lancet Countdown: tracking progress on health and climate change. Lancet 389:1151-1164.
Weiss, F. and A. Leip. 2012. Greenhouse gas emissions from the EU livestock sector: A life cycle assessment carried out with the CAPRI model. Agric. Ecosys. Environ. 149:124-134.
Whitworth, K.M., R.R.R. Rowland, V. Petrovan, M. Sheahan, A.G. Cino-Ozuna, Y. Fang, R. Hesse, Al Mileham, M.S. Samuel, K.D. Wells and R.S. Prather. 2019. Resistance to coronavirus infection in amino peptidase N-deficient pigs. Transgenic Res. 28:21-32.
Wilfart, A., S. Espagnol, S. Dauguet, A. Tailleur, A. Gac, F. Garcia-Launay. 2016. ECOALIM: A dataset of environmental impacts of feed ingredients used in French animal production. PLoS ONE 11:e0167343. Doi:10.1371/journal.pone.0167343.
Wunderlich, S. and K.A. Gatto. 2015. Consumer perception of genetically modified organisms and sources of information. Adv. Nutr. 6:842-851.
Zinsstag, J., L. Crump, E. Schelling, J. Hattendorf, Y.O. Maidane, K.O. Ali, A. Muhummed, A.A. Umer, F. Aliyi, F. Nooh, M.I. Abdikadir, S.M. Ali, S. Hartinger, D. Mäusezahl, M.B.G. de White, C. Cordon-Rosales, D.A. Castillo, J. McCracken, F. Abakar, C. Cercamondi, S. Emmenegger, E. Maier, S. Karanja, I. Bolon, R.R. de Castañeda, B. Bonfoh, R. Tschopp, N. Probst-Hensch and G. Cissé. 2018. Climate change and One Health. FEMS Microbiol. Lett. 365:1-9. Doi:10.1093/femsle/fny085.

K2

Antimicrobial resistance and antimicrobial stewardship in food producing animals

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Antimicrobial resistance (AMR) is a global concern. Over the last years, more quantitative data have become available related to the transmission of resistant bacteria (or resistance genes) between humans, animals and the environment. Most obvious is the transfer of AMR from animals to humans with food borne pathogens such as resistant *Salmonella* spp. (e.g. *S.* Typhimurium DT104) and *Campylobacter* spp. (e.g. fluoroquinolone resistance). Regarding livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA), it is without discussion that occupationally exposed people have a considerable chance to test positive for LA-MRSA. For transfer of other resistance markers (e.g. Extended Spectrum Beta-Lactamases) the transmission between animals and humans is more difficult to quantify as this is not simply dependent of the transmission of bacteria but also dependent on the transmission of plasmids containing the resistance genes. In the Netherlands, following the One Health approach, a consortium combined all recent ESBL-data from humans, animals and the environment. In this study it was estimated that between 1-10% of ESBLs in humans has a (direct) source in animals. It should be noted however that this only a specific type of resistance in a very specific context (a highly developed country at a time when AMU was decreasing) and these are estimates because transfer of AMR is very complex. In the presentation more examples will be presented as well as the actions undertaken to reduce veterinary AMU in the Netherlands with an emphasis on pig farming. These actions have led to an almost 60% reduction in AMU in pig farming. New initiatives are currently aiming to reduce AMU on the higher than average antimicrobial users via tailored interventions.
AMR is high on the political agenda. After the publication of the WHO-Global Action Plan and the adoption of the resolution on containment of AMR by the General Assembly of the United Nations in 2016, countries were requested to prepare a National Action Plan (NAP) with a One Health approach to combat the emergence of AMR. A One Health approach is generally considered as essential for the containment of AMR, however, globally, hardly half of the countries has this One Health component in their NAP which underlines the urge for action.
One of the 5 pillars of the WHO-Global Action Plan requests for the implementation of surveillance systems for AMR and antimicrobial use (AMU) in all countries worldwide. Several countries have a reliable system implemented but there are clear gaps in data collection, in particular in Low and Middle Income Countries (LMICs). Therefore, there is limited information about AMU and AMR, particularly in these LMICs. Given the often unrestricted availability of antimicrobials without veterinary prescription, especially in rapidly growing economies with intensive livestock sectors, AMR is assumed to be high, which is confirmed by data from case-studies. With the global trade of food products and travel of people, it is of high importance to develop interventions for AMU and AMR not only in high income countries but also in LMICs.
Nowadays AMU/AMR is high on the political agenda of national and supranational organizations. There is however, still a considerable gap between policy and practice. Changes are urgently needed at practical level but this will only occur when there is a political will. It is therefore of utmost importance to use the currently existing political momentum.

K3

EU Pig welfare priorities: castration, tail docking and beyond

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Lately, it's all about the pig (and pig farmers and pig veterinarians)

2019 is the Year of the Pig, according to the Chinese lunar calendar. But so far there has been little to celebrate about this, not in China nor in many other countries. Pigs and pig farming have been at the center of the attention for many reasons.

The first is of course **African Swine Fever**, which is sweeping through many countries across the world, including in more than 8 European countries. In Europe, we saw it coming, prepared for it to the extreme, and still have great difficulties to keep the disease at bay. In the meantime, it led already to millions (if not billions) of domestic and wild pigs being culled and it is de-stabilizing the pig meat trade and even financial markets. At the time of writing, the disease has not yet entered Germany or Denmark, who are anxious doing what they can to avoid this at all cost.

The **fight against antimicrobial resistance** is another global and European priority. Under the perspective of "One-Health", it has become in Europe not only a scientific challenge but also a politically loaded one. While the political discussion is mainly around the human antimicrobial resistance burden, the use of antimicrobials for animals have come under great scrutiny. Although science show us that transfer of AMR from animals or animal products is only responsible for a smaller part of resistance problems in humans, the impact of the use of antibiotics in animals cannot be ignored. This has especially its repercussions in the pig sector, which has traditionally been an antibiotic dependent sector. Already in 2016, the European Medicines Agency urged all European countries to limit colistin use (much used in the pig sector) to a minimum. At the same time, many efforts were already done and are continuing to reduce the total amount of use of other antibiotics in pig farming and especially critically important antibiotics to an absolute minimum.

Another issue getting more and more attention in Europe and worldwide are **environmental issues and climate change**. For environmental reasons, by 2022, Europe's pig producers have to know how to wean pigs without using zinc oxide. After this date, zinc oxide can only be used in pigs as a nutritional component, at levels of 150 ppm. Looking at the impact of animal farming on our climate, pigs also do not score very well. At the comparison of the full life cycle of greenhouse emissions from animal products, pork comes out only as the second worst, after cattle, in most calculations.

And last, but not least, the **welfare of farmed pigs** has become under close scrutiny in the European Union. Enforcing legislation on the welfare of pigs, such as in respect to tail docking, live animal transport and slaughter of pigs, is currently one of the European Commission's priorities in the area of animal welfare.

EU legislation in respect to the welfare of pigs The EU has strict legislation in respect to the welfare of pigs. In 1986, the Council of Europe adopted its first recommendation on pig welfare, which was revised in 2004. The first European Union rules on pig welfare were established in 1991 with Directive 91/630/EEC. The 1991 Directive has been amended several times and substantially updated by Directive 120/2008/EC (hereby called the Pig Directive). In addition to these specific pig welfare legislations, European Union cross-sector animal welfare legislation exists such as in respect to transport and slaughter. Legislation adopted at a European Union level, has to be transposed into national legislation by all EU Member States. This was done quickly and efficiently for the Pig Directive. However, one particular aspect of the Pig Directive continues to cause difficulties in respect to implementation and enforcement, namely the ban on routine tail docking and the need to always give pigs suitable and sufficient enrichment materials. In my presentation, I will mainly focus on pig welfare issues in relation to tail docking, provision of enrichment materials and only shortly touch on pig castration.

Already since 1994 (25 years ago!), EU legislation stipulated that tail docking could not be done routinely and that, to prevent tail biting, enrichment materials such as straw or other suitable materials should be provided to satisfy the behavioural needs of pigs. Among other provisions, and compared to previous legislation, the Pig Directive specifies the measures that must be undertaken before a farmer can resort to tail docking (i.e., addressing management, stocking density, and providing specific enrichment materials). Nevertheless, tail docking is still practiced routinely in many European countries, in violation of these provisions. In Finland and Sweden, due to stricter national rules compared to EU legislation, tail docking is no longer allowed. Outside the EU, in Norway and Switzerland less than 5% of the pigs are tail-docked. Routine tail docking is also carried out in many countries outside Europe.

In the last 5 years, great efforts have been done by European decision makers to assist Member States to facilitate better implementation and enforcement of the relevant rules regarding tail docking and provision of enrichment materials. These include financing research projects regarding pig welfare (e.g., EuWelNet, FareWellDock [37]), study visits, recommendations, audits, factsheets and many others.

In 2017, the European Commission launched an EU action plan and asked all Member States to develop national action plans as the main tool to improve compliance with the Pig Directive. Despite all these efforts, non-compliance remains high. Mid-2019, the EU action plan to facilitate the rearing of pigs with intact tails will end; it will be evaluated and a future approach needs to be decided. Potential future approaches include 1/ to prolong the action plan and continue with a guidance-based approach, or 2/ to start infringement procedures.

One other much debated pig welfare issue is around pig castration. In 2010, the 'European Declaration on alternatives to surgical castration of pigs' was agreed. The Declaration stipulates that from January 1, 2012, surgical castration of pigs shall only be performed with prolonged analgesia and/or anaesthesia and from 2018 surgical castration of pigs should be phased out altogether. Despite the support and efforts of many, the deadlines of January 1, 2012 and 2018 were far from being met. The opinions on the animal-welfare-conformity and the practicability and efficiency of the alternatives to surgical castration are widely dispersed. Although countries using analgesia/anaesthesia routinely found this method practical and effective, only few countries seem to aim at meeting the deadline to phase out surgical castration completely.

Let's be clear on one point: concerns about pig welfare will not go away in Europe. Just in 2018, an EU-wide public facing campaign collected over 1 million signatures from European citizens calling upon the European Commission and Member States to fully enforce this 25-year old legislation and to also tackle the problem of surgical piglet castration without pain relief. Numerous European Petitions and European Parliament questions keep being raised in respect to pig welfare. Farm animal welfare is and will remain of paramount importance for European citizens, as shown by the results of the last special Eurobarometer on Animal Welfare.

Never waste a good crisis

ASF, need to reduce antibiotics, welfare concerns regarding pig farming, etc., counting it all up it is clear that the pig sector is going through a crisis moment. And as said by several prominent world leaders: never let a good crisis go to waste.

The EU has a self-sufficiency of about 111% for pig meat and exports about 13% of its total production. The EU is the first global exporter of pig meat worldwide and arguably wants to maintain this position. Now is the time to reflect how we can make European pig production future proof. How can we make pig farming more sustainable, more fair and secure for pig producers, less sensitive to diseases or disasters, less antibiotic dependent and more welfare friendly and societally accepted?

The European livestock farming sector is concerned that EU animal welfare legislation is causing a competitive disadvantage for EU products on the global market. However, this is not true, as shown by a 2018 Commission report looking at the impact of animal welfare on the competitiveness of European livestock producers. The overall costs of compliance with animal welfare standards are very low compared to other production costs (such as the cost of labour and feed) and on the contrary, better welfare increases the image (and price given for) the product.

Need for increased veterinary expertise!

Since the beginning of the farm animal welfare debate half a century ago, the focus has been on the negative side of animal welfare, with most research studying the harms induced by modern husbandry to animals and how to prevent them. The same is true for diseases, much efforts have been put on how to prevent and treat specific diseases. The time has come to change this approach, looking more at how can we promote positive welfare and robust animals. The role of the veterinarian has never been of greater importance to achieve this. Veterinary expertise is needed to make sure animal breeding is focusing on robust animals and to prevent diseases entering the farm. 'Prevention is better than cure'.

We need to look at keeping piglets healthy and happy from all possible angles. Animals that are well cared for and appropriately housed, will experience a better welfare, be less prone to infections and will need fewer antibiotics.

Veterinarians also can act as 'gatekeeper' to ensure correct use of antibiotics and advise their clients on how to prevent diseases, thereby limiting the need to treat animals with antibiotics. Veterinarians in addition can help educate and raise awareness among their clients to ensure correct and responsible use of antibiotics in animals.

Experience learned from the fight against antibiotic resistance, shows us that the success heavily depends on the commitment and willingness of all actors in the field to work together and to take actions. Farmers and the veterinary profession play a key role in this. Veterinarians should continue to strengthen their advisory role and competencies to support and educate farmers. The profession should continue to strengthen the position of the veterinarian, such as through having mandatory animal health plans with a contracted responsible veterinarian per farm.

Through a close farmer and veterinary relationship, more efforts can be done to prevent animals becoming sick and to ensure animals are kept in good welfare. Regular animal health visits by veterinarians to farms are obligatory under the EU 'Animal Health Law'. Many countries already partly introduced regular veterinary visits, however, the frequency, topics covered and type (obligatory or voluntary) of visits very much differs. For pig farms, in 15 out of 24 analysed European Union countries it is mandatory to have preventive animal health farm visits. Most of these visits (93,3%) only focus on national health control programs. There is huge potential to improve the value of these preventive veterinary visits, working through a whole sector approach with two-way information between the farm, the private and official veterinarian, the slaughterhouse and by covering a holistic approach looking at animal health, medicines use, biosecurity, animal welfare and food safety.

References

European Commission. African Swine Fever https://ec.europa.eu/food/animals/animal-diseases/control-measures/asf_en

[ii]J. 'O Neill. Tackling drug-resistant infections globally: final report and recommendations. https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf.

[iii]https://www.wur.nl/en/newsarticle/Chance-of-ESBL-contamination-via-livestock-farming-is-small.htm

[iv] European Medicines Agency. Sales of veterinary antimicrobial agents in 30 European countries in 2016. https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-30-european-countries-2016-trends-2010-2016-eighth-esvac_en.pdf.

[v]FAO. Global Livestock Environmental Assessment Model (GLEAM). https://www.ema.europa.eu/en/documents/scientific-guideline/updated-advice-use-colistin-products-animals-within-european-union-development-resistance-possible_en-0.pdf.

[vii] http://www.fao.org/gleam/results/en/

[vii]Council of Europe. Standing Committee of the European Convention for the protection of animals kept for farming purposes (T-AP). Recommendations concerning pigs. https://www.coe.int/t/e/legal_affairs/legal_co-operation/biological_safety_and_use_of_animals/Farming/Rec%20pigs%20rev%20E%202004.asp.

[viii]European Union. Council Directive of 19 November 1991 laying down minimum standards for the protection of pigs (91 / 630 /EEC). 1991. Official J European Union 1991, L 340 / 33.

[ix] European Union. Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs (Codified version). 2009. Official J European Union 2009, L47:5–13.

[x]European Union. Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations. 2005. Official J. European Union 2005 L 3, 5.1.2005.

[xi] European Union. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. Official J. L 303, 18.11.2009, p. 1–30.

[xii]De Briyne, N.; Berg, C.; Blaha, T.; Palzer, A.; Temple, D. "Phasing out Pig Tail Docking in the EU - Present State, Challenges and Possibilities." Porc. Heal. Manag. 2018, 4 (1), 1–9.

[xiii]17. European Food Safety Authority EFSA. The risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. 2007. EFSA Journal 611, 1-13.

[xiv]EUWelNet. Understanding environmental enrichment and tail docking requirements for finisher pigs in accordance with EU Directive 2008/120/EC, 2013. http://pigstraining.welfarequalitynetwork.net/Pages/0.

[xv] FareWellDock, 2019. https://farewelldock.eu/.

[xvi]European Commission. *Overview Report Study Visits on Rearing Pigs with Intact Tails*. 2016, DG Health and Food Safety.

[xvii]European Union. Commission Recommendation (EU) 2016/336 on the application of Council Directive 2008/120/EC laying down minimum standards for the protection of pigs as regards measures to reduce the need for tail-docking. Official J European Union, 2016, L62: 20.

[xviii]European Commission, Directorate Health and Food Audits and Analysis. Audit Reports. http://ec.europa.eu/food/audits-analysis/audit_reports/index.cfm.

[xix]European Commission. Cutting the Need for Tail-Docking; 2016, pp.14. https://ec.europa.eu/food/sites/food/files/animals/docs/aw_practice_farm_pigs_tail-docking_eng.pdf

[xx]European Commission. European Declaration on alternatives to surgical castration of pigs. https://ec.europa.eu/food/sites/food/files/animals/docs/aw_prac_farm_pigs_castalt_declaration_en.pdf

[xxi]De Briyne, N.; Berg, C.; Blaha, T.; Temple, D. Pig Castration: Will the EU Manage to Ban Pig Castration by 2018? Porc. Heal. Manag. 2016, 2 (2016), 1–11.

[xxii]www.endpigpain.eu

[xxiii]European Parliament Plenary, Written Questions, Parliamentary Terms 2009-2014 and 2014-2019. http://www.europarl.europa.eu/plenary/en/parliamentary-questions.html?tabType=wq#sidesForm

[xxiv]European Commission. Special Eurobarometer 442. Report: Attitudes of Europeans towards Animal Welfare. 2016. http://ec.europa.eu/COMMFrontOffice/publicopinion/index.cfm/ResultDoc/download/DocumentKy/71348

[xxv]European Commission, Agriculture and Rural Development, EU Market Observatories, EU Meat Market Observatory. Pig Meat. https://ec.europa.eu/agriculture/sites/agriculture/files/market-observatory/meat/pigmeat/doc/pig-market-situation_en.pdf

[xxvi]Copa-Cogeca. The Reaction of European Farmers and European Agri-Cooperatives to the EU Strategy for the Protection and Welfare of Animals 2012-2015; 2015.

[xxvii]European Commission. Report from the EC to the EP and the Council on the Impact of Animal Welfare International Activities on the Competitiveness of European Livestock Producers in a Globalized World, 2018.

[xxviii]FVE. 2015. Relationship between antibiotic use and animal welfare. https://www.fve.org/cms/wp-content/uploads/063-FVE_AWW-Position-on-resistance-and-animal-welfare_final.pdf

[xxix]Beemer, F. 2010. What would be the effects of decoupling the prescription and sale of veterinary medicines by veterinarians? Berenschot Report for the Ministry of Agriculture, Nature and Food Quality, The Netherlands.

[xxx]European Union. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law').

[xxxi] Nyhof E. 2017. Developing recommendations for preventive animal health farm visits in the EU. https://dspace.library.uu.nl/handle/1874/356529

K4

Animal welfare at transport and slaughter

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An increasing number of reports on serious violation of animal welfare in pig slaughterhouses has had significant impact on consumer behaviour concerning meat products in the past year. NGOs have been documenting the violation of animal welfare and cruel handling of animals in slaughterhouses throughout Europe and media has been reporting on these issues. This has led to shutdowns of plants and the prosecution of food business operators and veterinarians in some cases. Due to the broad publicity of the "scandals", governments responsible for inspections in slaughterhouses are being urged to take action. Why did inspections carried out by official veterinarians in these slaughterhouses fail? Do veterinarians on the plants receive adequate support by their competent authorities to stand up to the pressure of food business operators (FBOs) concerning animal welfare issues? Which steps will increase FBOs understanding to recognise the relevance of animal welfare during slaughter and carry responsibility?

Legal responsibility for animal welfare in slaughterhouses

The responsibility for complying with animal welfare requirements on behalf of the FBO has been strengthened by the European Hygiene Regulations (EG) No. 853 and 854/2004 and Regulation (EG) No. 1099/2009 on the protection of animals at the time of killing. FBOs are obliged to take the necessary measures to avoid pain and minimise distress and suffering for the animals in the slaughterhouses. The appointed animal welfare officer (AWO) defines the sensitive areas and operations dealing with animals according to Standard Operation Procedures (SOPs). He/She ensures that staff has an appropriate level of competence for its occupation and is trained concerning to the SOPs. In general, large companies can assess the relevance of employing qualified AWOs and the importance of SOPs. The risk of the companies` image being damaged by negative press is too high. Small slaughterhouses or butcheries however often lack understanding for these issues. They officially define SOPs and formally appoint an AWO, but finally the responsibility of keeping animal welfare under surveillance remains to the official veterinarian on the plant. Unfortunately the official veterinarians are often understaffed and are not in an adequate position to ensure compliance with animal welfare without support of the FBOs and the competent authorities.

Official veterinarians in slaughterhouses in Germany

European legislation does not differentiate between official veterinarians that mainly work in district offices and those working in slaughterhouses full- or part-time. The European food hygiene regulations refer to "official veterinarians" in general. In the German version official veterinarians are called "beamtete/r Tierarzt". German national legislation, however, differentiates between "Amtstierarzt" (ATA) and "amtlicher Tierarzt" (amtl. TA). The ATA works in a competent authority and has usually successfully passed training including exams in veterinary legislation and its enforcement. The ATA carries out assignments for the competent authority. One of these are inspections of slaughterhouses at least once a year.

The veterinarians working in slaughterhouses and on meat plants on a daily basis are called "amtliche TA" (amtl. TA). They are employed by district offices and their field of activity is restricted to specific slaughterhouses or meat plants. According to European hygiene legislation, the requirement for amtl. TA to work in slaughterhouses is 200 h of experience for inspections. The amtl. TA have usually not been trained specifically for European hygiene or animal welfare legislation and possible enforcement measures and consequently depend on their district offices for the prosecution of offenses. The amtl. TA can be payed according to two specific wage agreements. One of the agreements determines that payment is continued only for 6 days after the shutdown of a plant. Consequently, this agreement can influence the willingness of the amtl. TA to report infringements to their competent authorities as they may be sawing off their own branch.

Another reason for amtl. TA in slaughterhouses not to report offenses is a certain proximity to the food business operators. Depending on how well they are backed by their district offices and integrated in the structures, the veterinarians working on the plants inevitably develop proximity to the FBO. Additionally, district veterinary offices at times do not prosecute violations the amtl. TA report on, which does not encourage reporting offenses. At times, the exertion of influence against penalising animal welfare offenses originates from a higher political level within the competent authorities so that hands are tied for all veterinarians involved.

How can the animal welfare situation in slaughterhouses be improved?

In order to improve animal welfare standards in slaughterhouses a package of measures must be implemented. The FBOs must primarily take more responsibility for animal welfare in the slaughterhouses according to the regulation (EG) Nr. 1099/2009. AWOs and the staff require a high level of knowledge and competence for their occupation. The FBO must ensure this by regular training and suitable staff in the areas dealing with livestock. The official veterinarians, the FBOs and the AWOs need to communicate about animal welfare issues on a regular base. All official veterinarians dealing with animal welfare in the context of slaughterhouses must be well trained to identify and prevent and if necessary penalise the offenses in the field. Data collected on relevant findings for animals arriving at the slaughterhouse and the relevant findings acquired during post mortem inspections thus must be put at the centre of evaluation in terms of animal welfare indicators. After all, the situation in the stocks of origin and the handling of the animals during transportation are relevant for risk assessments in slaughterhouses.

The number of official veterinarians needs to be matched in a risk-oriented way to the specific demands for animal welfare on the plant. A higher percentage of offenses e.g. should lead to an increase. In order to assess the risk, the veterinarians on the plants and in the district offices need to communicate about animal welfare issues in context with slaughterhouses on a regular base. They also need to be technically equipped in such a way that they ca to monitor and evaluate animal welfare adequately.

The wage agreements and the contracts need to be adapted to the European body of rules in order to provide more clarity and transparency for amtl. TA concerning their responsibilities. They require reliable support by their district offices and reporting offenses to their authorities must no longer be stigmatised.

In general, training for official veterinarians working in slaughterhouses needs to be re-assessed in terms of legal requirements and demands. And finally, the mandatory installation of closed circuit television (CCTV) cameras would have a strong impact on the standard of animal welfare in slaughterhouses and such support official veterinarians. In Great Britain the mandatory installation of CCTV cameras has improved the standard of animal welfare during slaughter significantly. It therefore can be seen as a chance both for the FBOs and the authorities to significantly improve the standard of animal welfare in slaughterhouses. Mandatory CCTV could prevent bad press and such improve the reputation of slaughterhouses and the official veterinarians.

The **single biggest problem in communication is the illusion that it has taken place** (Bernard Shaw).

K5

Meat inspection and interventions to control biological hazards in pig abattoirs in the European Union

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Background

Traditional meat inspection developed in the 19th century was used practically unchanged throughout the 20th century. It focused on controlling classical zoonoses which, however, became eradicated or rare in modern times. Currently, the main food safety risks associated with carcasses of slaughtered pigs include bacterial pathogens faecally excreted by healthy pigs. Because these "invisible" agents are undetectable by traditional meat inspection, it has been recognised that official meat inspection needs to be revised regarding better protection of public health *via* meat including pork. Accordingly, in the EU, a set of new legislation was introduced in 2004 ("food hygiene package"), which adopted novel key principles for modernised meat inspection. They focused on the use of risk assessment-based systems, verified through auditing mechanisms, as they have better potential to protect public health than traditional inspection. To help that and better link different players in the meat chain in achieving the common ultimate goal, safe meat, the use of Food Chain Information (FCI) was introduced. The main responsibility for meat safety was placed on the food business operator (FBO). Subsequently, to further improve the concept and the legislation, the EU Commission indicated its intention to use a generic framework, including appropriate indicators (criteria), which would allow Member States (MSs) to conduct their own risk analysis and adapt, where needed and possible, the most appropriate meat inspection methods. Accordingly, the European Food Safety Authority (EFSA) implemented a large scientific effort to identify directions for improvements to meat inspection, with clear focus on carcass meat safety and with the ultimate goal of better public health protection. In 2011, this resulted in EFSA's key scientific recommendations for improved meat inspection of pigs, to be achieved through a risk-based, comprehensive and coordinated carcass meat safety assurance system targeting the most relevant (priority) meat-borne hazards. The scope of this contribution is to overview the scientific principles, current status and perspectives of the work towards such improvements in the EU.

Need for and use of visual-only meat inspection

Published studies quantifying how much palpation and incision during examination of pig meat/organs mediate microbial cross-contamination of meat with e.g. *Salmonella* and *Yersinia* are lacking, but based on most fundamental food hygiene principles it can be assumed that it is happening. EFSA's scientific opinion stated that the public health risk generated by palpation/incision of carcasses from non-suspect animals is likely higher than the public health risk posed by the abnormalities found by those techniques. Moreover, the abnormalities found are largely of animal health relevance or quality issues rather than pork safety concerns. Therefore, omitting palpation/incision during *post-mortem* inspection of non-suspect pigs is considered as providing overall microbial meat safety benefits, although in suspect animals, the use of those manual techniques may be needed. A number of studies conducted in different countries indicated that the hands-off approach is justified and enabled a gradual shift from traditional inspection to visual-only pig meat inspection. They also showed that the change from traditional to visual-only inspection method poses negligible, or low at most, increase of the public health risk. Accordingly, the EU Meat Inspection Regulation 854/2004 was amended with Regulation 2018/2014 and visual-only inspection became the standard pig meat inspection method in the EU. The most recent EU Meat Inspection Regulation 2018/2014 does not differentiate between pig age or production systems, and allows visual-only inspection for all categories of pigs. Nevertheless, because manual examination continues to be relevant in the case of suspect/high-risk pigs and when required by international trade partners, alternative techniques aimed at avoiding cross-contamination caused by palpation/incision have been investigated. The proposed solutions include, for example, disassembled slaughtered-scalded pigs from outside-in as well as using imaging (vision) technology to detect and differentiate abnormalities on carcasses and organs including contamination. It has to be kept in mind that visual-only inspection has been introduced to improve control of public health hazards, but omitting palpation/incision can reduce the sensitivity of detecting some animal health/welfare hazards. Hence, further work is needed regarding the contribution of meat inspection to the overall surveillance/monitoring of pig health and welfare.

Need for and use of Food Chain Information

The main intention with the Food Chain Information (FCI) in the risk-based pig meat inspection system is evidence-based risk categorisation of incoming pigs regarding their hazard burden (i.e. farms of origin) as well as of the slaughterline processes regarding their risk-reducing capacity (i.e. abattoirs). Then, an informed decision on the best way to achieve targeted pig meat safety of the final carcasses can be made. For proper use of FCI, systematic collecting, recording, reporting and analysing of the necessary data are required. That can include historical hazard-testing data (on-farm and at-abattoir); production practices and risk-reduction interventions applied, data from Hazard Analysis and Critical Control Point (HACCP) verification, historical meat inspection data, and harmonised epidemiological indicators (HEIs) related to individual hazards in pigs (on farm) and meat (at abattoirs). In the EU, FCI is defined in Annex II of EU Regulation 853/2004 which relates mainly to the animal herd and its owner. The HEI is a relatively new concept, proposed and generically outlined by EFSA, in which a range of data from hazard testing in animals and carcasses and/or from auditing the farming and transport-lairage practices can be used. For that, each HEI's purpose, methodology, criteria separating acceptable from unacceptable, practicality and cost-benefit have to be determined. This requires good coordination along the meat chain and harmonisation of the regulatory system. With forward flow of FCI, the main benefits would include dividing incoming pigs at the abattoir based on their hazard status, e.g. level of *Salmonella*, which would enable the abattoir to choose and focus on the most beneficial control measures for those particular pigs. However, for that, a surveillance program is required, but currently it is run only in relatively few EU MSs, probably due to financial and practical constraints. With backward flow of FCI, the main benefits would include improving on-farm pig health, as information from abattoirs includes the various abnormalities/lesions found at *post-mortem* inspection. Nevertheless, the intended on-farm benefits are not always achieved, because of variations in how the abnormalities during meat inspection are categorised and recorded, and because some producers do not fully convert the feedback received into actions for improvements. Overall, while the potential of the FCI system has been recognised, it remains not fully developed and is underutilised in practice at present. The main reasons for that might include unclearness of what information is required from and insufficient/inaccurate information provided by individual players in the meat chain, as well as using FCI disjointedly from other control strategies with which it is supposed to go hand-in-hand. Some surveys indicate that FCI works noticeably better in the meat chains that are more integrated and comprise larger FBOs than in less integrated chains with smaller businesses, which seems logical. Further work is needed to identify the FCI system's specific objectives more clearly and translate them into meaningful parameters on which all the meat chain players can act efficiently. Further work is also needed on other existing aspects of FCI, e.g. improving the abnormality-recording system in meat inspection, fully developing HEIs, and evaluating some potentially useful novel tools (e.g. multi-serological/microarray herd profiles for priority hazards and potential use of Acute Phase Proteins levels in serum).

Generic framework for risk- and food chain-based meat safety assurance in pig abattoirs

For effective control of the priority hazards on pig carcasses, a range of measures need to be applied through a comprehensive, coordinated and risk-based carcass meat safety assurance system. In EFSA's scientific opinion on revision of pig meat inspection from 2011, a generic framework for such a system was outlined. Its main aspects include utilisation of the following data in a coordinated way: identification and traceability of pigs and meat; FCI focused on risk-reduction performances of farms and abattoirs to risk categorise both businesses; hazard control measures applied through Good Manufacturing Practice and Good Hygienic Practice (GMP/GHP)- and HACCP-based programs; and control measures through meat inspection *per se*. In such a system, well regulated, measurable meat safety targets and incoming animal-related safety targets are both needed. Assurance that each abattoir's system works as expected is provided through official verification and auditing, meaning the targets are met.

Control of priority bacterial hazards

Whether and how much pig carcass meat will be contaminated with *Salmonella* and/or *Yersinia* is primarily dependent on: a) the presence and level of these hazards in/on pigs delivered for slaughter (hence, the performance of the farms of origin), and b) the extent the hazards are transmitted from pigs and the abattoir environment onto the meat (hence, the hygiene performance of the abattoir). On the farms, *Salmonella* and *Yersinia* shed by asymptomatic pigs can be spread *via* feed-animal, animal-animal and animal-environment-animal routes, and total elimination of these hazards is possible but difficult to achieve. To minimise the risk, a range of on-farm control measures can be considered, and it is up to the risk managers to decide, within their specific situation and conditions on their farm, how much emphasis and what resources should be put on any of the individual options to achieve the targeted outcome.Contamination of carcasses with priority bacterial hazards (*Salmonella* and *Yersinia*)

in abattoirs occurs mainly due to direct or indirect contamination by faeces from those animals shedding the hazards. Hence, control measures in abattoirs aim at improving the process hygiene and effective cleaning-sanitation regimes. Because abattoirs differ regarding technology/equipment, extent of standardisation, available expertise, hygiene training and application, and motivation of staff and management, they can be categorised in respect of their risk reduction capacity. This can be achieved through the Process Hygiene Criteria (PHC) which include the maximum values for indicator bacteria (total viable count and *Enterobacteriaceae* count) and prevalence of *Salmonella* on the final carcasses. If these PHC are not met, the abattoir processes must be improved, but the product (meat) is not withdrawn from the market. For *Yersinia*, no such PHC exist in the current EU legislation. Information on risk category of each abattoir is useful, as it is unlikely that, for example, a high-risk abattoir (i.e. with low risk-reduction capacity) would handle incoming pigs posing high *Salmonella/Yersinia* risk effectively enough to reduce to an acceptable level the risk of pathogens being present on carcasses. The main process hygiene-based control measures regarding priority bacterial hazards on pig carcasses include: effective sanitation of trucks and lairage, logistic slaughter (low-risk/sero-negative pigs first), proper scalding in clean water (e.g. at 62°C), effective cleaning-sanitation and optimal design of de-hairing machines, ensuring good quality singeing (at 1300-1500°C) of the skin after de-hairing, making sure subsequent skin polishing does not negate the desirable effects of singeing, hygienic evisceration (sealing the rectum, prevention of gut content spillage), handling-removal of the tongue without cross-contaminating the carcass, preventing aerosol-mediated cross-contamination during carcass washing, and effective chilling of carcasses (≦7°C). Various carcass decontamination treatments can be used to further reduce priority bacterial hazards on pig carcasses, when the meat safety target cannot be achieved through process hygiene measures only. Note that carcass decontamination is an additional measure but is not a replacement for proper process hygeine-based control measures. Information on the effects of decontamination specifically on *Salmonella* and *Yersinia* on pig carcasses is relatively scarce. Rather, the large majority of published studies reported the achieved reductions of general microbiota including fecal indicators. Nevertheless, it was shown that various hot water treatments (e.g. 80°C/15 sec) can reduce *Salmonella* counts on pig carcasses. Care must be taken when selecting temperature-time regimes as some meat discoloration can occur in the process, either temporary/reversible or more permanent. Also, 2% lactic acid treatment can reduce *Salmonella* prevalence by two-fold. Higher acid concentrations are more effective, but detrimental effects on meat colour/flavour were observed in such cases. To enhance the antimicrobial effects, organic acid treatments can be combined with pre-treatment of carcasses with hot steam. Other carcass decontamination treatments reported include combinations of steam (130°C) and ultrasound (30-40 Khz), or steam and vacuum. Quantitative information on *Salmonella* reductions achieved by decontamination of pig carcasses is very limited, and for *Yersinia* it is lacking, but based on measuring other bacteria on pig carcasses or carcasses of other red meat animal species it seems that 1-2 log reductions of these pathogens could be achievable. It should be noted that a risk assessment conducted by EFSA in 2010 indicated that a reduction of two logs (99%) of *Salmonella* numbers on contaminated carcasses would result in more than 90% reduction of the number of human salmonellosis cases attributable to pig meat consumption, while a reduction of one log (90%) would lead to >80% reduction of human cases.

Control of priority parasitic hazards

In pigs on-farm, the presence/levels of *Toxoplasma gondii* and *Trichinella* are affected by zoo-sanitary conditions (e.g. biosecurity), whether the farm is an intensive or extensive system, if it is indoor or outdoor farming, and whether the pigs are fattening or breeding animals. Generally, in pigs raised in intensive, indoor farming systems, the occurrence of both parasites is lower than in smaller, outdoor farming systems, which can be utilised to differentiate farms/herds into lower and higher risk in the context of FCI. In addition, historical parasite testing data from the same farms/herds, monitoring, and epidemiological situation/geographical risks, including HEIs, can be fed into the FCI. According to the current EU Directive 2015/1375, only pigs from farms with low biosecurity are required to be tested for *Trichinella*, while those from farms with high biosecurity (controlled housing) are exempt, and the requirements for a herd to be officially recognised as a holding or as part of a controlled housing compartment are enlisted in Annex IV of the *Trichinella* Directive. The compliance is assessed through regulatory or independent third-party auditing. At the abattoir, if incoming batches of pigs are categorised as *Trichinella and/*or *T. gondii* low-risk (based on FCI and historical testing data), they do not have to be tested for these parasites or subjected to any parasite-inactivation treatments. Otherwise, for detection of *Trichinella*, the artificial digestion method is used. Currently, pig carcasses are not mandatorily tested for the

presence of *T. gondii*. The reasons include, as indicated in EFSA's scientific opinion from 2018, difficulties with methods differing in respect to their characteristics (e.g. discrimination between viable and non-viable parasites) and performance (i.e. sensitivity and specificity), which make the current methods unsuitable for routine testing of meat. When necessary, PCR and mouse bioassay are the most commonly used direct detection methods, followed by microscopy and cat bioassay. Moreover, *Toxoplasma* can be inactivated by effective heat-treatment (e.g. 58°C/9.5 min), freezing (e.g. -12°C/2 days) or curing (e.g. 3.3% salt in brine/3 days/20°C) of the meat.

Concluding Remark

It is envisaged that the future full development and implementation of a new meat safety assurance system in the EU will include stepwise improvements, adjustments, harmonisation and fusion of the existing meat safety systems under the new system's principles indicated in the generic framework recommended by EFSA.

K6

Zoonotic pathogens in the pork supply chain – what should be the responsibilities of the preharvest sector?

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A farm to table approach to food safety implies all participants along the food supply continuum, from production through to consumption, will bear some responsibility for mitigating the risk of foodborne illnesses. It is hard to disagree that all sectors should do what they can to contribute to a safer food supply. But at a practical level, how do we define and incentivize specific practices or changes in behaviour, which almost invariably will come at some cost. Conceptually, an ideal 'farm to table' program would define the set(s) of interventions throughout the continuum that optimizes the safety of food at the lowest overall cost. Furthermore, the optimal strategy may well differ among geographic regions, and over time, as the relative importance of different foodborne pathogens varies. To date there has been no articulated strategy for coordinating and incentivizing preferred interventions to optimize pork safety across different segments of the pork supply chain. The 1990s was an era of much optimism regarding the potential for 'preharvest food safety' to address foodborne disease risks, and this momentum spawned the 'Safepork' experience. Then, as now, *Salmonella* was the premier concern in pork safety in developed countries, and the Safepork community was born in 1996 when Dr. Paula Fedorka-Cray convened a meeting in Ames, Iowa, titled the "Ecology of Salmonella in Pork Production". Unequivocally, preharvest control has delivered societally impactful successes in reducing the risk of parasitic (*Taenia solium, Trichinella spiralis, Toxoplasma gondii*) and chemical (e.g., antibiotic residues) hazards in pork (Davies, 2011). However, almost a quarter of a century after our founding event, I suggest that progress in preharvest control of enteric bacterial pathogens (e.g., *Salmonella, Campylobacter, Yersinia, Listeria, E. coli*) remains remarkably unremarkable. An unsurprising conclusion is that the feasibility, effectiveness, and affordability of preharvest interventions for reducing the risk of zoonotic foodborne pathogens is highly variable, and is a function of the ecology and epidemiology of the respective pathogens along the supply chain.

There is no question that a preharvest strategy to 'attack the problem at its roots' is conceptually appealing, particularly to consumers. However, for enteric bacterial pathogens (EBPs), it is clear that effective, affordable preharvest control is much easier to talk about than to achieve! A fundamental question is whether current knowledge and 'the state of the art' of preharvest interventions for EBPs are sufficient to underpin effective control programs that actually deliver reduced risk to consumers. In the USA, the HACCP/Pathogen Reduction Act of 1996 made major changes to the regulation of food safety for meat and poultry in the USA. At the time, the inclusion of preharvest measures was much debated, but ultimately was not adopted. As we commented at the 3rd 'Safepork' meeting in 1999, 'the likely wisdom of that decision resides in the fact that current epidemiologic knowledge of most foodborne pathogens in animal populations is inadequate to enable reliable and cost effective control measures to be mandated' (Davies and Funk 1999). Two decades later at this 13th Safepork meeting, is there any EBP for which we have reached the point that we can mandate preharvest interventions that will reliably reduce foodborne risk to consumers, or even at the point of harvest of pigs? Are there EBPs for which current knowledge indicates that investing resources in preharvest control may be a fool's errand? And how should we prioritize EBPs so that research resources are focused to deliver the 'holy grail' of effective and reliable preharvest control where it is deemed important. The following discussion highlights several pathogens whose differing epidemiology points to different courses of action.

1.The case against preharvest research (*Campylobacter, Listeria, S. aureus*)The rationale for preharvest food safety is founded on two premises: a) Modifying farm (or farm supplier) practices will reduce the likelihood of foodborne hazards occurring in animals at the point of harvest; and b) This reduction will be sustained after harvest to the point of consumption and thereby reduce the incidence of foodborne disease (Davies et al. 2004). The ultimate misallocation of resources for preharvest control must be for pathogens for which pigs rarely, if ever, are the source of human infection. Foodborne risk cannot be directly inferred merely from the prevalence of putative pathogen in a livestock species (unless it is zero!). Unlike parasitic pathogens that exist in host tissues as part of their life cycle, presence of EBPs in pork products occurs due to contamination events during harvest or at subsequent steps in the supply chain. However, the ultimate risk to consumers due to this contamination is largely influenced by the abilities of pathogens to survive and thrive in post harvest environments. There are marked differences among EBPS in their ability to survive events in the processing of pigs, such as blast chilling (Nesbakken et al., 2008). Pathogens that are capable of multiplying at refrigeration temperatures (e.g., *Listeria* and *Yersinia*) pose additional challenges compared to pathogens that do not. Listeria can survive and multiply under a broad range of environmental conditions and form biofilms, while *Campylobacter* appear to have limited ability to multiply outside the host. Such fundamental differences between pathogens dictate that optimal control strategies must be different.

Campylobacter are among the most prevalent agents of human bacterial enteritis globally, with *C. jejuni* responsible for over 90% of human cases in developed countries (Sheppard and Maiden 2015). *Campylobacter* spp. are ubiquitous as part of the normal intestinal flora of pigs, with *C. coli* usually comprising 90–100% of isolates. Despite this high prevalence in pigs, numerous studies over decades have consistently indicated that pigs and pork play a negligible role in the epidemiology of human campylobacteriosis (Newell et al, 2016; Tyson et al., 2016; Thepault et al., 2017). As such, it is hard to make a case for investing effort to define specific interventions to control *Campylobacter* in pigs or pork, and particularly in the preharvest sector. Even in poultry, where considerable research investment has been warranted, effective control measures in the preharvest sector are conspicuously absent beyond generic measures such as improved biosecurity to prevent introduction into negative flocks (Wagenaar et al, 2013). This should not surprise us, as *Campylobacter* are widely distributed in nature and constitute part of the normal commensal microbiota of many thermophilic species. Particularly in mammals (vs. poultry, where hatcheries can facilitate exclusion of bacteria from birds), perhaps it is naïve to expect that simple changes in farm management can easily and reliably disrupt host–commensal relationships that have evolved over millennia. Accepting that pigs and pork play a negligible role in human campylobacteriosis, can we infer that they play a similarly negligible role in the epidemiology of antibiotic resistant campylobacter infections in humans (Alban, Nielsen, and Dahl 2008)? Certainly there is an argument for ongoing surveillance of *Campylobacter* across the food supply and in environmental reservoirs to advance epidemiological understanding. But it is difficult to make a case that *Campylobacter* research in swine is important for improving pork safety, and even more so that this should involve the preharvest phase.

Listeria monocytogenes was first identified in 1926, but emerged much later (>1983) to be an important foodborne pathogen. This relatively recent emergence has been attributed to changing eating habits and large-scale industrial food processing (Camargo et al., 2016). *L. monocytogenes* is considered ubiquitous in nature, and a normal inhabitant of the distal intestine of many species. It is also a recognized pathogen of several farm animal species, including pigs, but is of greatest importance in ruminants. Foodborne listeriosis has been linked to a vast range of food items, including pork products (Duranti et al., 2018).Clinical listeriosis is relatively rare in pigs, but one outbreak in pigs was linked to poor quality silage, which is a well established risk factor in ruminants (Stein et al., 2018). Beyond this, there appears to be negligible information related to control of *Listeria* in swine production. I would argue that this is a triumph, and that the situation need not be remedied! There is ample and recurring evidence of the problems in controlling *Listeria* in the post-harvest sector, and particularly in food processing (Awofisayo-Okuyelo et al., 2016). Notably, in many investigations *L. monocytogenes* has been isolated from ready to eat foods post processed in retail facilities, where inadequate procedures are also identified (Rodríguez-López et al., 2018). It is also established that *Listeria* are commonly found in domestic environments (Beumer et al., 1996) and that the final segment of the food chain cannot be ignored for Listeria and other foodborne bacteria (Azevedo et al. 2014). Given the demonstrated potential for *Listeria* contamination in downstream segments of the pork supply chain, I hold the view that resources should not be squandered in likely inefficient preharvest efforts. The food processing industry has been making considerable effort to reduce *Listeria* risk, particularly in 'ready to eat' foods (Jordan and McAuliffe, 2018). There are clear grounds for re-energizing efforts to reinforce education about the responsibilities of consumers and the considerable impact of their kitchen hygiene, food handling and refrigeration practices. Despite being among the longest recognized and most prevalent foodborne pathogens (Kadariya, Smith, and Thapaliya 2014), *Staphylococcus aureus* was not featured at Safepork until 2007, in the wake the first recognition of livestock associated ST398 MRSA (LA-MRSA) pigs in the Netherlands. The ongoing hysteria surrounding the public health risks associated with LA-MRSA in the media has distorted discussions of this agent in pigs. However, the focus of this paper is strictly on addressing risks of foodborne illness, and assessment of the arguments for preharvest control. Currently there is negligible evidence that the foodborne route plays a significant role in transmission of LA-MRSA to the broader community (Wendlandt et al., 2012; Larsen et al. 2015). We must remember that the importance of *S. aureus* as an agent of foodborne disease is due to production of enterotoxins, and antibiotics have no role in the treatment of staphylococcal enterotoxicosis. Indeed, staphylococcus enterotoxicosis is the major cause of

foodborne intoxications globally, and it is noted that the largest epidemiological reservoir of toxigenic *S. aureus* is the human nose (Szabo et al., 2012; Fetsch and Johler, 2018) Over 20 staphylococcal enterotoxins have been identified, but the vast majority of cases are due to the 'classical' enterotoxins SAE, SEB, SEC, SED and SED (Fetsch and Johler 2018). Although meat products (particularly processed) are often implicated in staphylococcal food poisoning events, it is recognized that improper handling and storage at elevated temperatures typically play a key role in enabling bacterial multiplication and enterotoxin production. Because *S. aureus* is part of the normal commensal flora of both pigs and humans, there is clear potential for the organism to enter the food supply anywhere on the continuum from the farm to the kitchen. Again, because *S. aureus* is part of the normal flora of healthy pigs, simple management interventions are unlikely to have significant impact on prevalence in the live animal population. Our investigations of *S. aureus* in swine in the USA indicated that MRSA was relatively uncommon compared with many European countries, that 3 MLST types (ST5, ST9 and ST398) comprised most isolates, and that classical enterotoxin genes were uniformly absent from these swine isolates (Sun et al., 2015). Other studies have also shown relatively low prevalence of enterotoxin genes in swine isolates compared with human isolates (Moon et al., 2015), and that enterotoxin genes are typically absent from ST398 MRSA (Zarfel et al., 2013). Current evidence suggests that the predominant source contaminating pork products with toxigenic *S. aureus* is humans involved in the post-farm sectors. Consequently, efforts to reduce (typically non-toxigenic) *S. aureus* prevalence on farms would likely have no impact on the risk of staphylococcal food poisoning. Given that consumers themselves are potential sources of contamination of food with *S. aureus*, consumer education about food handling and storage practices must remain a key focus for mitigating the risk of staphylococcal enterotoxicosis.

2.The case for redirecting preharvest research (*Yersinia, Salmonella*)Essentially two goals are available for preharvest control of foodborne pathogens. The first is the complete exclusion of the agent(s) from farms animals and their environment, which renders measures to control transmission within a herd unnecessary. The alternative is non-exclusion, where the presence of the pathogen(s) in the population is expected and tolerated, and interventions are implemented to reduce the prevalence of the agent(s), including interventions to reduce pathogens immediately before shipping. The choice between these options is pivotal. Along the lines of specific pathogen free (SPF) production, which excludes specific pathogens to protect animal health, exclusion should be the most appealing option. However, the feasibility and cost of establishing and maintaining pathogen free populations is highly variable, and governed by the epidemiology of individual organisms. For foodborne pathogens, the prime examples of successful exclusion are the foodborne parasites *T. solium*,*T. spiralis*, and *T. gondii* which have relatively simple epidemiology, coupled with negligible risk of downstream contamination in the food chain (Davies, 2011). The challenge of exclusion is clearly much more daunting for bacteria than parasites, and its feasibility is greatly influenced by production systems, and possibly geographic and climatic factors. For example, the challenge of excluding *Salmonella* and *Campylobacter* from cohorts of broiler populations hatched in hatcheries and raised in all-in/all-out facilities for 6 weeks, is vastly different to the scenario offarrow-to-finish swine facilities, or even cohorts of growing pigs reared over 6 months. There are prominent examples of successful exclusion of EBPs from food animal populations, but they have not proven easy to emulate. Most notably, the Swedish program for *Salmonella* control in swine and poultry (presented at the first 'Safepork' meeting in 1996) has been in place since the 1960's, and similar programs exist in Finland and Norway. In an overview of unresolved questions about *Salmonella* control in pigs at the 1999 'Safepork' meeting, we stated 'perhaps the most eloquent statement of the difficulty and cost of implementing the 'Swedish model' for *Salmonella* control is that, despite its apparent success, after some 40 years it has not yet been adopted by any major swine or poultry producing nation' (Davies and Funk 1999). Twenty years later, that has not changed. Highlighting the differences among production systems, it is instructive that goals of preharvest components of national *Salmonella* programs established in Denmark in the 1990s were exclusion for the broiler industry, but non-exclusion for the swine industry (Wegener HC et al., 2003).

Generally EBPs in swine and other species display enormous genetic diversity, and not all variants are of equal importance to human and animal health. In contemplating control programs, whether and how to prioritize the subsets of an EBP that are most significant to public health are also pivotal questions. I will discuss this issue in reference to *Y. entercolitica* and *Salmonella*. There are about 20 species in the genus *Yersinia*, of which 3 are pathogenic to people, including the foodborne pathogens*Y. enterocolitica* and *Y. pseudotuberculosis* (Drummond et al., 2012). Of these, *Y. enterocolitica* causes the majority of foodborne *Yersinia* cases,

and the pig is considered the major reservoir of pathogenic variants. The organism has broad genetic diversity, categorized into serotypes (>57) and biotypes (6), and the vast majority of variants are not pathogenic to humans (Peruzy et al. 2017). With respect to human disease, the most important biotype/serotype combinations are biotype 4/serotype O:3 (Europe, world-wide), biotype 2/serotype O:9 (Europe, world-wide), biotype 1B/serotype O:8 (USA), and biotype 2/serotype O:5,27 (USA/Japan/Europe) (Nesbakken, 2014). It would clearly be a fools errand to design any control program for the ubiquitous generic*Y. enterocolitica* rather than targeting the few variants implicated in human disease. Furthermore, geographic variability in the predominant pathogenic variants also means that prioritization of variants may need to be driven by local epidemiological data. A 2014 review of Y. *enterocolitica* within the pork production chain concluded that there is a need to reduce the occurrence and spread of the organism, and claimed it would be 'most effective' to control enteropathogenic *Yersinia* in the preharvest sector (Laukkanen-Ninios et al., 2014). However, the authors also noted that to date 'feasible intervention methods are lacking'. There are some data indicating that an exclusion strategy could be applicable for pathogenic Y. *enterocolitica*. Studies of SPF herds in Denmark and Norway have demonstrated most herds for free of pathogenic variants (Christensen, 1980; Nesbakken et al., 2007), and negativity was maintained in herds for over 10 years in Norway. Also, there is potential for effective herd classification using serology (Felin et al., 2019). Given the dearth of evidence on approaches to reduce prevalence in infected herds, and the relatively limited range of environmental reservoirs of pathogenic *Y. enterocolitica*, the exclusion approach may have the most potential for preharvest control this agent. To date, no country has embarked upon a preharvest control program for Y. *enterocolitica*. One explanation may be the relatively low incidence of human cases, compared to *Salmonella* and *Campylobacter*. In the USA, the annual incidence estimated (0.28 cases annually per 100,000 people) is orders of magnitude less that the incidence of *Salmonella* and *Campylobacter* (https://www.cdc.gov/foodnet/pdfs/FoodNet-Annual-Report-2015-508c.pdf). Furthermore, the FoodNet data show a 60% reduction in incidence over the last 20 years, and a similar reduction has been achieved in Denmark (Boes et al., 2018). Both are likely attributable to improved slaughter hygiene and perhaps altered consumer behaviours, but these substantial reductions occurred in the absence of any preharvest control efforts. Although there is some potential for preharvest control of Y. *enterocolitica*, particularly using exclusion, the relatively low impact of the disease on public health, coupled with the apparent effectiveness of post harvest interventions, poses the question of the cost-effectiveness of embarking on such a program. The costs and logistic challenges of translating a low herd prevalence of pathogenic *Y. enterocolitica* in a small number of SPF breeding herds into an industry wide prevalence reduction that would be sufficient to reduce risk to consumers should not be underestimated!. do not intend to plunge into the abyss of attempting to comprehensively discuss the vast body of work on preharvest control of *Salmonella* in swine, but to make some reflections and comparisons with pathogenic Y. *enterocolitica*. Firstly, *Salmonella* has a much more complex epidemiology in terms of host range and environmental reservoirs. Secondly, unless an exclusion model is adopted, I believe that we have not adequately resolved the question of whether preharvest efforts should address *Salmonella* generically or some sub-set of variants that are deemed of greatest importance to human health at a national level. This question is much more complex than for *Yersinia*, where a relatively small and stable subset of virulent variants have been defined. The emergence of previously unknown or rare *Salmonella* variants over time has been a feature of *Salmonella* epidemiology for decades (Cherubin, 2011), and likely centuries, supporting the traditional view of medical microbiologists that all *Salmonella* are pathogens. In contrast, the broiler industry in Australia saw the widespread emergence of the avirulent *S. sofia* across the industry (Duffy et al., 2012). There is little question that *Salmonella* can vary widely in pathogenicity for humans and animals, but currently there is no practical approach to capitalize on this in preharvest control programs. Obviously, serotype specific programs for prevalent S. Typhimurium, or S. Derby should bring some logistic advantages but may soon be undermined by emergence of other variants. One is the increased plausibility of vaccination, given that to date immunity appears to be relatively serotype specific. Despite our initial enthusiasm in the 1990s, and the modest promise of some interventions related to feeding practices (e.g. particle size and organic acids), we have fallen well short of the dream (Dahl 2013). Development of valid, reliable pre-harvest interventions to control EBPs remains the unconquered mountain in pork safety research. Over two decades of experience has indicated that epidemiological studies face great limitations beyond hypothesis generation, and that facile changes in management are unlikely to produce epidemiologically significant and sustained reduction in prevalence for most EBPs. 'Silver bullet' interventions have remained elusive, and are unlikely to be achieved by a simple 'trial and

error' approach. Fundamental studies to understand the biological phenomena of intestinal colonization may give better opportunities to deliver the 'holy grail' of cost-effective pre-harvest control of foodborne bacteria. At least for *Salmonella*, current understanding suggests that substantial reduction in prevalence must be achieved in animals entering harvest facilities before this will translate into reduced carcass contamination (Baptista et al., 2010; Dahl 2013). The lairage dilemma still exists and has great potential to negate incremental gains that are achieved in the preharvest sector, unless successfully adopted across most of the supply chain. Understanding and effectively communicating the obstacles to achieving substantial reduction of EBPs in the preharvest sector is an important responsibility in informing the debate as to how societal resources are best used to optimize food safety

References

Alban L, Nielsen EO, and Dahl J. A human health risk assessment for macrolide-resistant *Campylobacter* associated with the use of macrolides in Danish pig production. Prev. Vet. Med. 83:115-129 (2008).

Awofisayo-Okuyelo A, et al. An outbreak of human listeriosis in England between 2010 and 2012 associated with the consumption of pork pies. J. Food Prot. 79:732-740 (2016).

Azevedo I, et al. Food safety in the domestic environment. Food Control 37:272-276 (2014).

Baptista FM, Dahl J and Nielsen LR. Factors influencing *Salmonella* carcass prevalence in Danish pig abattoirs. Prev. Vet. Med. 95:231-238 (2010).

Beumer RR, et al. *Listeria* species in domestic environments. Epidemiol. Infect. 117:437-442 (1996).

Boes J, Nielsen B and Gerner-Smidt P. Epidemiology of *Yersinia enterocolitica* O:3 in humans and pigs in Denmark. In, Proceedings, International Conference on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork (Safepork), Foz de Iguacu, 2017, pp. 152-154.

Camargo AC, Woodward JJ and Nero LA. The continuous challenge of characterizing the foodborne pathogen *Listeria monocytogenes*. Foodborne Pathog. Dis. 13:405-416. (2016).

Cherubin CE. Antibiotic resistance of *Salmonella* in Europe and the United States. Rev Infect Dis 3:1105-1126. (1981).

Christensen SG. *Yersinia enterocolitica* in Danish pigs. J. Appl. Bacteriol. 48:377-382 (1980).

Dahl J. Controlling *Salmonella* in live pigs and at slaughter: the Danish experience. Vet. J. 197:529–530 (2013).

Davies PR and Funk JA. The epidemiology and control of *Salmonella* in pork – some of the questions. In, Third International Symposium on Epidemiology and Control of *Salmonella* in Pork. Washington, DC, pp. 1–11 (1999).

Davies PR. Intensive swine production and pork safety. Foodborne Pathog. Dis. 8:189–201 (2011).

Davies, PR, et al. The role of contaminated feed in the epidemiology and control of Salmonella enterica in pork production. Foodborne Pathog. Dis. 1:202-215 (2004).

Drummond N, et al *Yersinia enterocolitica*: a brief review of the issues relating to the zoonotic pathogen public health challenges and the pork production chain. Foodborne Pathog. Dis. 9:179–189 (2012).

Duffy LL, Dykes GA and Fegan N. A review of the ecology colonization and genetic characterization of *Salmonella enterica* serovar Sofia, a prolific but avirulent poultry serovar in Australia. Food Research International 89:1544-1548. (2012).

Duranti A. et al. A severe outbreak of listeriosis in central Italy with a rare pulsotype associated with processed pork products. J. Med. Microbiol. 67:1351-1360 (2018).

Felin E, et al. Assessment of the feasibility of serological monitoring and on-farm information about health status for the future meat inspection of fattening pigs. Prev. Vet. Med. 162:76-82. (2019).

Fetsch A and Johler S. *Staphylococcus aureus* as a foodborne pathogen. Current Clinical Microbiology Reports 5:88-96 (2018).

Jordan K and McAuliffe O. *Listeria monocytogenes* in Foods. in Advances in Food and Nutrition Research 86:181-213 (2018).

Kadariya J, Smith TC and Thapaliya D. Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Res. Int. (2014). ID827965

Larsen J, et al. Meticillin-resistant *Staphylococcus aureus* CC398 is an increasing cause of disease in people with no livestock contact in Denmark 1999 to 2011. Euro Surveill. 20 (37) (2015). doi: 10.2807/1560-7917.ES.2015.20.37.30021

Laukkanen-Ninios R. et al. Enteropathogenic *Yersinia* in the Pork Production Chain: Challenges for Control. Compr. Rev. Food Sci. Food Saf. 13:1164-1191 (2014).

Moon DC, et al. Identification of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* isolates in Korea and molecular comparison between isolates from animal carcasses and slaughterhouse workers. Foodborne Pathog. Dis. 12:327-334 (2015).

Nesbakken T, Eckner K and Rotterud OJ. The effect of blast chilling on occurrence of human pathogenic *Yersinia enterocolitica* compared to *Campylobacter* spp. and numbers of hygienic indicators on pig carcasses. Int. J. Food Microbiol. 123:130–133 (2008).

Nesbakken T, Iversen T and Lium B. Pig herds free from human pathogenic *Yersinia enterocolitica*. Emerg. Infect. Dis. 13:1860-1864 (2007).

Nesbakken T. Update on *Yersinia* as a foodborne pathogen: Analysis and control. In, Advances in Microbial Food Safety Volume 2, Ed. Sofos J. Elsevier - ISBN 1782421157, 9781782421153 (2014). doi:10.1533/9781782421153.1.33

Newell DG, et al. *Campylobacter* epidemiology - sources and routes of transmission for human infection. In, *Campylobacter*: Features, Detection, and Prevention of Foodborne Disease. Academic Press - ISBN 9780128036235. pp. 85-110. (2016).

Peruzy MF, et al. Evaluation of virulence genes in *Yersinia enterocolitica* strains using SYBR Green real-time PCR. Food Microbiol. 65:231-235 (2017).

Rodríguez-López P, et al. Current knowledge on *Listeria monocytogenes* biofilms in food-related environments: incidence resistance to biocides ecology and biocontrol. Foods 7, 85 (2018). doi:10.3390/foods7060085

Sheppard SK and Maiden MC. The evolution of *Campylobacter jejuni* and *Campylobacter coli*. Cold Spring Harb. Perspect. Biol. 2015;7:a018119.

Stein H, et al. Listeriosis in fattening pigs caused by poor quality silage–a case report. BMC Vet. Res. 14:362 (2018). doi:10.1186/s12917-018-1687-6

Sun J, et al. Prevalence and Characterization of *Staphylococcus aureus* in growing pigs in the USA. PLoS ONE 10(11): e0143670. (2015).

Szabo I, et al. Colonization kinetics of different methicillin-resistant *Staphylococcus aureus* sequence types in pigs and host susceptibilities. Appl. Environ. Microbiol. 78:541–548 (2012).

Thepault A. et al. Genome-wide identification of host-segregating epidemiological markers for source attribution in *Campylobacter jejuni*. Appl. Environ. Microbiol. 83(7). pii: e03085-16 (2017).

Tyson GH, et al. Molecular subtyping and source attribution of *Campylobacter* isolated from food animals. J. Food Prot. 79:1891–1897 (2016).

Wagenaar JA, French NP, and Havelaar AH. Preventing *Campylobacter* at the source: Why is it so difficult? Clin. Infect. Dis. 57:1600-1606 (2013).

Wegener HC, et al. *Salmonella* Control Programs in Denmark. Emerg. Infect. Dis. 9:774-780 (2003).

Wendlandt S, Schwarz S, and Silley P. Methicillin-resistant *Staphylococcus aureus* : A food-borne pathogen? Annu. Rev. Food Sci. Technol. 4:117-139 (2012).

Zarfel G, et al. Virulence and antimicrobial resistance genes in human MRSA ST398 isolates in Austria. Epidemiol. Infect. 141:888–892 (2013).

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Risk-based surveillance in the pork chain – requirements and challenges

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In the pork chain, there is a plethora of food-borne hazards for which there is a need of monitoring or surveillance: bacteria, parasites, viruses, toxic and pharmacological residues and drug-resistant microbes. In the European Union (EU) , *Salmonella* is currently number two, when it comes to the number of human cases, causing 91,662 human cases, and number one when focus in on cases ascribed to pig meat (EFSA/ECDC, 2018). Parasites – and in particular *Taenia solium* - play a large a devastating role on the African continent (FERG, 2015). Moreover, there is an increasing attention on antimicrobial resistant bacteria on pig meat without much knowledge about the full implications of human exposure– see e.g the annual reports from the Danish DANMAP surveillance on https://www.danmap.org/.

However, resources are scarce among veterinary services. Likewise, the food business operator – irrespective of whether this is the farmer or the abattoir–is preoccupied about maintaining a profit to be able to remain in business, also in the future. Still, customers and trade partners expect that meat products placed on the market are safe to consume and do not bear any risks of causing disease outbreaks. In this situation, risk-based surveillance systems may offer a solution by applying risk analysis principles to set priorities and allocate resources effectively and efficiently through a focus on a high cost-effectiveness ratio in sampling.

Risk-based surveillance and control was originally introduced into veterinary public health by Stärk et al. (2006). Since then, experience has been gathered, and the methodology has been further developed. In the following paragraphs, relevant steps to move towards a risk-based surveillance system are described and discussed.

First, it should be assessed where there is a need for surveillance, why, and which kind of knowledge is expected to be provided by the surveillance. Often, it starts with a risk that needs to be dealt with. In the present context, risk is seen as the product of probability and consequences. if a high capacity to cope with perturbations is judged as vital, indicators of consequences might be required as part of the surveillance. All this constitutes the strategic part of the analysis.

A government in collaboration with a livestock sector may have ambitions for improving animal and human health and/or expand the access of e.g. pork to the export market. If improvement of the national health is the objective, information about the burden of different diseases is the basis, for humans as well as animals. The FERG Report may come in useful for human health as it contains an assessment of the human burden of different foodborne diseases in the world, divided into regions (FERG, 2015). Next, a source account is needed, whereby the contribution of each kind of food consumed is assessed. For example, if the highest burden of foodborne disease is ascribed to poultry meat, then the value of surveillance in pig meat would be limited. That would be the case for *Campylobacter*. For animal health, disease may also be considered a good indicator or productivity, in particular in low-income countries where a sophisticated registration of production data is not feasible.

Requirements for trade resilience is also a part of this step. Hence, even though the outcome of a burden of disease assessment and a source account may show that the need for a given surveillance is negligible in a given population, it may still be needed to access or stay on a certain market. *Trichinella* in pig meat is an example of the latter (Alban & Petersen, 2016). Moreover, a country may decide to implement certain food safety standards for a part of its production – e.g. farms delivering to selected, large abattoirs – to be able to export to the EU, USA or similar countries with a high level of food safety. Once the relevant indicators have been identified, then technical and operational considerations should be made regarding how to design the surveillance. Here, the surveillance objective should be further defined, and surveillance designers should discuss which kind of surveillance is needed to meet the objective.

Surveillance involves that some pre-planned action is taken, when positive samples are found or when the prevalence gets above a certain threshold. In theory, monitoring differs from surveillance in the sense that no actions are necessarily taken immediately after results are made available (Hoinville et al., 2013). Antimicrobial resistance programmes might in some cases be considered as operating as monitoring programmes: Every year, the Danish DANMAP program publishes a report showing what has been found. For most findings, there is no immediate associated action, but if an unexpected finding is made, which is also considered as worrying, actions will be taken. The microbiological criterion for *Salmonella* in minced meat intended to be consumed raw is an example of a surveillance, where immediate action is taken, if *Salmonella* is found in just one out of five 25 g samples from a batch–as required by the EU legislation (Anon., 2005). Likewise, if *Salmonella* is found on the carcasses above the defined threshold of 3 out of 50 carcasses, actions must be taken immediately related to improvements of the slaughter hygiene and the process controls. This may also imply the biosecurity measures applied on the farms of origin of the delivered animals (Anon., 2014).

During the design of surveillance, design tools may be used. One example is the RISKSUR surveillance design tool, which guides the user through key elements such as 1) objectives and expected outcome, 2) surveillance components, 3) actions related to suspects and positive findings, 4) preventive actions, 5) testing protocol, 6) study design, 7) sampling strategy, 8) data sampling process (https://www.fp7-risksur.eu/). Such a standardized approach ensures that all elements are carefully considered before being decided.

Information about the biology of the hazard may come in useful in the process of designing surveillance or monitoring. This includes the prevalence of infection in different animal species, knowledge about risk factors, and ways of spreading. All this information may be used to identify where the risk is, implying that sampling is intensified in subpopulations that harbour the highest risk (Stärk et al., 2006). As described above, in the context of risk-based surveillance, risk is seen as the product of probability and consequences. Therefore, the highest risk is either found in the population strata with the highest expected prevalence of the hazard – or the strata, where the implications of having the hazard may be highest. For *Trichinella*, this means that sampling of outdoor-raised pigs is preferred to wildlife sampling, although wildlife may have a higher prevalence of *Trichinella* than outdoor-raised pigs.

Likewise, for meat, surveillance may be focusing on meat originating from animals raised outdoors and not indoors – if outdoor-raising is perceived as a risk factor for the hazard of concern. Moreover, one should have a view on the intended use of the meat. If the hazard is eliminated during ordinary processing, then there will be no need for surveillance in that part of the production, but there may be a need in another part of production. This implies that a pork value chain perspective is useful as it would offer novel opportunities for risk-based sampling.

A value chain perspective should also be used for *Toxoplasma gondii*, where data show that the prevalence is low in indoor raised finishing pigs, medium in outdoor raised finisher pigs, and high in sows (Kofoed et al., 2017; Olsen et al. 2019). Freezing and heat treatment eliminates the parasite, whereas curing requires that the meat product is subjected to high saline concentrations over a longer time to be effective (Dubey et al., 1997). This implies that there are only few pig meat products which will contain viable parasites at the time of consumption. All such information may be used when designing surveillance and mitigation measures to decrease the exposure of humans to *T. gondii* due to consumption of pig meat.

Feasibility of sampling and the related economics are also important to consider. In general, sampling at the abattoir is easier and cheaper than sampling on the farm. Choice of laboratory methods requires considerations regarding whether a high sensitivity or a high specificity is needed – and whether more methods should be used and interpreted, in parallel or in series. Regarding choice of sampling material (matrix) to use in the laboratory, meat may be easier to collect than blood. However, care should be taken before deciding, because the laboratory method may have been validated for one matrix and not for another.

In 2014, the EU legislation adopted a risk-based approach for *Trichinella spp.* in pigs (Anon., 2015). This implies that the official requirement for testing is applied only to pigs raised in the low-biosecurity compartment, which is called non-controlled in the EU and mainly implying outdoors or backyard production. This is due to data showing that *Trichinella spp.* is absent in the controlled housing compartment. This has moved focus from testing of each pig to auditing of biosecurity on-farm. Such indirect measurements are much cheaper than testing all pigs for the presence of the parasite. The compliance with the requirements for controlled housing should be checked at regular intervals. These requirements are described in detail in Annex IV to the EU *Trichinella* Regulation (Anon., 2015). Either the veterinary authorities or a third-party independent auditor may do the auditing. The latter is undertaken as part of a private standard, building on top of national and international legislation. Such private standards are common in many parts of the world and it may be expected that they will increase further in importance. Despite the EU legislation on *Trichinella* allowing no testing of pigs raised indoors, extensive testing is still taking place in the EU, because of trade requirements from countries outside the EU (Alban & Petersen, 2016). This shows the importance of international harmonization on the most common animal health and food safety issues–as it could lead to a more effective distribution of resources spent on assuring food safety and animal health and welfare.

There are several advantages of using risk-based surveillance systems: targeted efforts resulting in a low cost-effectiveness ratio, if planned well. Such systems require that there is knowledge about risk factors. However, in many cases it can be difficult or even impossible to get sufficient data regarding

the exact size of a risk factor. One example may be presence of residues of antimicrobial origin in pig meat. Detailed studies of the cases seen in Denmark indicate that primarily injectables are the cause and that a high within-herd prevalence of chronic pleurisy may be a risk factor. However, the number of cases in Denmark is so low that it disables a precise estimate of this risk factor. Here, a comparison with Dutch data helped to estimate the relative risk (Alban et al., 2016). Still, prudence should be used to avoid over-confidence, and assessments of the impact of uncertainty on the risk to be estimated should be made to ensure resilience of the system. Currently, the EU Residue Directive 96/23 is being discussed – the next version of the Directive will consider risk-based principles for surveillance and control. The challenge is that the perception of the importance of minimizing presence of residues in meat varies between the European countries. In Switzerland, which has no export of pig meat, the main objective is to show compliance with EU legislation. In contrast, Denmark and the Netherlands have a large export to protect and therefore perceive surveillance for residues as more important. In this case, a balance between flexibility and harmonization should be sought, e.g. regarding the minimum number of samples to take and analyse as well as handling of suspects (Alban et al., 2018).

Livestock farming is not static; and major shifts in pig production has been observed in Europe in the last decades. This implies fewer and larger farms and a specialization into breeding, growing or finishing farms, resulting in a change in the trade flows (Marquer et al., 2014). Moreover, the preferences of the consumers are not stationary. Therefore, changes in risk distribution should be foreseen and incorporated into surveillance e.g. as an early warning system. An example is when livestock is raised in new ways or areas, where there might be an increased exposure to certain hazards, compared to the traditional production. Outdoor-raising of pigs may be an example of this – and here, an increase in the preference for pink pork may imply a higher exposure to *T. gondii* than seen before. Similar considerations should be made regarding climatic changes, which may lead to presence of infections or vectors of infection not previously seen in the area. For both examples, focus should be on the capacity of the livestock system to cope with perturbations.

In this paper, risk-based surveillance to ensure safe meat has been the focus. Still "safe meat" may have different meanings to the consumers, and some may be willing to take a risk for the taste, e.g. for tartare (raw beef). This implies that resilience as well as risk and risk evaluations may vary at different levels of the consumer and production cycle. In line, one group of consumers may perceive pigs raised outdoors as associated with high animal welfare as well as a more resilient form of production compared to indoor pig production. For others, outdoor pig production may be perceived as a risk for animal welfare because of the climate and as a risk of introduction of African swine fever. In response, the authorities in collaboration with the food business operators may need to look more carefully into how we may frame risk, production and consumption in a way where we can satisfice the various aspects rather than optimize one or two these matters (e.g. risk and price).

Risk-based surveillance systems require that many kinds of information are gathered and carefully evaluated. This implies an opportunity to undertake a better surveillance compared to using a random approach. However, it also encompasses a weakness, because such systems are not well-known to the trade partners and the veterinary authorities in the importing country (Stärk et al., 2006). To ensure confidence in risk-based systems it is important that the design of the surveillance is transparent and evidence-based, and to have in mind that trust is built up gradually but can be destroyed fast. It may be confusing, if each country defines their own risk-based surveillance for a given hazard, and some level of harmonization would be useful. To obtain this, open access to information about surveillance systems would be helpful for the process of identifying the systems that work best, depending on the settings. In case of sensitive issues, a controlled disclosure could be used.

Moreover, a collaboration between authorities, academia and food business operators should be encouraged. In many cases, HACCP is in place for a given production and data are collected routinely. Also, livestock producers or the abattoirs have risk-mitigating actions in place, carefully selected based upon experience, feasibility and economics. Such a collaboration might make it possible to develop an effective surveillance for a given hazard or indicator.

Regular evaluation of surveillance is recommendable. This will among others ensure that the latest technical achievements are incorporated, the objectives are met, and the cost-effectiveness is maintained. Tools developed for evaluation should preferably be used, e.g. the RISKSUR surveillance tool described above. A broader evaluation framework to consider has been developed by the Network for Evaluation of One Health (NEOH). It is intended for the evaluation of any initiative addressing the health of people, animals and the environment. The framework provides a basis for assessing the integration of knowledge from diverse disciplines, sectors, and stakeholders through a systematic description of the system at stake and standardised sets of indicators. It illustrates how cross-sectoral, participatory and interdisciplinary approaches evoke characteristic One Health operations, i.e., thinking, planning, and working, and require supporting infrastructures to allow learning, sharing, and systemic organisation. It also describes systemic One Health outcomes, which are not necessarily possible to obtain through sectoral approaches alone (e.g. trust, equity, biodiversity etc.), and their alignment with aspects of sustainable development based on society, environment, and economy (http://neoh.onehealthglobal.net/).

Several other tools are currently available for evaluation of surveillance. A comparison of such tools is currently undertaken in an international project called "Convergence in evaluation frameworks for integrated surveillance of AMR: Moving towards a harmonized evaluation approach" (Co-Eval-AMR), where focus is on surveillance systems for antimicrobial resistance. The intent is to identify which systems are good at evaluating what and – if possible – to move towards a harmonized evaluation approach. In conclusion, risk-based surveillance systems offer a way to address situations, where there is a need for surveillance, but few resources are available. Risk-based surveillance-and-control is based on risk analysis framework and it helps to identify needs, set priorities, and allocate resources. First, a strategic decision should be made regarding what to prioritize. Next, operational decisions should be made regarding how to set up surveillance, and here feasibility and costs of sampling are evaluated together with a view on the entire supply chain. Similar considerations should be made for risk management. Focus should be on high cost-effectiveness ratio in surveillance/control, and here, it is advantageous to think about biology and look at the entire supply chain, while using direct or indirect measurements. Then, collaboration with the food business operator should be considered by identification of common interests, sharing of data and joint action. Finally, the surveillance system should be evaluated in a systematic way on a regular basis to ensure that the resources spent are providing value for money. Surveillance and control can be considered a continuous, iteratively adaptive process, which can respond to changing risk patterns, consumer behaviours and trade conditions. It is therefore important that the surveillance is set up to make control timely and easy.

References

Alban, L., Petersen, J.V., 2016. Ensuring a negligible risk of *Trichinella* in pig farming from a control perspective. Vet. Parasitol. 231, 137-144. http://dx.doi.org/10.1016/j.vetpar.2016.07.014

Alban, L., Rugbjerg, H., Petersen, J.V., Nielsen, L.R., 2016. Stochastic scenario tree modelling of costeffectiveness of risk-based antimicrobial residue monitoring in Danish pork. Prev. Vet. Med. 128, 87-94

Alban, L., Léger, A., Veldhuis, A., Schaik, G.V., 2018. Modernizing the antimicrobial residue monitoring programs for pig meat in Europe—the balance between flexibility and harmonization. Food Control, 86, 403-414 https://doi.org/10.1016/j.foodcont.2017.11.040

Anonymous, 2005. Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF

Anonymous, 2014. Regulation (EC) No 217/2014 amending Regulation (EC) No 2073/2005 as regards *Salmonella* on pig carcases. http://eurlex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32014R0217&rid=1

Anonymous, 2015. Commission Implementing Regulation (EU) 2015/1375 laying down specific rules on official control for *Trichinella* in meat. http://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32015R1375&rid=1

Dubey, J.P., 1997. Survival of *Toxoplasma gondii* Tissue Cysts in 0.85-6% NaCl solutions at 4-20ºC. *Journal of Parasitology*. 83, 946-949.

EFSA/ECDC, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 16, 12, 5500. FERG, 2015. WHO estimates of the global burden of foodborne diseases. https://www.who.int/foodsafety/areas_work/foodborne-diseases/ferg/en/ WHO, Geneva, Switzerland.

Hoinville, L.J., Alban, L., Drewe, J.A., Gibbens, J.C., Gustafsson, L., Häsler, B., Saegerman, C., Salman, M., Stärk, K.D.C., 2013. Proposed terms and concepts for describing and evaluating animal health surveillance terms. Prev. Vet. Med. 112, 1-2, 12 pp. http://dx.doi.org/10.1016/j.prevetmed.2013.06.006

Kofoed, K.G., Vorslund-Kiær, M., Nielsen, H.V., Alban, L., Johansen, M.V.A., 2017. Sero-prevalence of *Toxoplasma gondii* in Danish pigs. Veterinary Parasitology: Regional Studies and Reports. 10, 136-138.

PROCEEDINGS

Marques, P., Rabade, T., Forti, R., 2014. Pig farming in the European Union: considerable variations from one Member State to another. Statistics in focus 15. Eurostats. https://ec.europa.eu/eurostat/statistics-explained/pdfscache/3688.pdf

Olsen, A., Berg, R., Tagel, M., Must, K., Deksne, G., Enemark, H., Alban, L., Johansen, M.V., Nielsen, H.V., Sandberg, M., Lundén, A., Stensvold, R., Pires, S.M., Jokelainen, P., 2019. Seroprevalence of Toxoplasma gondii in domestic pigs, sheep, cattle, wild boars, and moose in the Nordic-Baltic region: a systematic review and meta-analysis. Parasite Epidemiology and Control, 3, e00100 https://doi.org/10.1016/j.parepi.2019.e00100

Stärk, K.D.C., Regula, G., Hernandez, J., Knopf, L., Fuchs, K., Morris, R., Davies, P., 2006. Concepts or risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. BMC Health Services Research. 6, 20.

NOTES

ONE HEALTH

01

Review of biological and chemical health risks associated with pork consumption in Vietnam: major pathogens and hazards

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Introduction

The global burden of foodborne illness undermines the safety and development of people and nations, particularly in low- and middle-income countries [1, 2]. In Vietnam, local food systems are experiencing a period of rapid change [3], and government efforts to regulate the food sector have had limited impact [4-6]. Socioeconomic development has also driven increased demand for meat, with pork becoming increasingly prominent in Vietnamese diets [5, 6]. Available public health data suggests that biological hazards represent the largest source of foodborne illness nationally, though chemical hazards are also present in food [5]. Thus, there is an urgent need to develop context-appropriate, effective, and low-cost solutions to food safety challenges in Vietnam [1, 5, 7]. In reviewing the scope and burden of different pork-borne health risks in the literature, this paper looks to identify high-value targets for future food safety objectives, highlighting relevant pathogens and hazards, as well as gaps in the current research.

Material and Methods

A search for suitable literature was undertaken using the PubMed database with articles published between 2008 and 2018 considered as relevant for inclusion. Due to limited levels of research specific to Vietnam, studies from comparable contexts in wider Southeast Asia were included. Included literature demonstrated a direct health risk to humans through pork consumption as a result of the conditions in which pigs were raised and/or slaughtered. Articles discussing pathogens that can be contracted directly from pork consumption were excluded if consumption of pork meat was not explored as a potential route of infection.

Results and Discussion

A variety of risks to the health of Vietnamese pork consumers were identified through the literature. Biological risks to consumers that were detailed in the literature included *Salmonella* spp., *Streptococcus suis* bacteria, as well as *Taenia solium* and *Trichinella* spp. parasites. However, these organisms do not represent the full scope of known pathogens associated with pork consumption. The omission of other appropriate pathogens is a result of gaps in the available research specific to Vietnam and comparable countries of Southeast Asia. Chemical hazards detailed in the literature included antibiotic residues, particularly sulfamethazine, and heavy metal contaminants.

Pork-associated pathogens detailed in the literature illustrate the presence and impacts of gradients of development in Vietnam. For instance, disease as a result of parasitic infection is more frequently documented in regional areas of Vietnam, particularly the northwest mountainous regions. Here, populations have lower relative levels of sanitation infrastructure, reduced access to healthcare, and livestock may be permitted to roam in order to graze.

A multitude of studies included in this review implicated raw pork consumption as a prominent risk factor in the development of disease from biological pathogens. However, epidemiological trends indicate that raw or undercooked pork dishes are part of prominent sociocultural events such as celebrations and funerals.

The epidemiological data explored across the foodborne pathogens included in this study reflected relatively consistent patient demographics. Various studies have reported that males are affected by pork-related foodborne illness at a significantly higher rate than females across Southeast Asia. Age was also positively associated with infection and disease following pork consumption in some studies. However, trends in the gender and age of patients may be a reflection of other underlying risk factors, such as lifestyle behaviours, health comorbidities, or age-associated immunodeficiency.

References

1. Havelaar, A.H., et al., World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. 2015. 12(12): p. e1001923.
2. WHO, WHO estimates of the global burden of foodborne diseases. 2015.
3. World Bank, Vietnam food safety risks management: challenges and opportunities - policy note (English). 2017, World Bank Group: Washington, D.C.
4. Hoang, H.G., Farmers´ responses to VietGAP: a case study of a policy mechanism for transforming the traditional agri-food system in Vietnam: a dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Agricultural Systems and Environment at Massey University, Palmerston North, New Zealand. 2018, Massey University.
5. Nguyen-Viet, H., et al., Food safety in Vietnam: where we are at and what we can learn from international experiences. 2017. 6(1): p. 39.
6. Rich, K.M., et al., Quantitative value chain approaches for animal health and food safety. 2018. 75: p. 103-113.
7. Wertheim-Heck, S.C.O., S. Vellema, and G. Spaargaren, Food safety and urban food markets in Vietnam: The need for flexible and customized retail modernization policies. Food Policy, 2015. 54: p. 95-106.

02

Evaluation of the implementation of one health in Kenya: a case study of the zoonotic disease unit

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Kenya became one of the first country in Africa to institutionalize One Health (OH) and operationalized it on 1st March 2012 as a cross-sectoral collaboration. It created a cross-sectoral One Health unit called the Zoonotic Disease Unit (ZDU) that establishes and maintains active collaboration at the human, animal and ecosystem interface towards better prevention and control of zoonotic diseases in Kenya. Using Network for Evaluation of One Health's (NEOH) standardized One Health evaluation framework a process evaluation of the ZDU was conducted to appraise its effectiveness and impact. The NEOH tools helped in identifying the drivers and outcomes of One Health, as well as necessary operations and infrastructure to implement an integrated approach. The evaluation included a description of the context and the initiative, illustration of the theory of change, identification of the expected and unexpected outcomes and assessment of OHness. The latter is the sum of characteristics that defines an integrated approach and includes OH thinking, planning, working, sharing infrastructure, learning infrastructure, and systemic organisation. Data for the analysis were gathered in 27 face-to-face key informant interviews using the Bristol Online Survey, 1 focus group discussion and a desktop review of literature. Qualitative data was thematically analysed using NVivo Pro version 12 while quantitative data was through SPSS v23 and the One Health Index. ZDU attained a One Health Index of 0.8261 with a score of 0.44 in One Health planning, 0.58 in One Health learning, 0.72 in One Heath working and 0.71 in One Health thinking. The Unit was praised for its elaborate strategic implementation strategy, vast network of stakeholders, and its relevance to address imminent One Health challenges in Kenya. Shortcomings were identified regarding duplication of efforts with no framework to harmonize activities by different institutions and weak institutional structures that facilitate sharing of resources between the animal and human health agencies. The critical application of the NEOH evaluation tools allowed identifying advantages and shortcomings in the processes of the ZDU that can be used by its coordinators to improve impact. We recommend that the next evaluation to focus on the assessment of impact and economic efficiency in line with the developed theory of change.

03

Serological prevalence of human trichinellosis and cysticercosis in Hoa Binh province of Northwest Vietnam

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Introduction

In Vietnam, parasites that use pigs as intermediate hosts are a multifaceted concern, which we often call "neglected tropical diseases", typically trichinellosis and cysticercosis. According to the joint report of FAO and WHO in 2014, Taenia spp. ranks 1st and Trichinella spp. ranks 8th of 24 food-borne parasites assessed [1]. Trichinella spiralis has worldwide distribution and five outbreaks were recorded in Vietnam between 1970 and 2012, affecting between 20 and 36 people each [2-4] while there has been little recent research into rates of human taeniasis and cysticercosis in Vietnam due to the accurate national baseline figures do not exist [5]. Those diseases can vary across region and there are about 0,1-12,0% human affected by trichinellosis and cysticercosis according to estimated [6, 7] and indigenous pig was found with high antibody of T. Spiralis and T. solium of 12,5% and 28,5% respectively [8].

Exposure to those parasites primarily occurs through the consumption of raw or undercooked pork products [9-11]. However, transmission can also occur through the consumption of wild or omnivorous animals such as boars, dogs and rats [3, 10]. In addition, the driven factor could also be poor sanitation, pigs are allowed to roam freely (free-range), or meat inspection is absent or inadequate [12, 13]. Da Bac, Hoa Binh Province located in northwest Vietnam with the great amount of indigenous pig and the consumption of raw/uncooked pork has been quite ubiquitous in this area. By the aforemention reasons, this study was undertaken to assess the prevalence of and associated risk factors with human trichinellosis and cysticercosis.

Methods

A cross-sectional study was conducted in September 2018 in Da Bac district, Hoa Binh province. Six communes were randomly selected for sampling including Muong Chieng, Giap Dat, Doan Ket, Trung Thanh, Tan Minh, Cao Son. Those included in the study were member in household, aged between 18 to 65 years old and those who consented to have a blood sample taken as part of the research. Samples of serum were approximately 3-4 millilitres in volume, kept at 4 degrees Celsius during transportation and were preserved at -20oC in the laboratory. An enzyme-linked immunosorbent assay (ELISA) was utilised to identify the presence of antigens in samples. The formula to calculate results for each test were based in manufacturer's instructions.

Results

There are total two positive and four suspected cases of trichinellosis (2.0%), alongside two positive and one suspected case of cysticercosis (1.0%). Positive and suspected diagnoses in this study were relatively equal between genders, despite survey results indicating that men engaged in higher levels of risk behaviours, including the consumption of wild animals and undercooked pork. Noticeably, five of nine positive or suspected cases reported at Tan Minh commune (Table 1)

Discussion and Conclusion

On the one hand, the seroprevalence of trichinellosis (0,67%) and cysticercosis (0,67%) positive cases were in line with previous study in Vietnam [6, 7, 14] and Slovakia [15]. Low infection rates suggest that the disease may be circulating in the community but may also be the result of past infections, since antibodies produced may exist in the body for several years after being infected [16]. Moreover, positive and suspected cases concentrated mainly in Tan Minh commune, which is a warning sign of future outbreaks may occur surround this area. It poses urgency that in the future the commune authorities should have solutions such as human and pig screening and conduct treatment for positive cases.

On the other hand, the consumption of raw/uncooked pork has been quite ubiquitous in this area, which could facilitate the likely of Trichinella spiralis infection 3.5 times [17] and increase the risk of other parasitic diseases. In addition, less access to adequate sanitation such as not having toilet can increase the likely of cysticercosis 5.9 times [17] due to the fact that worm eggs from infect human and animal can be excreted through the feces to the environment. Improving hygienic condition can be a potential solution to prevent the spread of diseases.

Table 1: Risk factors associated with positive and suspected cases of trichinellosis and cysticercosis

	Trichinellosis						Cysticercosis		
Status (Positive/ Suspected)	Pos	Pos	Susp	Susp	Susp	Susp	Pos	Pos	Susp
Sex	F	M	M	F	M	F	M	F	M
Age	36	39	59	34	39	50	31	40	36
Ethnic minority	X	X		X	X	X	X	X	X
Access to adequate sanitation	X	X		X	X				X
Livestock producer	X	X	X	X	X	X	X	X	X
Consumed wild animal		X	X		X	X	X		X
Consumed raw vegetables		X	X	X	X		X		X
Symptoms in the last three months	X	X		X	X	X	X	X	X

References

1. FAO/WHO, Multicriteria-based ranking for risk management of food-borne parasites. 2014.
V2. an De, N., et al.,Trichinellosis in Vietnam. Am J Trop Med Hyg, 2015. 92(6): p. 1265-70.
3. Rostami, A., et al., Meat sources of infection for outbreaks of human trichinellosis. 2017. 64: p. 65-71.
4. European Union, Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella inmeat. 2015: Official Journal of the European Union.
5. Trung, D.D., et al., Assessing the burden of human cysticercosis in Vietnam. 2013. 18(3): p. 352-356.
6. Somers, R., et al., Taenia solium taeniasis and cysticercosis in three communities in north Vietnam. Tropical Medicine & International Health, 2006. 11(1): p. 65-72.
7. Van De, N., et al., Current status of taeniasis and cysticercosis in Vietnam. The Korean journal of parasitology, 2014. 52(2): p. 125-129.
8. Unger, F., Chau Thi Minh Long and Nguyen Viet Khong, Prevalence of trichinellosis and cysticercosis in indigenous pigs from ethnic minorities for selected communes in the Central Highlands (Dak Lak). 2016, Nairobi, Kenya: ILRI.
9. Thi, N.V., et al., Trichinella infection in wild boars and synanthropic rats in northwest Vietnam. Veterinary Parasitology, 2014. 200(1): p. 207-211.
10. Vu Thi, N., et al., The hidden burden of trichinellosis in Vietnam: a postoutbreak epidemiological study. 2013. 2013.
11. Taylor, W.R., et al., Acute febrile myalgia in Vietnam due to trichinellosis following the consumption of raw pork. Clin Infect Dis, 2009. 49(7): p. e79-83.
12. Garcia, H.H., et al., Taenia solium cysticercosis. Lancet, 2003. 362(9383): p. 547-56.
13. Bern, C., et al., Magnitude of the disease burden from neurocysticercosis in a developing country. Clin Infect Dis, 1999. 29(5): p. 1203-9.
14. Vu Thi, N., et al., Anti-Trichinella IgG in ethnic minorities living in Trichinella-endemic areas in northwest Vietnam: Study of the predictive value of selected clinical signs and symptoms for the diagnosis of trichinellosis. Acta Tropica, 2014. 139: p. 93-98.
15. Antolová, D., et al., A Community-Based Study to Estimate the Seroprevalence of Trichinellosis and Echinococcosis in the Roma and Non-Roma Population of Slovakia. International Journal of Environmental Research and Public Health, 2018. 15(2).
16. Harms, G., et al., Trichinosis: A Prospective Controlled Study of Patients Ten Years After Acute Infection. Vol. 17. 1993. 637-43.
17. Okello, A.L., et al., Integrating market chain assessments with zoonoses risk analysis in two cross-border pig value chains in Lao PDR. Asian-Australasian journal of animal sciences, 2017. 30(11): p. 1651-1659.

04

Pork consumption habits and occurrence of trichinellosis and cysticercosis in communities of Southern Laos

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Introduction

Parasitic pork borne diseases (PPBD) are of major public health importance globally. FAO/WHO recently listed the ´Top Ten´ food-borne parasites of global concern, which included cysticercosis and trichinellosis, both expected to be endemic in Laos. While some study exists for Northern Laos updated information for Southern provinces is lacking. This study aimed to determine the prevalence of cysticercosis and trichinellosis in pigs and humans and related risk factors in communities of Champasak province, Laos.

Material and Methods

Champasak, an emerging business hub in Southern Laos, was chosen purposively due to its high pig population, considerable cross-border trade with neighbouring countries and involvement in another project. Two-hundred and seventy pig samples were collected randomly from 14 villages across three districts. In addition 238 villagers present in the same villages were ask to provide blood samples. Pig owners and villagers were interviewed on PPBD knowledge and pork consumption behaviour. Survey tools included questionnaires including Likert scales and focus group discussions. *Trichinella spiralis* IgG ELISA, DETRIG0480 (pig serum) or BioFisher (human serum) and apDia Cysticercosis Antigen ELISA (humans serum) were used to confirm the presence of Trichinella and cysticercosis respectively. Due to high cross-reactivity with other Taenia spp. Cysticercosis ELISA was not performed for pig sera.

Results

Out of 270 pig samples analysed 79 (29%) were tested positive for Trichinella. ELISA testing for cysticercosis in pigs wasn't performed due to high cross reactivity with other taenia spp. Sero-prevalence for Trichinella were higher in older pigs (> 1year). Results for Trichinella and cysticercosis in humans indicate a prevalence of 17% (40/238) and 3.4% (8/238) respectively. Positive serological responses for Trichinella were higher in males than females. Results also showed that the most villagers are aware of health risks when consuming raw or undercooked pork but they continue to do so as they like certain dishes containing raw or undercooked pork e.g. fermented sausages. This finding shows that past public health campaigns may have increased awareness of villagers on PPBD but consumption behaviour remains often unchanged. Therefor socio-cultural aspects for behaviour and its change should be further explored. Policy level (national and provincial) and community feedback was provided through a previously established one-health multi-institutional platform. The platform consists of 6 ministries namely: Health, Agriculture, Tourism, Communication, Education and Defence.

Discussion and Conclusion

While results of this study for trichinellosis and cysticercosis in humans were considerably lower than those reported for the neighbouring province of Savannakhet (Holt et al. 2016) both parasitic zoonoses still pose a considerable risk to villagers in the study area. As we also observed risky consumption habits of villagers it is crucial that public health campaign also cover socio-cultural aspects of communities to be more effective in the future. Follow up activities are planned for 2019 will focus on more in-depth diagnoses procedures for cysticercosis in pigs and may include dissection of carcasses in an attempt to get more reliable information on the presence of cysts in pigs. Furthermore the multi-institutional platform will be further engaged and linked to a recently established one health platform to facilitate dissemination of results to relevant stakeholders and informative materials to villagers.

Acknowledgement

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Reference

Holt, H., Inthavong, P., Khamlome, B., Blaszak, K., Keokhamphet, C., Somoulay, V., Phongmany, A., Durr, P., Graham, K., Allen, J., Donnelly, B., Blacksell, S., Unger, F., Grace, D., Alonso, S., Jeff, Gilbert, J. (2016), Endemicity of Zoonotic Diseases in Pigs and Humans in Lowland and Upland Lao PDR: Identification of socio-cultural risk factors. PLoS Negl Trop Dis., 10(4): e0003913. doi:10.1371/journal.pntd.0003913

AMR

05

Reduction of antimicrobial use in the pork food chain – did it reduce antimicrobial resistance?

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Introduction

Antimicrobial use (AMU) selects for antimicrobial resistance (AMR) in bacterial populations. However, what happens to AMR when selection pressure declines due to a reduction of AMU in animal husbandry? The purpose of this study was to evaluate whether the reduction of antimicrobial use in pig husbandry in Germany was followed by a reduction in antimicrobial resistance. In 2014, Germany changed its drug legislation (Arzneimittelgesetz, AMG). The purpose of this legal change was the reduction of antimicrobial use in meat producing animals. Among other animal populations, the regulation addressed piglets ≤30 kg body weight and fattening pigs >30 kg body weight. The general principle of the regulation was a benchmarking approach. Data on antimicrobial use were collected in a systematic manner using treatment frequency as a measure. This measure was compared between farms housing the same type of animal populations, i.e. therapy frequency in pigs ≤30 kg in a farm was compared to therapy frequency in pigs ≤30 kg on the other farms. From all farm level therapy frequencies of the same type of population, the median and the 3rd quartile were determined twice a year. Farms with a therapy frequency above the 3rd quartile need to present a catalogue of measures aimed at reducing antimicrobial use to the respective local competent veterinary authority. Those above the median had to identify the reasons for the AMU above average.

The median and the 3rd quartile were published twice a year by the Federal Office for Consumer Protection and Food Safety (BVL). Figures for pigs up to and above 30 kg of body weight showed a substantial decline of both values indicating an overall substantial reduction of AMU in fattening pigs in Germany over the years.

Material and Methods

Based on the substantial reduction of antimicrobial use in pigs we compared AMR in commensal *Escherichia coli* from pigs from several years to determine whether the reduction in antimicrobial use also caused a decline in antimicrobial resistance. Antimicrobial resistance was observed in isolates of commensal *E. coli* from pigs over the years using harmonized methods based on technical specifications provided by EFSA (1). Minimum inhibitory concentrations of the antimicrobials for *E. coli* were evaluated according to epidemiological cut off values fixed in Commission Implementing Decision (CID) 2013/652/EU. Statistical analysis was based on logistic regression analyses on overall AMR (i.e. proportion of isolates that were susceptible to all tested antimicrobials and proportion of isolates resistant to more than three of the substances) and on AMR to specific antimicrobials. Isolates of *E. coli* were collected from randomized samples on farm and at slaughter, both reflecting domestic production only. AMR was determined using broth microdilution in line with the above mentioned CID.

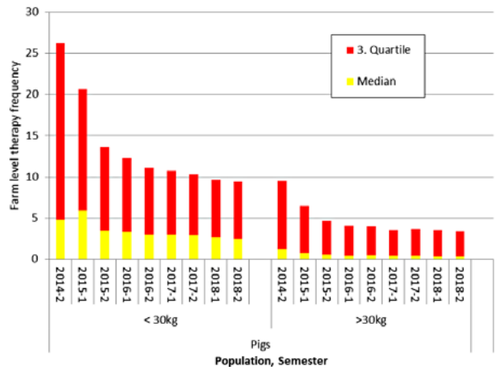

Figure 1: Development of median and 3rd quartile of farm level therapy frequency per half year as calculated according to § 58c of the German Medicine Act and published by the Federal Office of Consumer Protection and Food Safety

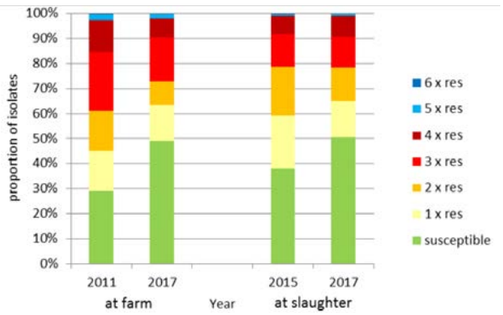

Figure 2: Antimicrobial resistance of E. coli from fattening pigs at farm (left two columns), caecal samples of pigs at slaughter (right two columns)

Results

Reduction of antimicrobial use in pigs can be seen in a decrease of the median and 3rd quartile of the therapy frequency as defined by the AMG (Fig. 1). Values for the 3rd quartile decreased from 26 treatment days per semester to less than 10 days in piglets ≤30 kg. They decreased from 9.5 to 3.7 days in pigs >30 kg. Overall, antimicrobial resistance decreased in isolates of *E. coli* from pigs that had been collected on farm and at slaughter between 2011 and 2017 (Fig 2). The proportion of fully susceptible isolates increased from around 30 % in 2011 to around 50 % in 2017 in fattening pigs at farm. In pigs at slaughter, samples had only been collected in 2015 and 2017. However, again, the proportion of fully susceptible isolates was higher in 2017 than in 2015.

With respect to individual antimicrobials a reduction in AMR of *E. coli* was observed for tetracycline, ampicillin, sulfamethoxazole and trimethoprim (Fig. 3). Resistance to ciprofloxacin, cefotaxime, gentamicin and colistin did not decrease. However, resistance to these substances had been on a low level from the start.

Discussion

Our results confirm a reduction of AMR in fattening pigs that goes along with the reduction of antimicrobial use in the German pig population based on data collected in the framework of a national monitoring program. The reduction was most obvious for the five substances that had the highest resistance level in 2011, i.e. tetracycline, sulfamethoxazole, ampicillin, trimethoprim and chloramphenicol. As use data were only available on an overall therapy frequency level, the share of the reduction that is taken by these substances is not known. However, tetracyclines, penicillins and sulfonamides are the substances with the largest quantity sold to veterinarians in Germany (2) and they are also known to be frequently used in pigs in Germany (3).

Selection pressure can on the one hand be exerted by the individual substance. On the other hand, co-selection is frequent as resistance determinants are often genetically linked. That means that use of one substance may indirectly select for resistance to another substance. This may for example have contributed to resistance to chloramphenicol despite the ban of chloramphenicol for food producing animals in the early 1990s.

Conclusion

Results indicate that a reduction in AMU in the population has beneficial effects on the AMR situation in the population especially to those antimicrobials that had high resistance rates in the first place. Further in-depth studies are needed to investigate the differences between the substances and to try to establish a dose-response relationship between AMU and AMR reduction.

References

EFSA. Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in Salmonella, Campylobacter and indicator Escherichia coli and Enterococcus spp. bacteria transmitted through food. EFSA Journal. 2012;10(6):2742.

Wallmann J, Bender A, Bode C, Köper LM, Heberer T. Abgabemengen antimikrobiell wirksamer Stoffe in Deutschland 2016. Auswertung der nach DIMDI-AMV eingereichten Daten 2016 und Vergleich mit den Daten aus den Vorjahren. Deutsches Tierärzteblatt. 2017;65(12):1650-9.

van Rennings L, von Munchhausen C, Ottilie H, Hartmann M, Merle R, Honscha W, et al. Cross-sectional study on antibiotic usage in pigs in Germany. Plos One. 2015;10(3):e0119114.

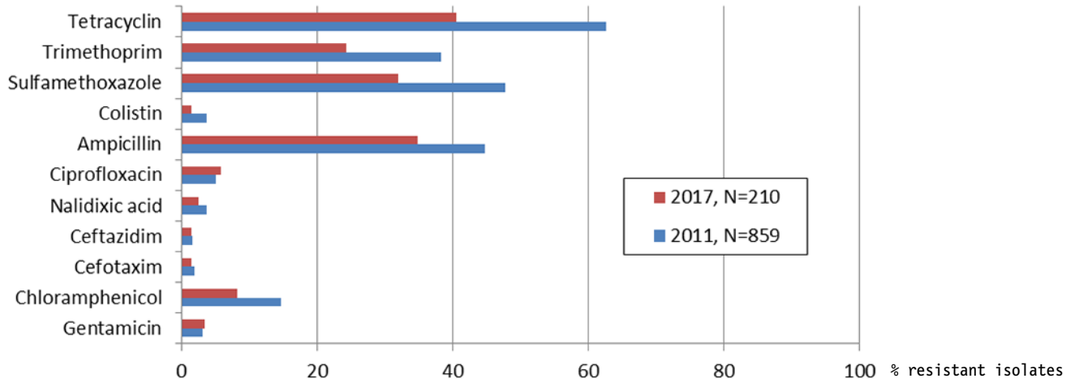

Figure 3: Resistance of E. coli from fattening pigs at farm to antimicrobials in 2011 and 2017

06

Evaulating average MIC over time using a Baysian latent class mixture model: examples from a Salmonella enterica serovar Typhimurium and S. enterica serovar 4,[5],12:i:-

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The minimum inhibitory concentration is a measure of resistance to an antibiotic that is used commonly to describe the resistance of an isolate. As these data arise from a serial dilution experiment, the average MIC cannot be calculated using the standard average formula. As a consequence, MIC data often dichotomized based on a threshold that splits the population into two (resistant and non-resistant) or three categories (susceptible, resistant, intermediate) and the changes the proportion of bacteria in the population evaluated. This approach although valid can result in a less complete picture of the patterns of MIC seen in the bacterial communities, in particular, gradual increases (MIC creep) and decreases (MIC decline) below the threshold of resistance. We used data from MIC results from 15 antibiotic and two Salmonella: Salmonella enterica serovar Typhimurium and S. enterica serovar 4,[5],12:i: to evaluate patterns of mean MIC and the proportion of the population in the resistant population using the Bayesian latent class mixture model.The results of the analysis demonstrated that for some antibiotic there appears to be evidence of MIC creep in the non-resistant population which would otherwise go undetected, as no significant changes in the proportion of the bacterial community in the resistant population are detected. Also, the results document that when the serial dilutions are severely truncated, that application of the Bayesian latent class mixture model might be unsuitable. The use of Bayesian latent class model has the potential to add an additional dimension to the analysis of MIC data obtained from surveillance programs. However, a limited the spectrum of MIC dilutions is also discussed can limit the application for some antibiotic.

07

Characterization of a multidrug-resistant (MDR) Salmonella enterica serovar I 4,[5],12:i:- isolate associated with a 2015 foodborne outbreak from pork

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Introduction

Nontyphoidal *Salmonella* is a leading cause of bacterial foodborne disease in humans. *Salmonella enterica* serovar I 4,[5],12:i:- has emerged as the fourth most frequent cause of human salmonellosis in the U.S. based on 2015 National Antimicrobial Resistance Monitoring System (NARMS) data, and the most common multidrug-resistant (MDR; resistance to 3 or more antimicrobial classes) serovar with ~68% of isolates being considered MDR (CDC, 2018). An MDR serovar I 4,[5],12:i:- outbreak was linked to pork in 2015 with 188 infections and 30 hospitalizations (Kawakami, et al. 2016); 523,380 pounds of pork were recalled. The pork outbreak-associated isolates were resistant to ampicillin, streptomycin, sulfisoxazole, and tetracycline (R-type ASSuT). Colonization and pathogenesis of a pork outbreak-associated serovar I 4,[5],12:i:- isolate in swine was consistent with trials conducted with virulent serovar Typhimurium, indicating that the increased prevalence of serovar I 4,[5],12:i:- is not due to an increase in pathogenesis (Shippy, et al. 2018). We sought to characterize strain FSIS1503788 associated with the pork outbreak and its derivatives using genomic, transcriptomic, and phenotypic analysis.

Material and Methods

Salmonella serovar I 4,[5],12:i:- strain FSIS1503788 was recovered from the cecal contents of a pig postslaughter during investigation of the 2015 outbreak by FSIS. Strains SX240 (isolated from swine ileocecal lymph node following passage) and BBS 1270 [*Salmonella* Genomic Island 4 (SGI-4) deletion mutant] are derivatives of strain FSIS1503788. Recombineering was used to delete SGI-4 from FSIS1503788 by insertion of *neo*, resulting in a kanamycin resistant phenotype for BBS 1270.

The genome sequence of strain FSIS1503788 was assembled using PacBio sequencing reads and error corrected using Illumina data. Growth of strains FSIS1503788 and BBS 1270 were evaluated using Biolog Phenotype MicroArrays to determine metal tolerance. Transcriptional analysis using RNA-Seq was determined for FSIS1503788 and BBS 1270 following 60 minutes of growth during early log-phase in the presence or absence of 5 mM copper sulfate.

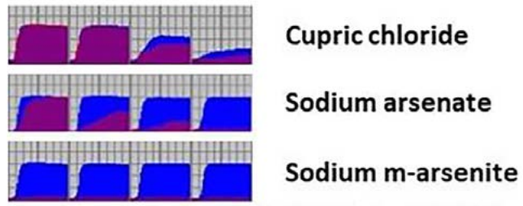
Swine were administered a diet with or without zinc oxide (2,000 mg/kg) and copper chloride (200 mg/kg; n=10/group) for 4 weeks prior to and 3 weeks post-inoculation with 8 x 10^7 CFU of SX240 via intranasal route. Fecal shedding of serovar I 4,[5],12:i:- was monitored at 2, 7, 14, and 21 days post-inoculation (dpi) with SX240. Colonization of the cecum, cecal contents, ileocecal lymph nodes, and Peyer's Patches region of the ileum by SX240 was determined at 21 dpi.

Results

Genome analysis of pork outbreak-associated serovar I 4,[5],12:i:- isolate FSIS1503788 indicates 2 large insertions compared to serovar Typhimurium. An ~28 kb module contains antimicrobial resistance genes for R-type ASSuT and mercury tolerance genes; the insertion deletes ~15 kb of the *fljB* genomic region, resulting in the monophasic phenotype. *Salmonella* genomic island 4 (SGI-4) is ~80 kb and encodes genes for metal tolerance (copper, silver, and arsenic) and DNA mobilization and transfer.

Growth in Biolog Phenotype Microarrays (Figure 1) indicate that strain FSIS1503788 has increased tolerance to copper and arsenic compounds compared to BBS 1270 (SGI-4 deletion mutant) or serovar Typhimurium (data not shown).

Exposure of SX240 to 5 mM copper for 60 minutes resulted in significant differential expression of 1,635 genes including transcriptional induction of metal tolerance genes for copper, arsenic, silver, and mercury; copper tolerance genes in both the core genome and SGI-4 were induced.



Growth comparison for FSIS1503788 (blue) and BBS 1270 (red) grown in the presence of metal compounds. Growth overlap (i.e. similarity) is shown in purple.

Figure 1. Reduced metal tolerance of BBS 1270 (SGI-4 mutant) compared to wildtype FSIS1503788.

Inclusion of copper and zinc as an antimicrobial in the swine diet did not significantly reduce quantitative fecal shedding (2, 7, 14, or 21 dpi) or intestinal tissue colonization (21 dpi; data not shown) of SX240 compared to control pigs receiving a diet without metals. A strong trend towards a significant increase (P=0.0572) in fecal shedding of SX240 was seen at 21 dpi in Zn/Cu fed swine compared to control pigs (Figure 2).

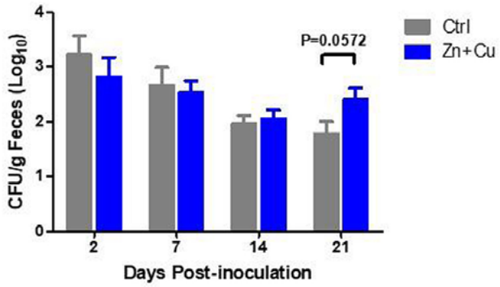


Figure 2: Swine fecal shedding following Salmonella serovar I 4,[5],12:i:- inoculation

Discussion and Conclusion

Salmonella serovar I 4,[5],12:i:- has emerged as a common cause of human salmonellosis and the most frequent MDR *Salmonella* serovar in the U.S. Serovar I 4,[5],12:i:- strain FSIS1503788 has 2 large DNA insertions conferring antimicrobial resistance (R-type ASSuT) and metal tolerance (copper, silver, arsenic, and mercury); this 2015 pork outbreak-associated isolate is genetically related to other MDR serovar I 4,[5],12:i:- strains that are globally distributed (Europe, Australia, and Japan).

Exposure of serovar I 4,[5],12:i:- to 5 mM copper for 60 minutes resulted in a metabolic shift with significant differential expression of >1,600 genes including induction of metal tolerance genes present in the core genome, SGI-4 (copper, silver, and arsenic), and the antimicrobial resistance module (mercury). This suggests that exposure of serovar I 4,[5],12:i:- to copper may co-select for the MDR phenotype of this strain due to induction of mercury tolerance genes located on the antimicrobial resistance module.

The inclusion of copper and zinc in the diet did not reduce swine fecal shedding or intestinal tissue colonization of serovar I 4,[5],12:i:-, and at 21 dpi, a strong trend for increased fecal shedding in pigs administered the metals was observed. The use of copper and zinc in swine feed as alternatives to antimicrobials to limit microbial pathogens may have the unintended consequence of selecting for the persistence of MDR *Salmonella* serovar I 4,[5],12:i:- in swine production.

The prevalence of MDR *Salmonella* serovar I 4,[5],12:i:- has increased globally and the combined presence of multiple antimicrobial resistance and metal tolerance genes may be beneficial for swine colonization or environmental survival.

References

CDC. (2018) National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Surveillance Report for 2015 (Final Report). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC.

Kawakami, V.M., et al. (2016): Outbreak of multidrug-resistant *Salmonella* infections linked to pork-Washington, 2015. Morb. Mortal. Wkly. Rep., 65:379.

Shippy, D.C., et al. (2018): Porcine response to a multidrug-resistant *Salmonella enterica* serovar I 4,[5],12:i:- outbreak isolate. Foodborne Pathog. Dis., 15:253.

08

Resistance to colistin and production of extended-spectrum β-lactamases and/or AmpC enzymes in Salmonella isolates collected from pigs in NW Spain between 2008 and 2009

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Introduction

In the pig industry, the nursery is a critical production period as piglets are susceptible to a variety of enteric infections after weaning, and antimicrobials are commonly used as prophylactics to control Gram-negative (GN) infections. Colistin has been traditionally used to prevent post-weaning diarrhoea. Until recently, prevalence of colistin resistance (CR) was considered low and associated with chromosomal mutations of *pmrA* and *pmrB* genes (Adams et al., 2009). The recent detection and spread of new plasmid-mediated CR-associated genes (Lima et al., 2019), prompted the WHO in 2017 to declare colistin as a ´reserve´ drug against multidrug resistant (MDR) infections in human. In 2015, its use as prophylactic had been banned in Europe. In Spain, the use of colistin remained high (31.4 mg/PCU) until 2015. In that year, a voluntary plan to reduce colistin use in pigs resulted in a significant drop in colistin use (9 mg/PCU)[1].

β-lactam antibiotics have become some of the most used in pig production against GN bacteria (van Rennings et al., 2015). Resistance to these antibiotics is mediated by a wide range of genes coding for β-lactamase enzymes, which are associated with mobile genetic elements (Michael and Schwarz, 2016). The emergence of resistance to these antimicrobials in *Salmonella enterica* has been reported worldwide (Michael and Schwarz, 2016).

We estimate and characterize the prevalence of CR on a collection of *Salmonella* strains isolated from slaughtered pigs in Spain between 2008–2009, that is, much before the official policies on colistin reduction in animals. We also tested a subset of these strains for the detection of extended-spectrum β-lactamases (ESBLs) or AmpC enzyme production.

Methods

A total of 625 *Salmonella* isolates from mesenteric lymph nodes (MLN) from slaughtered pigs were tested for CR by the broth microdilution method (ISO 20776-1:2006), and the epidemiological cut-off (ECOFF) value of >2 mg/L was considered[2]. To assess the possible chromosomal origin of CR, *pmrA* and *pmrB* genes from resistant strains were sequenced and compared to the reference *Salmonella* strain LT2 using BLAST. The presence of the plasmid-mediated CR genes *mcr-1* to *mcr-4* was tested by PCR (García et al., 2018) on all strains with MIC>1mg/L.

A subset of 271 isolates were analysed for ESBL/AmpC production (Total ESBL+AmpC Confirm kit; Rosco Diagnostica, Denmark). At least one isolate of each serotype found in each *Salmonella*-positive herd was selected. Genetic characterization of ESBL/AmpC production was further assessed by PCR (Dallenne et al., 2010).

Results

Six (0.96%) *Salmonella* isolates from 4 different pig farms located far apart showed CR (4 *S.* 4,5,12:i:-, one *S.* Enteritidis, and one *S.* 9,12:-:-). The *mcr-1* gene was detected in all *S.* 4,5,12:i:-, 3 belonging to the same herd. In one strain (*S.* 9,12:-:-) polymorphisms producing protein variants in *pmrAB* were observed. The resistance detected in *S.* Enteritidis is still under characterization. Only one (0.37%) *Salmonella* (*S.* Bredeney) showed AmpC production, which was associated with the bla_{CMY-2} gene.

Discussion and Conclusion

The *mcr-1* gene was identified in *Salmonella* strains isolated one year earlier than the first *Salmonella* and *E. coli* strains reported to bear this gene in Spain (Quesada et al., 2016). Despite its presence, the prevalence of CR in *Salmonella* isolates from pigs exposed to colistin was low. Three of the *mcr-1* positive *Salmonella* isolates belonged to the same farm, suggesting a clonal spread, but the transmission of the *mcr-1* gene among *Salmonella* isolates might not be so frequent. *mcr-1* was detected only in *S.* 4,5,12:i:-, supporting the idea that *S.* Typhimurium and *S.* 4,5,12:i:- are the most common serotypes harbouring *mcr* genes (Lima et al., 2019). Most of resistant strains belonged to zoonotic serotypes, thus a potential transmission of CR to humans is possible. All *Salmonella* isolates harbouring the *mcr-1* displayed MDR (i.e. to aminopenicillins, phenicols, aminoglycosides, sulphonamides and tetracyclines), which may contribute to the co-selection of CR (Lima et al., 2019).

Resistance to 3rd generation cephalosporins was lower (0.37%), and within that observed in Europe for those years (Seiffert et al., 2013), likely because cephalosporin use in food animals was limited at that time (Hornish and Kotarskias, 2002). AmpC production was found in a S. Bredeney and related to the presence of the bla_{CMY-2} gene. This gene was first detected in Spain in 1999 (Navarro et al., 2001) and, although is usually associated with mobile genetic elements (Seiffert et al., 2013), has been scarcely found in *Enterobacteriaceae* from pigs in Spain (Dandachi et al., 2018). Indeed, to the author´s knowledge, this is the first time this gene is detected in a S. Bredeney isolated from pigs in the country. However it has been previously detected in S. Bredeney isolates associated to human cases (González-Sanz et al., 2009; de Toro et al., 2013) indicating its zoonotic potential. This isolate also displayed a MDR pattern, supporting the idea that the emergence/maintenance of resistance to 3rd generation cephalosporins in animals may be related to the co-selective pressure applied by the over usage of non-beta-lactams (Dandachi et al., 2018).
In conclusion, between 2008 and 2009 the prevalence of chromosomal and plasmid-based CR in *Salmonella* from pigs was low in Spain. ESBL/AmpC production was low as well. Both resistances were coded by genes associated with mobile genetic elements and involved zoonotic serotypes.

References

Adams MD, et al. (2009). Antimicrob Agents Chemother, 53:3628-34.
Dandachi I, et al. (2018). Front Microbiol, 28;9:2299.
Dallenne C, et al. (2010). J Antimicrob Chemother, 65: 490–495.
Det Toro et al. (2013). Int J Antimicrob Agents, 42: 167-172.
García V, et al. (2018). Int J Antimicrob Agents, 52:104-108.
González-Sanz et al. (2009). J Antimicrob Chemother, 64, 1181-1186.
Hornish RE, Kotarski SF (2002). Curr Top Med Chem, 2, 717:731 717.
Lima T, et al. (2019). Microorganisms, 19;7(2). pii: E55.
Michael GB, Schwarz S (2016). Clin Microbiol Infect., 22:968-974.
Navarro et al. (2001). J Antimicrob Chemother, 48: 383-389.
Quesada A, et al. (2015). J Antimicrob Chemother., 70:71-4.
Seiffert SN, et al. (2013). Drug Resist Updates 16:22-45.
van Rennings L, et al. (2015). PLoS One., 10(3):e0119114.
WHO. (2017): Report of the 21st WHO Expert Committee on the Selection and Use of Essential Medicines. 27–31 March 2017, pp. 13.
[1]http://www.resistenciaantibioticos.es/es/publicaciones/informe-anual-2016-2017-plan-nacional-frente-resistencia-antibioticos
[2]http://www.eucast.org

09

Quantitative investigation of ESBL resistance in the Danish pork meat chain with estimation of the full burden of ESBL resistance carried in other bacteria than E. coli

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During 2015 to 2018 Danish the pork chain has been investigated qualitatively and quantitatively for ESBL resistance in E. coli and Enterobacteriaceae. The level of resistance carried by animals into slaughter was measured on caecal content (N=266). The contamination of the carcass at slaughter (N=266) was measured from carcass swabs of 1400 cm^2, and the contaminations at cutting (N= 288) and retail (N=529) were measured from meat cut samples of 100 cm^2 . Extended Spectrum Cephalosporinase (ESC) producing E. coli and Enterobacteriaceae were quantified by direct plating on cefotaxime containing media. In feces, on carcasses , at cutting and at retail the observed prevalence of cefotaxime resistant E. coli was 32%, 2%, 1%, and 1%, respectively. The observed mean log concentrations were 2.3 log cfu/g, 2.4 log cfu/1400 cm^2, -0.4 log cfu/cm^2, and at retail it was below detection limit. To quantify the total bacterial population carrying specific resistances, qPCR was performed using primers specific for *tetA* , *tetB*, for all bla_{CTX} genes, and for *uidA* (E. coli). The regression of qPCR C_T values against E. coli cell counts was used to design standard curves, which enable to link a qPCR C_T value to a corresponding cell count. By this way concentrations of bacteria carrying bla_{CTX}, *tetA* and *tetB* genes were estimated. The total number bacteria carrying *tetA* in pigs (caecum) was estimated to be 30 times the number of *E. coli* carrying *tetA*. For ESBL we estimate that the total number bacteria carrying bla_{CTX} in caecum was 30 times the number of E. coli carrying bla_{CTX}. Maximum likelihood methods and Tobit regressions are used to determine quantitative levels of TET and ESBL resistant E. coli below the detection limit, which enables us to do a comparative assessment of *E. coli* ESBL and of total ESBL carrying bacteria in the meat at retail. To substantiate modelling at retail, the more solid data generated at slaughter is included in the analysis. A perspective of the study is to compare the information obtained from this project against the information acquired in the current surveillance system for antibiotic resistance, and to discuss the potentials for adjusting the current surveillance.

010

Antibiotic resistance in E. coli from pigs is associated with their antibiotic treatments and with resistance in E. coli from their dams

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Introduction

A recent European study involving nine countries showed that 88% of pig production batches receive antibiotics during their life, mainly beta-lactams, polymyxins, tetracyclines and macrolides (Sarrazin et al. 2018, JAC).

The purpose of our German longitudinal study was to follow pigs from birth to slaughter and to investigate the association between antibiotic treatment and resistance of fecal *E. coli* from the pigs with a focus on beta-lactams, tetracyclines, polymyxins and macrolides.
We evaluated
a) the antibiotic resistance in different production stages,
b) association between resistance of *E. coli* from these pigs and their dams and
c) potential risk factors (management of housing, feeding, hygiene, animal health, production performance) for antibiotic use at different pig production stages.

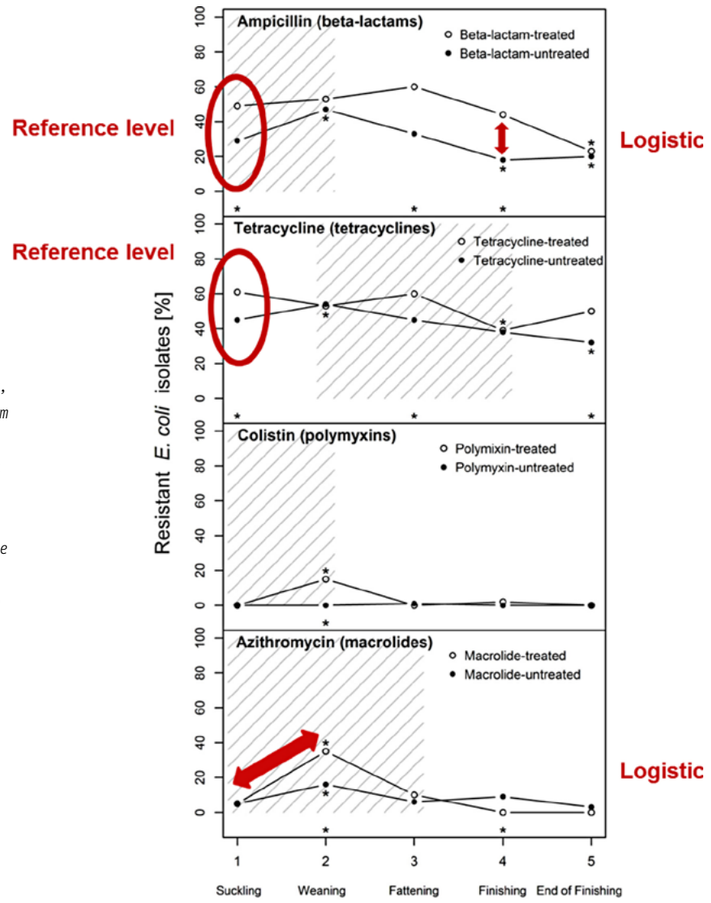

Figure 1: Proportions of E. coli resistant to ampicillin, tetracycline, colistin, azithromycin (representatives for penicillins, tetracyclines, polymyxins and macrolides) from treated and untreated pigs.
Asterisks close to marks indicate significant (p < 0.05) differences compared to first sampling in treated or untreated pigs; asterisks at the bottom of each graph indicate significant (p < 0.05) differences between isolates of treated and untreated pigs at the same sampling points in chi-squared tests for beta-lactams and tetracyclines, as well as in Fisher's exact test for colistin and macrolides

Methods

In each of 29 German breeding herds, two sows were selected. From each sow, seven piglets (in total 406) were followed from birth to slaughter. Antibiotic treatments were documented and fecal samples were collected from the sows around farrowing and from their progeny while suckling, after weaning, and three times during fattening. *Escherichia coli* were tested for their susceptibility to ampicillin (beta-lactam), tetracycline, colistin (polymyxin) and azithromycin (macrolide) by determination of the minimum inhibitory concentration (MIC; broth microdilution, Clinicial and Laboratory Standards Institute 2012, commercial testplates Sensititre, TREK Diagnostic Systems, UK) in accordance with Decision 2013/652/EU (European Commission 2013). The MIC were evaluated against the epidemiological cut-off-values provided by EUCAST (2015). The owners/ managers of the herds answered a questionnaire on relevant farm and animal related factors leading to 121 variables concerning the production stage of piglets, 123 variables concerning weaners and 133 for fattening. All factors were tested on herd-level for their significant effect on antimicrobial use in univariate (decision criterion p<0.2) and multivariate (p<0.02 as the threshold) logistic regression using SAS 9.4 (North Carolina).

Results

a) Resistance to ampicillin and tetracycline was already frequent before pigs were treated with beta-lactams or tetracyclines. Isolates were more likely to be ampicillin resistant in the fattening period if the pig was treated with a beta-lactam during suckling or weaning compared to not been treated (logistic analysis). After administration of macrolides, the risk for *E. coli* to be resistant to azithromycin increased (logistic analysis; Figure 1).
b) Isolates from piglets were more likely to be resistant to ampicillin or azithromycin if those from the dam were so as well (Figure 2).
c) Farm management factors identified for decreasing the risk for antibiotic use at specific production stages were professional rodent control at suckling stage, cleaning of the feeding system after weaning and cleaning of the water pipes with chlorine during fattening (in logistic regressions on herd-level).

Conclusions

The results hint towards the potential of improved hygienic measures to reduce antimicrobial resistance. Reducing antibiotic resistance in sows might also have a positive impact on the progeny. More longitudinal research is necessary.
Hatched area = period in which at least individual pigs received antibiotics.
Number of sampled piglets/*E. coli*: 403 at suckling, 386 at weaning, 339 at fattening, 313 at finishing, 258 at slaughter.

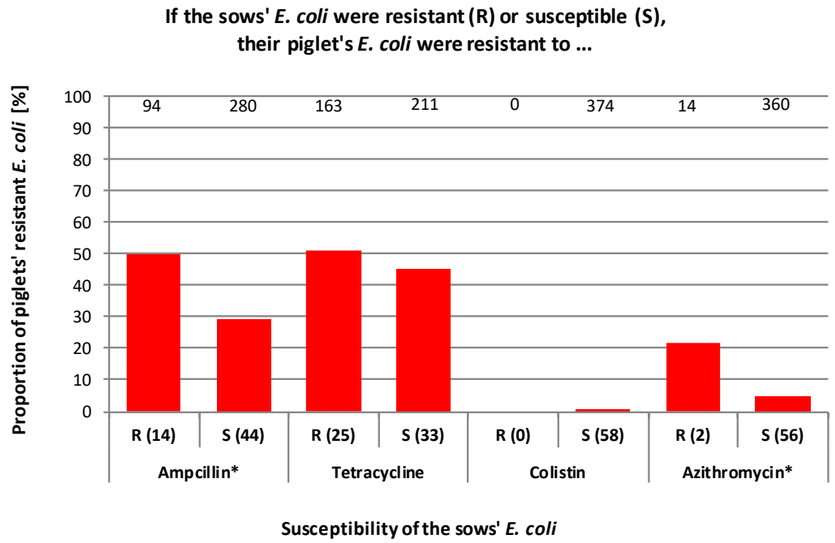

Figure 2: Proportion of resistant E. coli in the intestine of pigs originating from sows with resistant or susceptible fecal E. coli (numbers below bars are total numbers of E. coli isolates from sows; numbers above bars are total numbers of E. coli isolates from piglets; asterisk behind antibiotic indicates significant, p<0.05, association in fisher's exact test)

011

Patterns of antimicrobial use in heavy pig production

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Introduction

Pig farming is a concerning source of antimicrobial resistance (AMR) and reducing antimicrobial use (AMU) at farm-level represents an essential action against AMR spread. Furthermore, information on AMU at farm-level is crucial to develop tailored antimicrobial stewardship (AACTING, 2018). Several studies on AMU in pig farms are available worldwide; however, data on heavy pig production are scarce. Italy is both a major producer of heavy pigs and one of the highest consumers of antimicrobials in Europe (EMA, 2018).
The aim of this study was to investigate AMU patterns in Italian heavy pigs starting with the fattening farms.

Material and Methods

Data from 143 farms were collected retrospectively, covering the 2015 pig population (reared pigs and mortality) and AMU. Information on pig population was provided by the farmers. Data on AMU came from paper prescriptions and health logs. The sampled farms were located in the north of Italy, where most of the Italian pig production takes place. The farms were fattening farms, rearing heavy pigs from 20–30 kg to slaughter. All farms included were involved in the ClassyFarm system trials, a monitoring system under development by the Italian Ministry of Health. AMU was expressed as number of treatment days per 100 days (treatment incidence 100 (TI100)) (AACTING, 2018) using Defined Daily Dose Animal for Italy (DDDAit) as metric. Standard weight at treatment and days at risk were set, respectively, at 100 kg and 180 days. DDDAit were based on Italian summaries of product characteristics.
Associations between AMU, herd size, and mortality were examined using Spearman´s rank correlation, principal component analysis (PCA) and factor analysis (FA)

Results

On the sampled farms, a median of 4,362 fattening pigs were reared (range 1,014–43,159) yielding a total of 916,276 pigs. Median weight at slaughter was 169 kg (range 137–182 kg). Median TI100 was 10.7 (range 0.2–49.5). Tetracyclines was the most commonly administered class (27%), followed by lincosamides (22%), penicillins (13%), pleuromutilins (9%), and macrolides (9%). According to WHO's 2017 list, classes considered as highest priority critically important antimicrobials (HPCIAs) for human medicine represented 17% of the overall AMU. Figure 1 illustrates the distribution of HPCIAs by class.

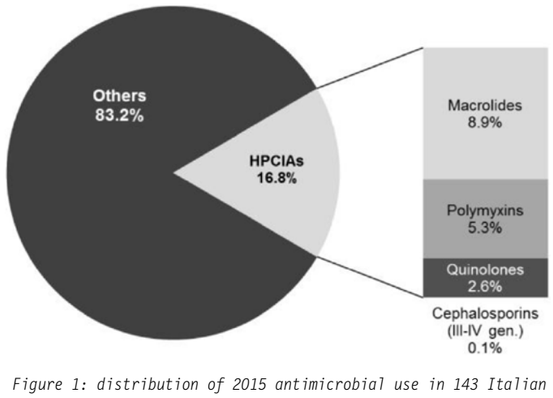

Figure 1: distribution of 2015 antimicrobial use in 143 Italian

In larger farms, AMU (ρ = -0.29, P< 0.001) and mortality (ρ = -0.23, P = 0.01) tended to be lower than in smaller farms. AMU was negatively correlated with use of injectables (ρ = -0.46, P< 0.001) and positively with use of oral products (ρ = 0.21, P = 0.01) and premixes (ρ = 0.26, P= 0.002). Correlation between AMU and mortality was low, but statistically significant (ρ = 0.18; P = 0.03). PCA and FA suggested four dimensions to explain the variance.

Discussion and Conclusion

Wide differences among farms in terms of AMU were found, similar to those described by several studies on pigs slaughtered at lower weights. Macrolides were frequently used which was expected considering how largely they are sold in Italy (EMA, 2018). Although macrolides consumption should be reduced, their prioritisation is still debated (EMA, 2019). The relatively high use of colistin may be explained by the low farmer awareness in 2015. Promoting the administration of injectable antimicrobials, whenever is feasible, could reduce overall AMU.
The negative relations between herd size and both AMU and mortality may suggest that larger farms are more careful on management and biosecurity. The impact of AMU on mortality was low. To better understand AMU in heavy pig production, potential preventive factors (e.g., biosecurity, vaccinations), AMU in other age groups (i.e., sows, sucking piglets, weaners), and production indicators shall be investigated among others using results of the PCA and FA. Hereby, positive examples for farmers can be developed and guiding policies for veterinary authorities can be set, providing a valid tool for rational management and AMU reduction. Nationwide monitoring systems are already successful implemented in several countries. However, developing such a system for a large nation is challenging. Therefore, starting with a sample of farms is a first step towards a nationwide system.

References

AACTING consortium (2018): Guidelines for collection, analysis and reporting of farm-level antimicrobial use, in the scope of antimicrobial stewardship (version 1.1). Available at http://www.aacting.org/swfiles/files/AACTING_Guidelines_V1.1_2018.03.23_39.pdf. Last accessed 24 April 2019.
European Medicines Agency (2018): Sales of veterinary antimicrobial agents in 30 European countries in 2016. Available at https://www.ema.europa.eu/documents/report/sales-veterinary-antimicrobial-agents-30-european-countries-2016-trends-2010-2016-eighth-esvac_en.pdf. Last accessed 24 April 2019.
European Medicines Agency (2018): Answer to the request from the European Commission for updating the scientific advice on the impact on public health and animal health of the use of antibiotics in animals - Categorisation of antimicrobials. EMA/CVMP/CHMP/682198/2017. Draft available at https://www.ema.europa.eu/en/documents/other/answer-request-european-commission-updating-scientific-advice-impact-public-health-animal-health-use_en.pdf. Last accessed 24 April 2019.
World Health Organization. (2017): Critically important antimicrobials for human medicine: ranking of antimicrobial agents for risk management of antimicrobial resistance due to non-human use. 5th revision, Geneva, Switzerland.

012

Handling of cases where a pig producer calls in regarding delivery of slaughter animals prior to the end of the withdrawal period

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Introduction

Withdrawal periods are set to ensure that the concentration of residues of legal medicinals is below the maximum residue limits (MRL) when animals are delivered to slaughter. Very few human cases are reported dealing with adverse effects related to consumption of meat with residues of antimicrobial origin. This is presumably related to the low prevalence as well as the low concentration of these substances at the time of consumption of the meat.
In the official Danish surveillance and the abattoirs' own check of veterinary medicinals, the carcass is withheld pending the test result. If residues are found >MRL, the carcass is condemned. Occasionally, a pig producer calls the abattoir to inform that - by mistake - an animal has been delivered prior to the end of the withdrawal period. If the producer calls in time, the abattoir finds the animal in the lairage and ensures that it is not slaughtered but euthanized and destructed. However, if the animal is slaughtered, it may be difficult for the abattoirs to find the carcasses. In line, the by-products may be mixed with by-products from animals slaughtered on the same day.
A case arose in Denmark in 2018, where a pig producer informed the abattoir that two pigs had been delivered to slaughter too early. The drug used was Ethacillin, a penicillin product with protracted effect. The pigs were slaughtered 28.8 hours after treatment, and the withdrawal period is 96 hours. When the abattoir was informed, the pigs were already slaughtered. An analysis showed that that the residue concentration was above MRL at the time of slaughter. The carcasses were identified and destroyed. The organs, blood and fat were mixed with similar tissue from the other pigs slaughtered on the same day. For blood and fat, a dilution had taken place whereby the concentration would have been below MRL. However, as the abattoir was unable to find the organs from the affected animals, all organs from the slaughterday were condemned due to a health concern because of the presence of the organs from the two treated pigs.
The decision to condemn should be seen in the context of the Danish interpretation of the residue programme as surveillance requiring action. In other EU Member States, the programmes are run mainly as monitoring implying that the carcasses are not withheld, but where follow-up visits are made to herds from which a positive animal (>MRL) is detected.
The abattoir and the pig producers have product responsibility insurances in place. The maximum amount which can be paid in relation to the insurance is €660,000, and the producer would have to pay around €5,000 as own risk. These maximum amounts were reached in the case which this paper deals with.
The question is how to balance between avoiding unnecessary food waste and complying with EU legislation to ensure consumer confidence. We suggest using Allowed Daily Intake (ADI) as an alternative to MRL, specifically for the situation where the producer contacts the abattoir to inform about slaughter animals delivered before the end of the withdrawal period.

Material and Methods

The two pigs were weighing 100 kg and had each been given 5 ml Ethacillin in a concentration of 300 mg per ml. Hence, they had each received 1500 mg Ethacillin. The pigs were slaughtered 28.8 hours after they had received the injections.
The amount of Ethacillin left was calculated based upon information about the half-life of the drug, which is around 9 hours. The amounts left were compared to EU MRL of penicillin which is 50 µg/kg in muscle, fat, liver and kidney.
Next, we estimated the amount of Ethacillin present in 1) 150 g meat and 2) 50 g sausage, if made from meat from the two pigs.
ADI is the maximum daily dose, which a person may consume without experiencing negative reactions. For penicillin, ADI is 0.03 mg (30 µg) (http://www.inchem.org/documents/jecfa/jeceval/jec_2002.htm and http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1938).

Results

Using a half-life of 9 hours implies that the amounts of residues left in the body at the time of slaughter was halved 3.2 times (28.8/9=3.2). Hence, $0.5^{3.2}$ = 11% of the original concentration was left - corresponding to 165 mg.
This amount was assumed to be dispersed evenly in the body, whereby the concentration would have been: 1.65 mg/kg (165 mg / 100 kg) or 1650 µg/kg, which is 33 times higher than the MRL.
If a person has consumed 150 g of meat or 50 g of sausages, the person would have been exposed to eight times the ADI (for meat) or three times (for sausages).

If the meat, organs, fat and blood had been used as category 3 animal by-products, then the processing involving chopping and mixing would have resulted in a concentration below MRL and ADI.
The amounts of residues in the sausage portion would have been below ADI, if the slaughter had taken place 40 hours after treatment, whereas 56 hours after treatment would have to pass for the amounts to be below ADI for the serving of 150 g meat.

Discussion and Conclusion

The present case shows that there are two threshold values that are of importance for the assessment of the food safety impact: MRL og ADI. Both represent an indicator of what humans can be exposed to every day over a long time without experiencing negative human effects. Moreover, in the establishment of MRL and ADI, safety factors are used. The current EU Directive 96/23 only operates with MRL. We suggest that both MRL and ADI are used in the handling of potential presence of residues of legal medicinals. First, information about the treatment should be obtained (time, product, volume, concentration, and way of administration). Next, the residue concentration at the time of slaughter is calculated. If the concentration is above MRL, then the intended use of the meat, organs, blood or fat should be considered by calculating the amount of drug present in a relevant serving size. The effect of dilution - through chopping and mixing - should be included.
In the case described above, the organs could for example have been used as category 3 animal by-products, because organs from the two pigs would have been chopped and mixed with similar organs from the same slaughterday. This view is in line with the risk assessment approach already taken in Denmark to the handling of blood and fat, since there are no concerns for toxicity and cancerogenic concerns for veterinary medicinals already approved for legal use. In EU Member States where the residue programme for legal veterinary medicinals is interpreted as monitoring, meat and organs from an entire slaughterday would all be used for human consumption, without any restrictions.
Disproportionate actions are creating a disincentive for producers to report. From a food safety culture perspective, reporting of mistakes should be encouraged, so we can learn and improve our practices. Moreover, it is the Good Farming Practices (GFP) - including marking and registration of treated animals - which ensure that the withdrawal periods are complied with, not the surveillance system.
A generic risk assessment model which includes intended use of meat or organs could be used as support for the local authorities and the abattoirs. Use of such a tool would lead to a systematic, science-based and objective decision, where harmonisation with EU legislation and various trade requirements should be ensured. Hereby, unnecessary food waste may be avoided.

ANIMAL WELFARE

013

Ethical implications of the alternatives to surgical piglet castration

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The changing human-animal-relationship

The human-animal-relationship has drastically changed in the last two decades: Animals are not any longer regarded as just objects that the owner can treat how he or she wants to, but as they are more and more seen as subjects, i.e. as sentient creatures, who deserve that people that own or care for animals have to guarantee their animals a decent life. A good description of a decent life of animals in human care is the concept of the "Five Freedoms" that describe the current European understanding of good animal welfare:

- Freedom from hunger and thirst, by ready access to fresh water and a diet to maintain full health and vigour.
- Freedom from discomfort, by providing an appropriate environment including shelter and a comfortable resting area.
- Freedom from pain, injury, and disease, by prevention or rapid diagnosis and treatment.
- Freedom to express normal behaviour, by providing sufficient space, proper facilities and company of the animal's own kind.
- Freedom from fear and distress, by ensuring conditions and treatment that avoid mental suffering.

Of course, keeping and using animals for human purposes is mostly connected with imposing on the animals some sort of stress, curtailing normal behavious and even pain and suffering. However, in the light of the growing understanding of the responsibility that humans have for the animals in their custody and/or use, there is the moral imperative that **only the mildest possible treatment** is allowed and that there must be **a strong justification for causing any pain or stress** to animals.

The responsibility of humans for the animals in their custody

In the light of this modern understanding, it is necessary to scrutinize many of the traditional treatments of animals that may be obsolete, since they can be replaced by better (more animal-friendly) methods to reach the same goal. One of these traditional treatments is piglet castration, especially the castration without anaesthesia and pain relief.

Castration of both male and female pigs has a long history: for centuries it was done first to prevent the commingling of pastured domestic pigs with wild boars, then for preventing the "boar taint" of pork produced from adult male pigs. Until today, most piglets in Europe are still castrated by the farmers without anaesthesia/analgesia, which until recently was not questioned, since the "strong justification" was to make sure that the killing (slaughtering) of the animals for food production is only given, if the meat of the slaughtered animals is afterwards indeed used for human consumption, which would not be true if the meat "stinks" and the meat would be discarded. So: the only justification for the castration of male piglets is preventing the "boar taint" of the meat from male pigs.

However, modern views on how to treat animals diminish more and more the acceptance of inflicting pain to animals, when this can be avoided. Thus, the questioning, why piglets are castrated without anaesthesia and pain relief started around the year 2000. In the following years, the following three alternatives to the painful traditional castration were discussed and Europe-wide legally approved:1. surgical castration with anaesthesia/analgesia; 2. raising entire males, and 3. Immunization against GnRH

What has been done and what not

However, there was and still is a lengthy debate about which of the alternatives should be applied - up to now only arguments from the farmers' community, the meat industry and the retailers about why this or this alternative cannot be accepted are exchanged. The 2010 European Declaration initiated by the EU Commission on the voluntary end of the painful castration throughout Europe by 2012 did not have a measurable effect. And even the German legal deadline of ending castration without anaesthesia, which was set by the German Welfare Act for the 31.12.2018, has been postponed by the German government.

The general argumentation is that all three alternatives have comparably equal pro's and con's and the various players in the food production chain cannot agree on which one alternative since they would be differently affected by the alternatives. The judgement that all three alternatives have the same amount of pro's and con's, is, however, from an ethical point of view simply wrong. Why: since there are different kinds of con's, namely on the one hand economic disadvantages for humans, e.g. additional costs and labour and/or for difficulties to market the meat of entire boars or difficulties to explain the consumer the vaccination at the point of sale; and on the other hand there are disadvantages for the animals due to e.g. loss of body parts by the amputation or increased stress and anxiety due to the fixation of the animals or due to increased ranking order fights. At this point of the considerations it is important to be reminiscent of the fact that the discussions to change the traditional castration method never were started for economic reasons, but solely for reasons that are in the interest of the animals. Thus, we have to rank the pro's and con's of the alternatives by strictly looking at the level of stress and anxiety that each method imposes on the animals. If we do this, then we have a clear ranking order from the method that charge the animals the "highest price" to the method that charges the animals the "lowest price" (see Tab. 1).

Of course, there is in case of the immunocastration, the method that imposes the lowest pain and stress level on the animals, a "price" to be paid by the farmers and the meat industry and to a certain extend by the retailers: the farmer has to buy the vaccine and to vaccinate the animals twice (the second time when the male pigs are already quite heavy), the meat industry must develop a method to recognize those animals that may not be vaccinated correctly, and the retailers have to properly explain the animal welfare advancement to the consumer to make sure that the vaccination is not confused with any hormone treatment.

Ethical assessment

There are interests of humans "against" interests of animals - and: ethics requires balancing conflicting values and interests. In the case of the alternatives to piglet castration, the moral judgement is quite easy: humans can handle the economic disadvantages of the alternatives that are "better" for the animals, animals can NOT handle the disadvantages that are imposed on them. Thus: it is a **moral obligation** of all stakeholders in the pork chain to **agree on the vaccination** against the boar taint, and to compensate the additional costs for the farmers, and to generate the acceptance of this **animal friendliest method** to prevent the boar taint.

Table 1: A synopsis of the Pro's and Con's of the 3 in Europe approved alternatives (disadvantages for the animals are printed in red, those for the humans are printed in green)

Method	Pro's	Con's
Surgical castration with anaesthesia/analgesia	Pain relief during and after surgery	Restraining pigs = stress Local anaesthesia is painful Castration = amputation
Raising entire males	No manipulating the animals No pain due to surgery No amputation	Injuries due to fighting males Soft fat Potential boar taint
Immunization against GnRH	Only two injections No pain due to surgery No amputation	Structural changes Additional work during finishing the pigs

MEAT INSPECTION

014

Pork safety assessment and first results from pilot interventions targeting slaughter and retail in selected provinces of Northern Vietnam

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Introduction

Pork constitutes 75% of the meat consumed in Vietnam with 80% of pork products produced by smallholders, slaughtered in small scale facilities and sold in traditional markets. Food safety is one of the most pressing concerns of Vietnamese consumers. In this research programme we address whether pork in Vietnam is safe to consume and investigate what mitigation options are feasible, acceptable and effective.

Methods and Methods

Research conducted since 2013 includes risk profiling, risk assessment for biological hazards, a cost of illness study and food safety performance assessment of a range of current pork value chains in 4 provinces of Northern Vietnam. The pork value chains studied include different production systems as well as modern and traditional retail, 'organic' food shops and pork originated from indigenous pigs. Data collection spanned the entire pork value chain using focus group discussions, key informant interviews, observations and biological sampling for *Salmonella* using a probabilistic sampling design.

Potential interventions focus on technical solutions e.g. use of mini-ozone units complemented by nudging to influence the behaviour of pork value chain actors.

Results

Results show that pork is not safe: 44%-80% of pork sampled was contaminated with *Salmonella*. A quantitative microbial risk assessment indicated that one to two out of 10 pork consumers are at risk of *Salmonella* poisoning annually (Sinh Dang et al. 2017). Meat in both modern and traditional retail was found to be highly contaminated with *Salmonella*. Various approaches to improving pork safety have been tried e.g.: Good Animal Husbandry Practices (GAP), traceability and modernising retail. Despite these efforts production and distribution of safe pork has not yet reached a significant share of the market in Vietnam. The key constraint to uptake was the lack of incentives for stakeholders in the chains. We propose gradual improvements to the food system in place. Potential mitigation options in the ongoing pilot testing phase are iron grids to avoid floor slaughter. A previous pilot trial has demonstrated that a tailored iron grid can reduce contamination but must go along with behaviour change (see *pilot trial example* and Photos 1 and 2) and explanations. Other mitigations are mini-ozone units to decontaminate surfaces, antimicrobial cutting boards or clothes at retail. Potential behavioural nudges are being explored to support technical interventions and behaviour change. First results from the nudge study indicate that value chain actors such as slaughter house workers or retailers consider the effect of colour on salience differently; e.g. red was considered dirtiest while blue the cleanest colours, respectively.

Photos 1 and 2: Workers at a pig slaughterhouse in Hung Yen province, Vietnam testing the use of an off-the-ground slaughtering rack, February 2016 (photo credit: Hanoi University of Public Health/Sinh Dang Xuan)

Pilot trial example:

The introduction of a low-cost, off-the-ground slaughtering rack (designed with the slaughterhouse owner) and other measures to reduce contamination in the treatment group (n = 10) significantly improved slaughter hygiene compared to the control using a business-as-usual approach (n = 10). The improvement in hygiene was indicated by lower coliform load (p = 0.002) on the carcass surface compared to the control. The pilot trial also demonstrated that technical solutions must go along with behavior change of butchers (Photos 1 and 2) (ACIAR, 2019).

Discussion and Conclusion

Pork was found not safe and public health implications for consumers have been quantified. Potential mitigations, currently piloted, require incentives and behaviour change of value chain actors.

Acknowledgment

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References

ACIAR 2019. Final *report*. Project. Reducing disease *risks* and improving food safety in smallholder *pig* value chains in Vietnam. Project number, LPS/2010/047. https://www.aciar.gov.au/file/105392/download?token=KsQUi5H4

Sinh Dang-Xuan, Hung Nguyen-Viet, Unger, F., Phuc Pham-Duc, Grace, D., Ngan Tran-Thi, Barot, M., Ngoc Pham-Thi and Makita, K. 2017. Quantitative risk assessment of human salmonellosis in the smallholder pig value chains in urban of Vietnam. *International Journal of Public Health* 62(Supplement 1): 93-102. http://hdl.handle.net/10568/77739

015

Handling of lesions indicative of prior septicemia in sows

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Introduction

During meat inspection, abscesses may be found which indicate that an animal has suffered from septicemia at an earlier stage in life. In Denmark, due to the national legislation such animals are sent to the rework area for an extended pyemia examination with incisions targeting the predilection sites for such abscesses. Tissue with abscesses found at this stage is removed from the carcass. Next, the carcass is sent for mandatory de-boning after which almost all carcasses are accepted, although all bones separated from de-boned carcasses are condemned and treated as category 2 animal byproducts. The food safety value of this approach has been questioned. It should be noted here that abscesses have no impact on food safety but are regarded as a quality issue only (Bækbo et al., 2016). Preliminary studies and analyses using register data from the largest sow abattoir in Denmark have shown that almost no abscesses are overlooked at the pyemia examination (Pedersen et al., 2017). However, routine recordings at meat inspection may not have been sufficiently detailed to address this issue. Hence, to elucidate whether and where abscesses could be found after the pyemia examination a prospective study was needed. Based upon the outcome, the examination could be updated, and the legislation amended.

Material and Methods

Therefore, a detailed study was undertaken, involving 100 sows destined for de-boning, following findings of lesions indicating prior septicemia. The study was undertaken in November and December 2018 at the largest sow abattoir in Denmark in a collaboration between the abattoir, the local and national veterinary inspection authorities, and the Danish Agriculture & Food Council. A recording scheme was designed to ensure systematic registration of findings during 1) the pyemia examination, 2) a supplementary examination and 3) the de-boning.

The pyemia examination and the supplementary examination consist of incisions, palpations and visual inspections at defined locations and predilection sites for abscesses:

Pyemia examination:

1) Inspection of the spine, 2) inspection/palpation of fore and hind legs, 3) loosening the bow including deep cuts along humerus, 4) loosening the inner thighs including deep cuts into and along femur, 5) loosening the tenderloin muscle from column, 6) inspection of sternum and ribs with special attention to the transition between bones and cartilage

Supplementary examination: 1) Incision along the thorn pins and inspection of the entire spine, 2) cutting off the head and toes, 3) in the thoracic cavity, incision of the transition between the cartilage and the bones of the ribs and inspection of the sternum and ribs, with special attention to the transition between bones and cartilage.

Results

The results show that additional abscesses related to pyemia were found at de-boning for seven carcasses. The location was: femur and humerus (n=1), humerus (n=3), hind side of costae (2), and scapula (1). For the abscesses in femur and humerus, the location was latero-proximal to the growth line.

Additional abscesses related to pyemia were found in seven carcasses in relation to the supplementary examination. Two of these were considered as related to the pyemia complex; one in the pelvis and one latero-proximal in the humerus. The remaining five abscesses were found in the neck or the midpart and were not considered as related to the pyemia.

Hence, abscesses related to pyemia were found in nine out of 100 carcasses - either during the supplementary examination or the pyemia examination. Moreover, neck abscesses related to injections were found in 54 in total out of 100 carcasses.

Discussion and Conclusion

The aim of the study was to collect data that could be used to update the current pyemia examination in sows. During the supplementary examination, seven sows with abscesses were found, of which a single sow had a pyemia-related abscess in the pelvis. During the supplementary examination combined with de-boning, a single sow was found with a pyemia-related abscess in the humerus. Hence, in total two sows with pyemia-related abscesses were found.

In 93 sows no abscesses were found during the supplementary examination. In these 93 sows, de-boning led to detection of pyemia-related abscesses in seven cases. Of these seven sows, four had an abscess in the humerus, one in the femur, one in the ribs, and one in the scapula. In total, six of the seven sows had one pyemia-related abscess, and one sow had two pyemia-related abscesses (humerus and femur).

The high prevalence of neck abscesses (54%) was expected, because sows are adult animals that have received many routine vaccinations and some antibiotic treatments throughout their lives. As no control group was included in the study, it is not to say whether sows destined for de-boning have a higher prevalence of neck abscesses compared with sows not destined for de-boning. In any case, such abscesses have no relation to the pyemia complex, and they are routinely handled by the slaughterhouse employees, as it is known that there may be neck abscesses in sows.

The sites of abscesses latero-proximally on the femur and humerus, and in the scapula (total of six carcasses out of 100) raised the question of whether it would be possible to add a latero-proximal incision on the four extremities on the hanging carcass. If these four incisions were added to the supplementary pyemia examination, most of the abscesses related to pyemia found in the present study would have been encompassed.

Subsequently, the possibilities of expanding the supplementary examination with specific deeper incisions on the forelimb´s and hindquarter´s muscle into the bones followed by inspection and palpation were elucidated. The inspection and palpation covered the epiphysis of femur and the humerus, as well as the area covered by the lateral epiphysis line for occurrence of abscesses originating here from. This was proved to be possible in practice on the hanging sow.

The study has led to an update of the official Danish pyemia examination to be done on the hanging sow upon suspicion of prior septicemia. This implies improved working conditions, and to a possibility of replacing mandatory de-boning with a supplementary examination to the pyemia examination. The new legislation came into force mid-April 2019 and has led to higher profitability, less food waste and better working conditions for the employees.

References

Bækbo, A.K., Petersen, J.V., Larsen, M.H., Alban, L., 2016. Food safety value of handling chronic cases of pyaemia in finishing pigs by use of de-boning. Food Control, 69, 177-184.

Pedersen, T.Ø., Bækbo, A.K., Petersen, J.V., Larsen, M.H., Alban, L., 2017. Evaluation of the food safety risk associated with de-boning of Danish sows with chronic purulent lesions indicative of prior septicaemia. Fleischwirtsschaft International, 2, 66-70.

016

Statistics of meat inspection: How to standardise the assessment of ante-mortem and post-mortem inspection of pigs nationwide? - Development of an educational concept for Germany

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Introduction
The evaluation of slaughter animals, carcasses and offal is a central task of the official ante-mortem and post-mortem inspection. The assessment is biased for several reasons: individual, administrative, or organisational. The data collected are of particular relevance to the food business operator, the competent authority, and the official meat inspection statistics. Consequently, a valid data collection by the competent authority requires quality control and quality assurance.
The aim of this project is the development of an innovative educational concept for the standardised assessment of pigs all over Germany.

Material and Methods
The focus is on creating digital teaching and training material (videos and eBooks) to recognise selected clinical findings, and to grade them as far as possible. These findings are relevant for animal health, meat safety, or animal welfare. The primary target groups are official veterinarians and official auxiliaries, followed by students of veterinary medicine. The raw material was recorded in two German pig abattoirs in 2018 and edited with Adobe® CC software. In 2019, the teaching material was provided for a first review exercise to the Federal Ministry of Food and Agriculture, other federal institutions, the veterinary authorities of the federal states, and the universities/ faculties of veterinary medicine. The revisions were discussed by an expert panel and approved by expert representatives of the federal states. The final teaching material will be distributed to all relevant institutions for implementation into their own didactic structures.

Discussion and Conclusion
Deviations among reviews demonstrate some heterogeneity in assessment and, hence, support the need for a nationwide standardisation. The extended communication at each step and the inclusion of all available experts are expected to allow for an overall acceptance. The actual impact will be visible in the coming years.

017

Interactive meat inspection: Do we all decide in the same way?

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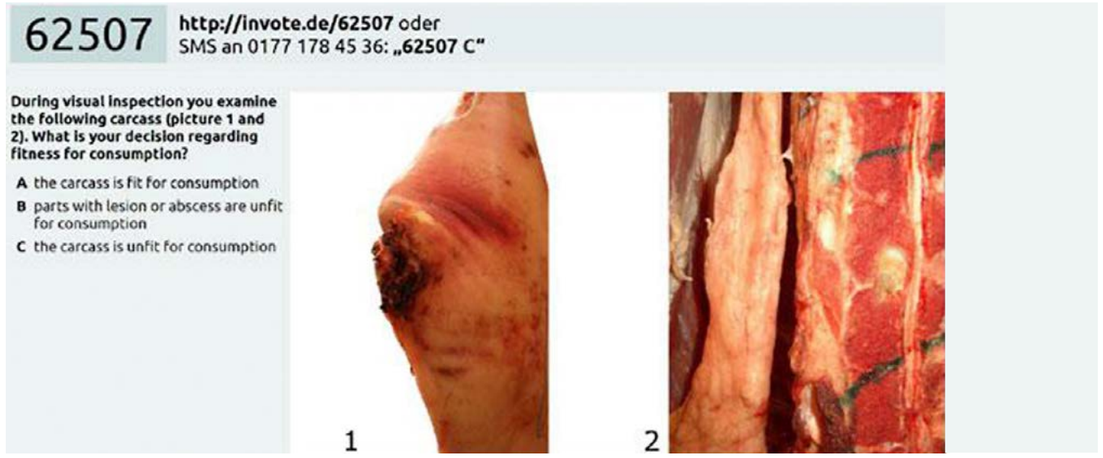
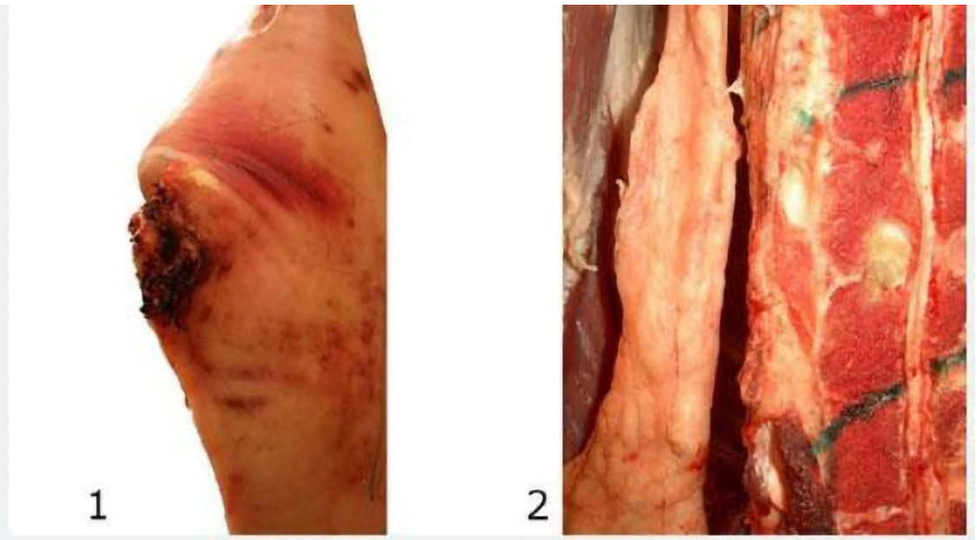
In the EU, the visual post-mortem inspection is the standard procedure for pigs at the slaughterhouse. Nevertheless, the official veterinarian has to expand the post-mortem inspection procedures by using palpation or incision if one or more of the following indicates a possible risk to public or animal health or animal welfare:
checks and analysis of the food chain information following Reg. (EC) No. 853/2004
findings of ante-mortem inspection
results of the verifications concerning animal welfare rules
findings of post-mortem inspection
additional epidemiological data or other data from the holding of provenance of the animals
Visual findings in pigs and the value at the current food chain information as required in Reg. (EC) No. 853/2004 are critically discussed topics since several years. In the presentation, we will focus on post-mortem findings and a few cases that have to be evaluated visually, as it is done day by day in the slaughter line in the EU. In this context, we will be dealing with the question: “Do we all decide in the same way?”

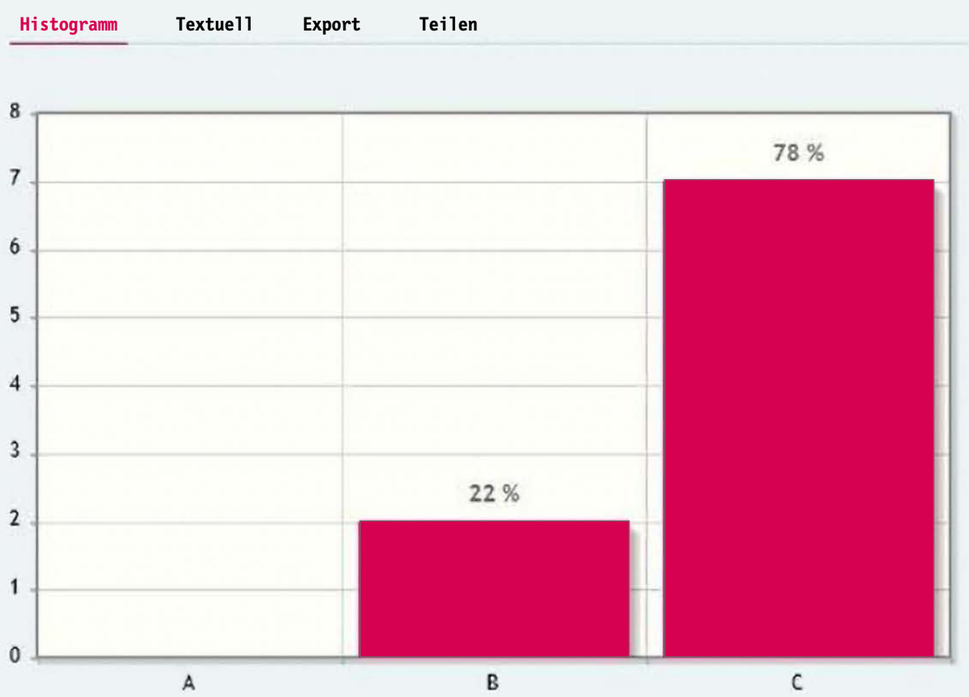
The intention of these interactive case series is to initiate a professional exchange between the expert auditorium and to discuss possible decisions in the visual post mortem inspection.

Via an online voting platform, all conference participants can take part in an anonymous vote. The evaluation of the voting will be directly available as a basis for an in-depth discussion of the available choices.
You can actively take part in the discussion by using the online-voting tool “Invote”.
Please use your smartphone or tablet to participate in our interactive presentation using the following link:
https://invote.de/62507
or scan the QR-Code:

Below you can see an example case with a short description and a question to be answered. One of the three possible choices must be selected and submitted:
Afterwards we would like to discuss the voting results together.
Example results after an evaluation of the example case:







A the carcass is fit for consumption
B parts with lesion or abscess are unfit for consumption
C the carcass is unfit for consumption

N=9

018

Assessing the food safety risk associated with federally regulated pork establishments in Canada using the Canadian food inspection agency's establishment-based risk assessment model

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Introduction

The Canadian Food Inspection Agency (CFIA) has developed a quantitative risk assessment model to help inform inspection resources' allocation for food establishments. This "Establishment-based risk assessment" (ERA) model takes into consideration risks associated with a specific food commodity, operation or manufacturing process, mitigation strategies implemented by the industry to control their food safety risks, as well as establishment compliance information (Racicot et al., 2018 and 2019; Zanabria et al., 2018). In 2014, a pilot project assessed the model's performance with 49 meat/poultry establishments resulting in a Spearman correlation coefficient of 0.64 ($p < 0.001$) between the model outputs (annual number of DALYs) and the assessment done by CFIA senior inspectors.

Materials and Methods

To assess the food safety risk of all federally regulated pork establishments across Canada, 689 meat establishments, including 59 facilities exclusively doing pork slaughtering and/or processing activities, attended WebEx information sessions along with their assigned inspectors. Using an Excel questionnaire, both provided inputs, from April to October 2017, on the inherent/mitigation factors associated with the establishments, which were analysed by the model algorithm along with up to 5 years-compliance data from CFIA's systems.

Results

Nineteen establishments (out of 689) were not considered in the analysis because they refused participating (0.7%), were not operating (1.6%), or were not processing/storing meat products (0.04%) at the time of data collection. Forty-nine percent (337) of the meat establishments reported processing only pork or pork and other meat species. From those, 111 (33%) establishments distributed products directly to vulnerable population, 204 (61%) applied several additional treatments to further reduce their risk (e.g., antimicrobials), and 336 (99.7%) applied specific controls for incoming supplies (Figure 1). Intact meat (e.g., raw cuts, carcasses) (60%), ready-to-eat cooked (15%), and offal or meat by-products (9%) were listed as the most common pork sub-products being processed (see Table 1).

The 337 establishments processing only pork or pork and other meat species (representing 33% of the total meat production volume) were responsible for 40% of the total meat risk. Among pork establishments, only 10 contributed to 44% of total risk related to the pork sector. This model helped categorizing pork establishments into 4 groups calculated based on their individual risk contribution to the overall meat risk. Then, considering its individual contribution to the overall food safety risk in the meat sector there were 0, 41, 150, and 146 for category 1 to 4 respectively, where 1 represents the highest risk and 4 the lowest, as of March 2019.

Discussion and Conclusion

By using scientific data and establishment specific information gathered from regulated parties the ERA model evaluates a facility and determines an establishment´s level of risk. How often an inspection occurs will be guided by where a facility falls in the four categories of risk assigned by the ERA model, i.e., higher risk establishments (categories 1 and 2) would require more oversight while lower risk establishments (categories 3 and 4) would require less oversight. These findings will be integrated in the Agency's work planning for risk-informed oversight, to proportionally allocate inspection resources based on the establishment risk contribution.

References

Racicot M, Zanabria R, Leroux A et al. (2018). Quantifying the impact of food safety criteria included in the Canadian Food Inspection Agency risk assessment model for food establishments through Expert Elicitation. Food Control. 92, 450-463. Friedrichstr./ Reichstagufer

Racicot M, Zanabria R, Paradis ME et al. (2019). Identification of risk factors to be considered for food establishments' risk assessment models. Microbial Risk Analysis, 11, 1-10.

Zanabria R, Racicot M, Cormier M et al (2018). Selection of risk factors to be included in the Canadian Food Inspection Agency risk assessment inspection model for food establishments. Food Microbiology. 75, 72-81.

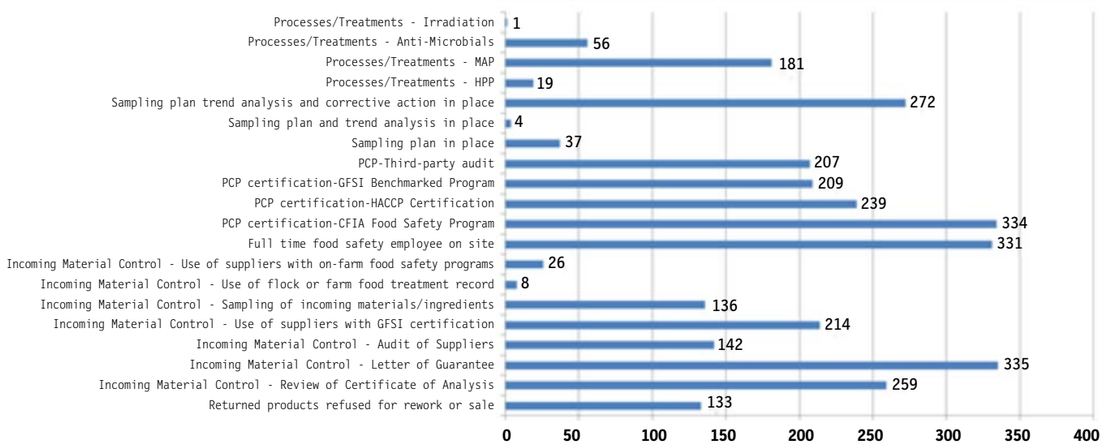

Figure 1: Number of Canadian pork establishments implementing strategies to reduce food safety risks (n=337)

Table 1: Pork Sub-products volume (processing/slaughtering) (includes establishments processing multi species)

Sub-product manufactured by the establishment	Domestic volume (millions of Kg)	% of total (domestic) pork sub-products	Export volume (millions of Kg)
Raw Non-Ready-To-Eat (non-RTE) comminuted meat: ground, finely textured, chopped, mechanically separated, flaked and minced	67.82	4.7	24.87
Raw Non-Ready-To-Eat (non-RTE) meat: Non-intact (tenderized, injected, restructured, etc.)	93.99	6.5	18.63
Raw Non-Ready-To-Eat (non-RTE) meat: Intact and/or commercial raw cuts (including carcasses)	865.13	60.3	971.76
Raw Non- Ready-To-Eat (non-RTE) meat: Offal or Meat By-Products	129.52	9.0	190.16
Ready-To-Eat (RTE) cooked meat	213.96	14.9	16.86
Ready-To-Eat (RTE) dried cured meat	8.60	0.6	0.99
Ready-To-Eat (RTE) dried fermented meat	19.42	1.4	0.44
Ready-To-Eat (RTE) canned (appertized) meat	14.31	1.0	0.34
Other	22.80	1.6	9.28

019

Safe pork or safer pork? What has been changed and is to be changed in the EU hygiene legislation?

Ernst E.[1]

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Since the adoption of the "hygiene package" in 2004 by the legal bodies of EU several modification and changes of the EU hygiene legislation has taken place or will enter into force as from 14th December 2019 (Control Regulation (EU) 2017/625 and Delegated Acts and Implementing Regulation according Art. 18). The key elements to produce safe pork subsequent to the primary production are the legal arrangements for the information exchange between farmer and slaughterhouse and official vet (food chain information and reports of the official veterinarian), (risk based) meat inspection, GHP and HACCP-based procedures, microbiological criteria and residue controls. As a member of the Commission working group on the hygiene legislation, the author likes to give an overview about the last and actual changes in hygiene legislation in relation to safe pork.

In 2009 the European Commission and the Member States (MS) and asked the European Food Safety Authority (EFSA) to give a scientific opinion to modernise meat inspection. Based on the recommendations Commission developed and adopted together with the MS several, more risk-based approaches to modify the legal requirements for meat inspection and meat production in domestic swine:

- introduction of the meat inspection method "visual only" as standard method in 2014 for domestic swine to reduce the cross contamination risk for zoonotic agents during the slaughter process
- modification of the process hygiene criteria (PHC) "Salmonella" from maximum 5 to 3 positive tested carcasses within the moving window of 50 tested carcasses within 10 weeks from 2015
- report of the results from the MS/competent authorities about the own controls results according Regulation (EC) No 2073/2005 (PHC) in relation to Salmonella beginning from the year 2015
- possibility to omit trichinella testing in 2015 under controlled housing conditions for domestic swine

In the course of merging of the Regulations (EC) No 882/2004 and 854/2004 to Regulation (EU) 2017/625 on official controls Commission was authorised to adopt delegated and implementing acts according Art.18 (7) und (8). The procedures on official controls in the field of meat productions had to be revised.
These new EU regulations on official controls to replace Regulation (EC) No 854/2004 will come into force from 14th of December 2019. The delegated and implementing acts were published in the Official Journal of the European Union (OJ) on 17th of May 2019 as Regulation (EU) 2019/624 and Regulation (EU) 2019/627. Major issues are:

- definition of small slaughterhouses as facilities with a slaughter throughput of less than 1.000 large cattle units per year and some derogations for them (meat inspection by official auxiliary)
- possibility to perform ante mortem inspection for all species at the holding of provenance
- "visual only" as standard meat inspection method for young bovines and lamps, other examination methods only risk based
- ante mortem inspection can be done by official auxiliary under the supervision of an official vet in slaughter houses when the animals alive show no abnormalities.
- relevant findings in meat inspection (human and animal health, animal welfare) are to be reported always to the competent authority responsible for supervising the holding of provenance
- more detailed specifications on auditing fresh meat establishments and measures in cases of noncompliance for official veterinarians and competent authorities
- emergency slaughter needs an official veterinarian for ante mortem inspection, other veterinarian are no longer allowed
- reduction of the theoretic training for official auxiliaries
- some other derogation for the official controls for the production of small amounts of meat (farmed game, reindeers, grouse)

The prominent aims of the last changes and revised versions of the EU hygiene legislation are in the first line more flexibility for small establishments and more efficiency and effectivity in official controls. The changes in 2014/2015 addressed particularly the salmonella risk in pork.
According the framework and responsibilities of the "hygiene package" from 2004, the involved food business operators have to put systems in place in such a way that relevant information to ensure food safety are available. These last changes supports these objectives of the hygiene package. On the other hand, the concrete requirements remains very

diffuse. For small establishment this might reduce the “bureaucratic burden”. However, it does not help to implement effective systems on food safety.
At several places in the EU hygiene legislation is the talk about “relevant information.” What are those “relevant information”?
Up to now, more than 99% of food chain information from farmers to slaughter houses in Germany are delivered using the standard form of Annex 7 of the German regulation for food from animal origin and testifying that there are no relevant information. There is no guidance document in Germany available, which tries to define “relevant information” according Annex II Section III of Regulation (EC) No 853/2004 for animals for slaughter.
For the producers of meat products with the need to use pork with low or risk profile according their processing methods there is no legal development towards a more specific or effective risk management of the meat industry. It is up its own risk management to deal with biological risk like Yersinia, Toxoplasma, Hepatitis-E-virus or Campylobacter.
In addition, it remains almost unclear what competent authorities can claim from the meat industry to fulfil the requirements of HACCP based procedures for RTE meat products without heat treatment.
Therefore, it is up to the retailer and other customers of the meat industry to demand safe pork and safe meat products. Or, let us say “safer pork”?

ZOONOTIC PATHOGENS

020

The successful control of Salmonella in pigs in Norway

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Introduction
The occurrence of *Salmonella* in domestic animals is in many countries considered the normality, and especially grain-based industrial production of pigs is linked to high levels of infections and corresponding common transfer to humans through the food chain (Davies et al., 2004). However, despite the fact that latent *Salmonella* infections were a problem in pigs thirty to forty years ago, Norwegian pig herds are virtually free from *Salmonella* today. Although the biology of *Salmonella* has been well known for decades, reports of the practical and efficient intervention of *Salmonella* in pig herds implemented at the national or regional level are rare. This paper demonstrates the unique and favourable situation which Norway shares with Finland and Sweden, in a global market with a significant *Salmonella* problem.

Materials and Methods
The data sources used in this paper consisted of:
- A compilation of historical data
- Data from the systematic Norwegian *Salmonella* Surveillance and Control programme (NSSCP)
- Data from serological testing presented in scientific reports
- Reported human cases caused by *Salmonella* based on the Norwegian Surveillance System for Communicable Diseases (MSIS).

Results
- Documentation from the 1950s up to the 1970s showed common latent *Salmonella* infections in Norwegian pig herds. As one example, Bøvre (1957) investigated ileocaecal lymph nodes from 4114 pigs reduced into 436 pooled samples, and *Salmonella* was isolated from 45 (10.3%) of the pooled samples and 27 (13.4%) of 202 herds.S. Typhimurium was isolated from 16 of the herds. In the early 1970s, Ween (1972) investigated ileocaecal lymph nodes from 540 pigs reduced into 54 pooled samples. *Salmonella* was isolated from 12 (22.2%). S. Typhimurium was isolated from 7 of the pooled samples. Two of the isolates were further characterized as variant Copenhagen.
- The number of positive faecal samples, lymph nodes, carcass swabs isolated in NSSCP since the start in 1995 has remained very low (below 0.1%) throughout the period, and S. Typhimurium dominants among the few isolates
- In the serological survey of serum from 2424 pigs representing 66 herds, 22 (0.9%) pigs were positive when a cutoff level of OD (Optical Density)% = 30 was used in the ELISA. The positive samples were distributed among 11 herds. A comparison between traditional micro-biological and serological testing was carried out in the survey of 1915 samples randomly selected from 18 slaughterhouses (Lium et al., 1998). The average OD% for the whole material was 1.1. S. Typhimurium was isolated from lymph nodes in two pigs
- Most cases of human salmonellosis in Norway (70-80%) are due to infection abroad, except S. Typhimurium, where about half of the cases are infected in Norway. Salmonellosis occurs most frequently during the summer, mainly due to increased travel activity during this period. Also, single domestic cases and outbreaks are often caused by imported foods.

Discussion and Conclusion
The fact that two historical articles within this topic had titles like “Latent *Salmonella* infection in slaughter animals in Norway” (Bøvre, 1957) and “Latent *Salmonella* infection in fattening pigs” (Ween, 1972), tells that the results were not considered arbitrary or unusual. There were, in other words, certain considerable problems related to *Salmonella* some decades ago in Norwegian pigs.
After implementing measures at herd level, *Salmonella* in farm animals hardly poses any risk for the meat industry and the human population of Norway today. It may be argued that the Norwegian success is linked to a husbandry structure with limited animal density. However, Rogaland (Jæren) in Norway represents one of the regions with the highest density of livestock in Europe. Climate and temperature may be limiting the spread and persistence of *Salmonella* in our pig production and environment. Our pig population has further been separated from pigs from other countries through an industry-driven system to limit the import of live animals.
S. Typhimurium is the most common *Salmonella* in pig herds in most countries, and this agent is known to be introduced into the herds by healthy carriers among the breeding animals and also by contaminated feed (Davies et al., 2004). Other types than S. Typhimurium are introduced by feed, and the most

common types do not survive in the environment. Strict biosecurity linked to imported feed, may also hinder the introduction in the pig production. There is an extensive list of additional risk factors connected to biosecurity that should be taken care of at herd level such as birds, rodents, insects, water, humans entering the piggery and environment (manure etc.).

In Norway, the traditional co-operation between the farmers, abattoirs and the food safety authority through many decades is also essential. The food safety authority follows up positive herds by preventing transmission to other herds, humans and food by prohibiting the purchase and transportation of animals and foods from infected farms. The food safety authority also demands sampling until the herd is documented free from *Salmonella*, and also sampling of herds which have been in contact with the infected herd.

We have experienced and accordingly support the view that starting with breeding animals free from *Salmonella* at the top of the breeding pyramid have been the most important measures. We do not believe that any country has to live with a high level of *Salmonella* infections in their pigs, but control of this agent is a continuous effort and the main elements linked to biosecurity, population management and feed control need to be focused all the time. There are other ways to achieve nearly *Salmonella*-free pig carcasses such as good slaughter hygiene and decontamination. A study by Goldbach & Alban (2006), suggests that post-harvest interventions such as hot-water decontamination seem to be more cost-efficient than a pre-harvest strategy for *Salmonella* in pork in Denmark. However, this issue is also linked to a sustainable and "clean" pig production from farm to fork also solving the general problems connected to the environment and willingness to work over many years to achieve this goal. The Norwegian experience and success story, together with similar stories from Sweden and Finland, is a good illustration of this issue.

References

Bøvre K (1957): Latent Salmonellainfeksjon hos slaktedyr i Norge (Latent *Salmonella* infection in slaughter animals in Norway). Norsk Veterinær Tidsskrift, 69, 855-867.

Davies PR, Hurd HS, Funk J A, Fedorka-Cray PJ and Jones FT (2004): The role of contaminated feed in the epidemiology and control of *Salmonella enterica* in pork production. Foodborne Pathogens and Disease, 1, 202-215.

Goldbach SG and Alban L (2006): A cost-benefit analysis of *Salmonella*-control strategies in Danish pork production. Preventive Veterinary Medicine, 77, 1-14.

Lium B, Hopp P, Hofshagen M, Tharaldsen J, Feld NC and Nesbakken, T (1998): Sammenligning mellom bruk av bakteriologi og serologi for overvåkning av forekomsten av salmonella i svinebesetninger (Comparison between the use of bacteriology and serology for monitoring the occurrence of *Salmonella* in pig herds). Proceedings. Husdyrforsøksmøtet (National lifestock research meeting) (pp. 186-190). Ås, Norway.

Ween J (1972): Latent salmonellainfeksjon hos slaktegris. (Latent *Salmonella* infection in fattening pigs). Norsk Veterinær Tidsskrift, 84, 9-10.

021

Applying Salmonella vaccination at the top of a UK pig production pyramid

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Introduction

Salmonella is widespread in pig farms, causing both disease in humans and economic costs for society, regulators and pig farmers. The reduction of zoonotic non-typhoidal *Salmonella* in animals at slaughter can improve the safety of meat and offal for human consumption, and reduce the risk of cross-contamination on the slaughter line. Previous UK studies have shown sow vaccination can reduce *Salmonella* prevalence (Davies at al., 2016; Smith et al., 2018). However, vaccination is unlikely to be cost-effective on most pig farms producing finisher pigs, as most infections are subclinical (Gavin, 2018). The continuing supply of infected pigs to breeding and rearing farms undermines the effectiveness of other interventions applied to reduce *Salmonella*. It has been proposed that reducing transmission at the top of a production pyramid might improve control throughout the pyramid whilst remaining cost-effective.

Material and Methods

This study used a single production pyramid, following a closed multiplier farm and 2-3 representative farms at each of the following levels: gilt mating unit and surplus breeding stock, breeding, rearing, and finishing farms. Following a baseline visit to the farm, sows and piglets in the multiplier herd were given a live attenuated vaccine against *S.* Typhimurium, according to the manufacturer's recommendations. Repeat visits to this farm were carried out 6, 9, 12 and 15 months after the start of vaccination. Farms directly receiving pigs from the multiplier (gilt mating unit and two surplus finisher farms) also received a baseline visit before vaccinated piglets arrived on these farms, then were visited 9 and 15 months after vaccination commenced. Baseline visits to three outdoor breeder farms and two rearer farms they supplied were carried out at around 6 months into the study, shortly before the vaccinated mated gilts were placed on the breeder farms, with follow-up visits at 12 and 18 months. The two finisher farms supplied were visited at the 6 and 18 month time points. Pooled and individual floor faeces and environmental samples were collected at each visit, ensuring sufficient samples were collected within each pig stage to allow for estimations of prevalence and serovar diversity within and between stages. Samples were cultured by a BPW, MSRV and Rambach agar method using a modification of the ISO 6579:2002 (Annex D) method, as described previously (Martelli et al., 2014). Positive isolates were serotyped using standard methodology (Jones, McLaren and Wray 2000). Typhimurium strains cultured from the multiplier farm and the farms directly receiving their weaned piglets (i.e. the gilt mating unit and the surplus breeding stock farms) were tested to differentiate the vaccine strain Typhimurium from wild-type. At each visit, data on farm management practices was also collected, to monitor any other changes that may have influenced the prevalence of *Salmonella* over time.

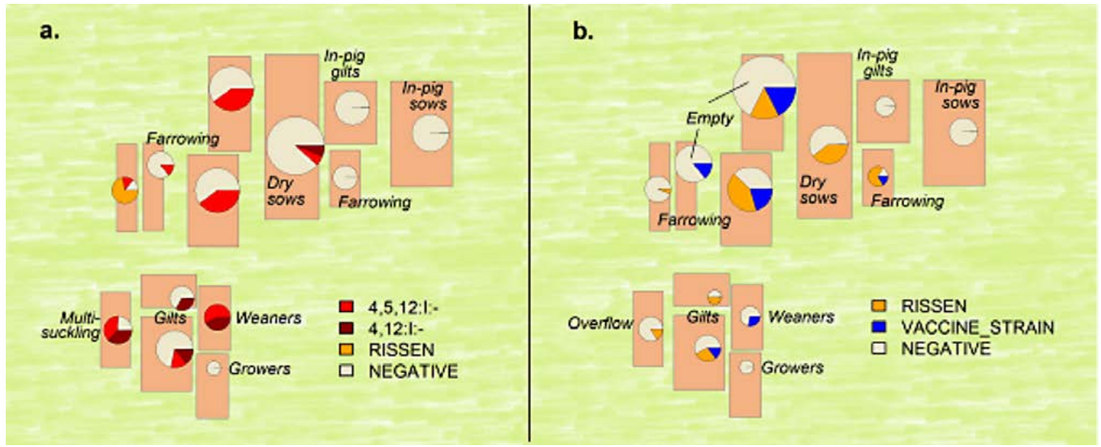

Figure 1: Distribution of Salmonella serovars scaled according to the number of samples per building

Results

At the initial visit to the multiplier farm, *Salmonella* prevalence in pooled samples was 38.2%, with mainly monophasic S. Typhimurium detected, plus a few S. Rissen isolates in a single farrowing shed (Fig 1a.). Following vaccination, the prevalence of monophasic S. Typhimurium steadily reduced and S. Rissen became the predominant serovar. Similar results were observed in the farms directly supplied by the multiplier. Clinically, the farmer reported a reduction in scouring in weaned pigs, and the gilt mating unit was able to stop the use of apramycin for prevention of enteric disease in weaners received from the multiplier unit. At the final visit to the multiplier farm, only vaccine-strain Typhimurium and S. Rissen were detected (Fig 1b.). Some reduction in monophasic S. Typhimurium was observed in other farms in the pyramid, although detection of other serovars, particularly S. Newport, increased and overall *Salmonella* prevalence did not decrease in these farms.

Discussion and Conclusion

Vaccination of sows and piglets on a closed multiplier farm demonstrated that the control of monophasic S. Typhimurium was achievable. The spread and persistence of S. Rissen, and the detection of this serovar at the final visit in empty sheds that had been cleaned and disinfected showed that, overall, biosecurity was sub-optimal. However, as this serovar is relatively non-pathogenic in pigs and people, the farm was satisfied with the results of the vaccination programme. The farms directly supplied with vaccinated weaners by the multiplier herd showed a similar change in the dominant serovars from monophasic S. Typhimurium to S. Rissen, highlighting the role that pig movements play in maintaining infection and environmental contamination on farms. Lower down the pyramid, in the outdoor breeder, rearer and finisher farms, some reduction in S. Typhimurium was also observed, although the fact that other serovars maintained *Salmonella* prevalence at a similar level to the pre-vaccination period suggests that eradicating these opportunistic infections from this environmental niche may not be realistic.
This study indicates that vaccination of pigs in a closed gilt multiplier farm was effective in reducing a serious zoonotic *Salmonella* serovar on this farm and also demonstrated improvements in herds further down the pyramid.

References

Davies, R., Gosling, R.J., Wales, A.D., Smith, R.P. (2016) Use of an attenuated live Salmonella Typhimurium vaccine on three breeding pig units: a longitudinal observational field study. Comp Immunol Microbiol Infect Dis. 46: 7-15.

Gavin, C., Simons, R.R.L., Berriman, A.D.C., Moorhouse, D., Snary, E.L., Smith, R.P., Hill, A.A (2018) A cost-benefit assessment of Salmonella-control strategies in pigs reared in the United Kingdom. Prev Vet Med, 160, 54-62.

Jones, Y.E., McLaren, I.M., Wray, C. (2000) Laboratory aspects of Salmonella, in: C. Wray, A. Wray (Eds.), Salmonella Domest. Anim., 1st ed., pp. 393-405. CABI Publishing, Wallingford, UK.

Martelli, F., Gosling, R., McLaren, I., Wales, A., Davies, R. (2014) Development and testing of external quality assessment samples for Salmonella detection in poultry samples. Lett Appl Microbiol 59, 443-448.

Smith, R.P., Andres, V., Martelli, F., Gosling, B., Marco-Jimenez, F., Vaughan, K., Tchorzewska, M., Davies, R. (2018). Maternal vaccination as a Salmonella Typhimurium reduction strategy on pig farms with a salmonellosis problem. J Appl Microbiol, 124, 274-285.

022

Effect of group vaccination of sows and gilts against Salmonella Typhimurium on Salmonella serology and excretion in sows and their offspring

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Introduction

Vaccination might be effective to control *Salmonella* infections at farm level. The present study evaluated the effect of group vaccination of sows and gilts against S. Typhimurium (ST) on *Salmonella* serology in sows and their offspring and the excretion in the offspring in three pig farms.

Materials and Methods

In each farm (A-B-C), all sows and gilts were vaccinated twice with an attenuated live vaccine (Salmoporc®, IDT Biologika) (3 weeks apart, subcutaneously, 1 mL/dose). From 3 months after the group vaccination onwards, all sows were given a booster dose 3 weeks before every farrowing. The farms were monitored serologically (sows and their offspring at slaughter age) and bacteriologically (fattening pigs of 18 and 26 weeks of age) one year before and one year after the group vaccination.

The presence of ST-field strain was evaluated based on ISO6579:2002, serotyping and distinguishing field/vaccine-strains using IDT Salmonella Diagnostikum®. Sera were analyzed by ELISA (IDEXX) and S/P-ratios were assessed. Data were analyzed using a logistic regression model (bacteriology) or a linear regression model (serology).

Results

After group vaccination, the mean S/P-ratios of the sows increased from 1.60 to 2.97 in farm A, from 1.58 to 1.85 in farm B and from 1.31 to 2.14 in farm C. The mean S/P-ratios of the offspring at slaughter age decreased from 0.99 to 0.72 in farm A, from 1.48 to 0.83 in farm B and from 2.69 to 1.57 in farm C. In the combined analysis of all farms, the increase in the S/P-ratios of sows and the decrease in the S/P-ratios of their offspring at slaughter age were both significant (p<0.001 and p=0.001, respectively). After group vaccination, the percentage ST-field strain positive fecal and overshoe samples decreased from 17% to 11% (p=0.242) and from 15% to 7% (p=0.092) in the fattening pigs of 18 and 26 weeks of age, respectively. None of the collected samples tested positive for the vaccine strain.

Discussion and Conclusion

Group vaccination of sows and gilts induced a serological response in sows and resulted in significantly lower S/P-ratios in their offspring at slaughter age, although the excretion of ST-field strains in the offspring of the sows did not significantly decrease.

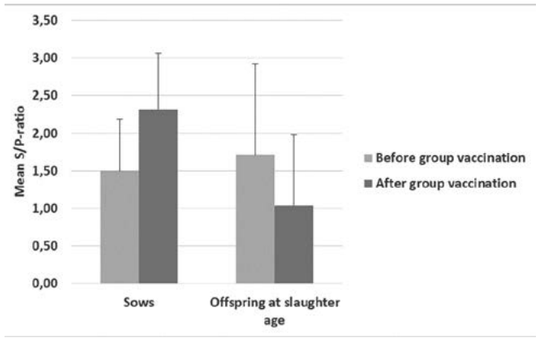

Figure 1: Mean S/P-ratios and standard deviations (SD) from the sows and their offspring at slaughter age before and after group vaccination on farms A, B and C.
The increase in the S/P-ratios of sows and the decrease in the S/P-ratios of their offspring at slaughter age were both significant (p<0.001 and p=0.001, respectively)

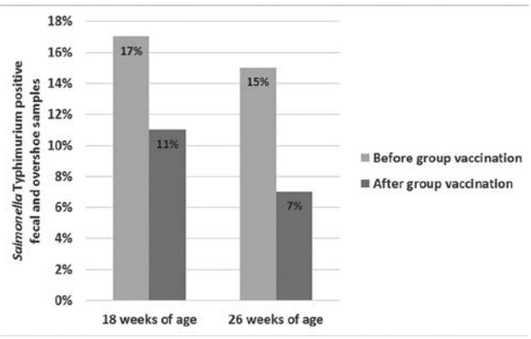

Figure 2: Percentage Salmonella Typhimurium positive fecal and overshoe samples collected from the offspring at 18 and 26 weeks of age before and after group vaccination on farms A, B and C.
The excretion of Salmonella Typhimurium did not significantly decrease

023

Assessment of the relative role of meat of domestic pigs, sheep, cattle, wild boars and moose for the exposure of humans to Toxoplasma gondii

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Introduction

Toxoplasma gondii is a zoonotic parasite prevalent worldwide. Meat from infected animals may contain tissue cysts with viable parasites and is therefore a potential source of infection for other hosts, including humans. Differences in consumption of meat and variation in the infection prevalence in animals between countries may be drivers of the geographical variation in seroprevalence observed in humans across the Nordic-Baltic region[1]. While consumption data are available, data on prevalence of *T. gondii* in different animal species used for human consumption are scattered, and no quantitative risk assessment studies have evaluated the risk of exposure of *T. gondii* through consumption of meat in the region. Therefore, the first objective of this study was to estimate the seroprevalence of *T. gondii* in domestic pigs, sheep, cattle, wild boars and moose in the Nordic-Baltic region. The next objective of the study is to develop a comparative exposure assessment (CEA) framework, and this is a work in progress. The CEA model will allow for the quantification and comparison of exposure to *T. gondii* parasites from various fresh and processed meat products consumed by different age-groups. This model will be applied to four countries.

Material and Methods

1. Systematic review (SR) and meta-analysis: To estimate the seroprevalence of *T. gondii* in domestic pigs, sheep, cattle, wild boars and moose in the Nordic-Baltic region, we conducted a systematic review and meta-analysis[1]. The apparent seroprevalence estimates retrieved from the individual studies were pooled using restricted maximum likelihood method with a random effects model to obtain a pooled seroprevalence estimate for each species.
2. CEA model framework: The framework for the CEA model was developed to estimate the annual risk of consuming one or more viable tissue cysts in different age-groups (≤4yrs, 5–14yrs, 15–24yrs, 25–44yrs, 45–64yrs, ≥65yrs) in Denmark, Finland, Norway and Sweden. The following steps were considered in the development of the CEA model framework:

a) Estimation of the true prevalence in the selected five animal species by country based on apparent seroprevalence collected in the SR and sensitivity and the specificity of the applied serological tests to detect infected animals. Where data on the sensitivity and the specificity of the serological tests were lacking, this information was extracted from the literature. If not available for specific animal species, surrogate data from other species were used. These sensitivity and specificity estimates were used as informative priors in a Bayesian hierarchical model to estimate the true prevalence for each species by country.

b) For each selected meat product originating from domestic pigs, sheep, cattle, wild boar or moose consumed in the region, we will use the food consumption survey data available for each country to estimate the average size of a portion of the meat product as well as the number of infected portions of each meat product consumed. We will do this by age-group and by country.

c) Conversion of true prevalence to number of tissue cysts per infected portion will be estimated from published data on both the number of bradyzoites in a portion from a true positive animal and the number of bradyzoites contained in a tissue cyst as described by Crotta et al[2].

d) Calculation of the probability that a portion contains one or more viable tissue cysts after salting, freezing or cooking will be based on reduction factors as described by Condoleo et al[3] adapted from Opsteegh et al[4]. The reduction factors will be applied at tissue cyst level; as the tissue cysts contain viable bradyzoites. We assumed that when the treatment is applied, all bradyzoites in a given tissue cyst will be assumed to either die or survive each treatment.

e) Estimation of the number of portions of each meat type containing viable tissue cysts consumed annually will be based on the number of portions consumed annually by each age-group by country and the probability that the portion contains viable tissue cysts.

f) Finally, relative comparison of exposure to viable tissue cysts by consumption of the different meat products, for source attribution of *T. gondii* infection will be performed for the four countries.

Results and Discussion

1. Systematic review and meta-analysis: The systematic literature review[1] included eight countries. Thirty-two studies qualified for the meta-analysis; 13 on domestic pigs, 6 on sheep, 3 on cattle, 6 on wild boars, and 4 on moose: Estimated pooled apparent seroprevalence of *T. gondii* was lowest in domestic pigs (6%, $CI_{95\%}$: 3–10%) and highest in wild boars (33%, $CI_{95\%}$: 26–41%)[1].
2. Preliminary framework for the CEA model: The preliminary true prevalence estimates to be used as one of the input parameters in the exposure assessment model are shown in Table 1. The present estimates from the Bayesian model is a work in progress and may therefore be further adjusted.

For the steps outlined in the flow diagram for the CEA model (Fig. 1), input values will be fitted using appropriate probability distributions during the model implementation stage.

Conclusion

The results of the systematic review and meta-analysis showed that a substantial proportion of animals raised or hunted for human consumption in the Nordic-Baltic region have been exposed to *T. gondii*. Therefore, meat of all the five animal host species are potential sources of infection in humans. The next step in this study is to implement the CEA model to quantify the importance of different meat products in *T. gondii* transmission through consumed meat in each of the four countries.

Table 1: Preliminary true prevalence estimates for domestic pigs, sheep, cattle, wild boars and moose from Denmark, Finland, Norway and Sweden

	TP (95% CrI) in pigs	TP (95% CrI) in sheep	TP (95% CrI) in cattle	TP (95% CrI) in wild boars	TP (95% CrI) in moose
Denmark	5 (3–8)	N/A	N/A	49 (33–67)	N/A
Finland	0.3 (0.002–1)	23 (17–31)	11 (6–19)	35 (23–51)	1 (0.05–5)
Norway	2 (0.1–4)	22 (16–29)	N/A	N/A	5 (0.3–1)
Sweden	7 (5–9)	N/A	N/A	65 (50–87)	16 (9–25)

TP = true prevalence; CrI = 95% credible interval; N/A = No data available for the species in the country

References

1. A. Olsen, R. Berg, M. Tagel, K. Must, G. Deksne, H. L. Enemark, L. Alban, M. V. Johansen, H. V. Nielsen, M. Sandberg, A. Lundén, C. R. Stensvold, S. M. Pires, P. Jokelainen, Seroprevalence of *Toxoplasma gondii* in domestic pigs, sheep, cattle, wild boars, and moose in the Nordic-Baltic region: A systematic review and meta-analysis, Parasite Epidemiol. Control 5 (2019) e00100.
2. M. Crotta, G. Limon, D. P. Blake, J. Guitian, Knowledge gaps in host-parasite interaction preclude accurate assessment of meat-borne exposure to *Toxoplasma gondii*, Int. J. Food Microbiol. 261 (2017) 95-101.
3. R. Condoleo, L. Rinaldi, S. Sette, Z. Mezher, Risk Assessment of Human Toxoplasmosis Associated with the Consumption of Pork Meat in Italy, Risk Anal. 38 (6) (2018) 1202–1222.
4. M. Opsteegh, S. Prickaerts, K. Frankena, E. G. Evers, A quantitative microbial risk assessment for meatborne *Toxoplasma gondii* infection in The Netherlands, Int. J. Food Microbiol. 150 (2–3) (2011) 103–14.

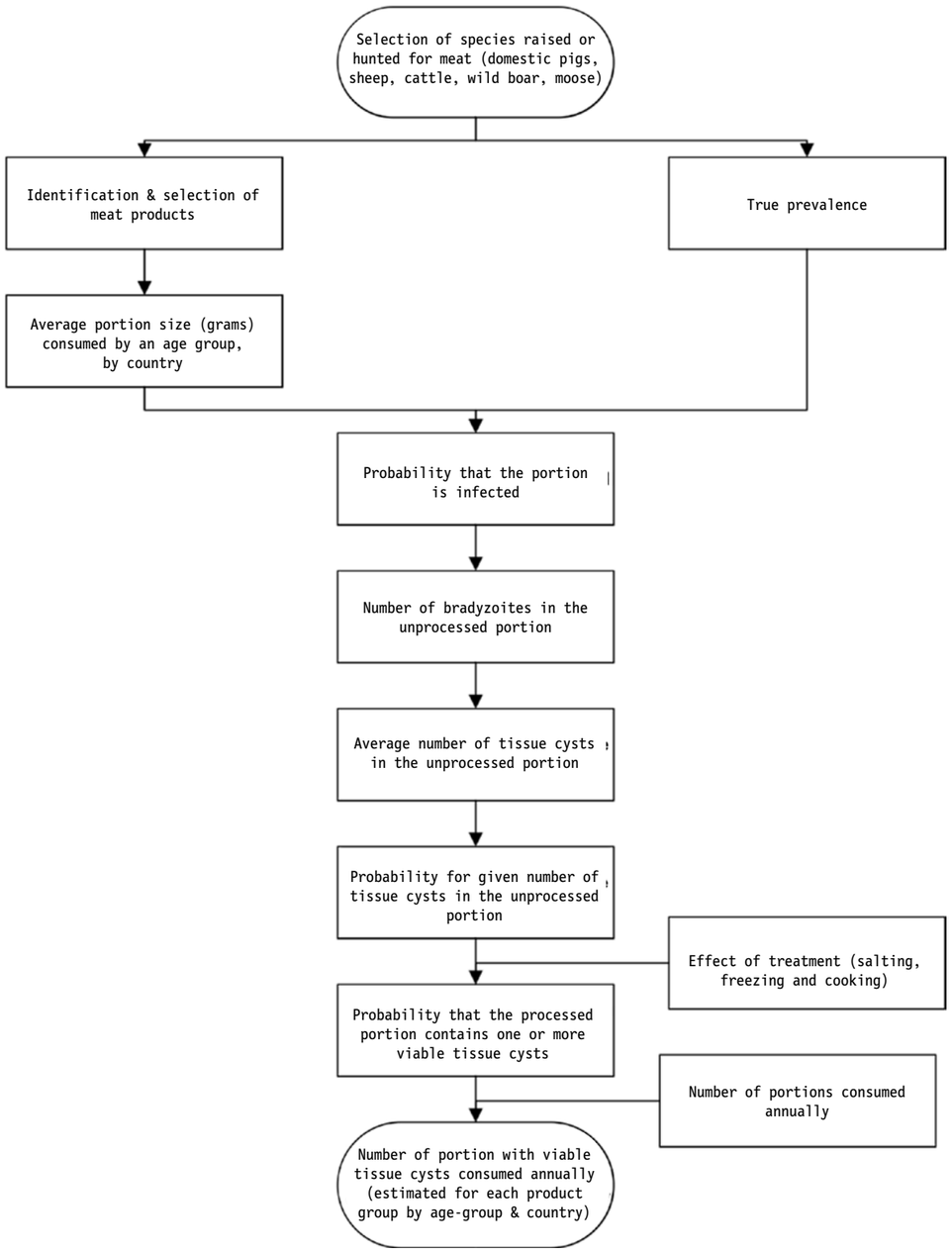

Figure 1: Flow chart of the comparative exposure assessment model to estimate the number of portions containing viable tissue cysts consumed per year by age-group and country

024

Risk factors for the occurrence of antibodies against Toxoplasma gondii in organic pig fattening farms in Austria and prospect for their control

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Introduction

In organic pig farms pigs are often exposed to various pathogenic agents that can cause important health problems and/or lead to zoonoses. One of these is the protozoan parasite Toxoplasma (T.) gondii that can infect humans by the incorporation of oocytes as well as by intake of raw or undercooked pork (Guo et al, 2016). Generally, the prevalence of antibodies against T. gondii in slaughter pigs is low (Steinparzer et al., 2015). In slaughter pigs raised on organic farms, however, the seroprevalence can be far higher (up to 50% of the farms) (Kreinöcker et al., 2017). The aims of the study were to identify risk factors for an infection with T. gondii in organic pig fattening farms, to develop strategies to control the infection and to test their efficacy.

Material and Methods

The study included 59 organic farms in Austria. A total of 1035 blood samples (approximately 17 per farm) were taken. All serum samples were tested for the presence of antibodies against T. gondii by ELISA (PIGTYPE® Toxoplasma Ab, INDICAL BIOSCIENCE, Germany). Additionally, on every farm a questionnaire including information about potential risk factors was completed. Through comparison of antibody positive and negative farms using Fisher's exact test and an estimation of the odds ratio, risk factors have been identified. All farms that had raised T. gondii antibody positive slaughter pigs underwent a farm visit to identify potential possibilities to reduce risk factors. The influence of the elimination of one or more basic risk factors on the prevalence of T. gondii antibodies was assessed after one year by re-testing the farms by blood sampling the slaughter pigs.

Results

In 29 farms (49%) antibodies against T. gondii were detected. These results have been published in Kreinöcker et al. (2017). The presence of cats on the farms had a significant influence on the prevalence of antibodies; although most farms had cats and the odds ratio had a wide confidence interval. The age of the respective cats, however, as well as the fact that the cats had access to the barns and the pig feed, had a significant influence on the prevalence with a high odds ratio. Pigs raised in farms with cats aged younger than one year were significantly more likely to be T. gondii antibody positive. Other factors such as piglet quarantine, access of wild birds or dogs to the pig housing had no significant influence on the seroprevalence. One year after the recommendation to reduce the risk factors (especially to reduce the number of cats and keep them from pigs and feed) 23 of the positive farms were re-tested. Twelve farms (52%) remained T. gondii antibody positive. In eight of these farms, none of the recommended measures had been implemented. On the other eleven farms (48%), no T. gondii antibodies were detected in the sampled pigs. Most effective was keeping cats away from the pig feed, but the introduction of effective rodent control with the use of rodenticides or traps also helped to reduce T. gondii antibody prevalence. Detailed results have been published in Sattler et al. (2019).

Discussion and Conclusions

Organic pig farming enjoys increasing popularity among consumers. Because of regulatory demands, fattening pigs raised in organic farms have increased contact with pathogens and zoonotic agents, including T. gondii. By reducing risk factors through simple measures such as restricting the access of cats to pig barns and feed and removing younger cats from farms, the prevalence of antibodies against T. gondii could often be reduced.

References

Guo M, Mishra A, Buchanan RL, Dubey JP, Hill DE, Gamble HR, Jones JL and Pradhan AK (2016): A Systematic Meta-Analysis of Toxoplasma gondii Prevalence in Food Animals in the United States. Foodborne Pathog Dis 13, 109-118

Kreinöcker K, Sattler T, Hagmüller W, Hennig-Pauka I and Schmoll F (2017): Vorkommen von Antikörpern gegen Toxoplasmen, Leptospiren und PRRSV sowie von Salmonellen und Ascaris suum in biologischen Mastschweinebetrieben in Österreich. Wien Tierärztl Monat - Vet Med Austria, 104, 221-228

Sattler T, Kreinöcker K, Hagmüller W and Schmoll F (2019): Risikofaktoren für das Auftreten von Antikörpern gegen Toxoplasma gondii in biologisch wirtschaftenden Mast-schweinebetrieben in Österreich und deren Beeinflussbarkeit. Wien Tierärztl Monat - Vet Med Austria, 106, 67-73

Steinparzer R, Reisp K, Grünberger B, Köfer J, Schmoll F and Sattler T (2015): Comparison of different commercial serological tests for the detection of Toxoplasma gondii antibodies in serum of naturally exposed pigs. Zoon Publ Health. 62, 119-124

025

Occurrence of Trichinellosis in indigenous pigs of ethnic minorities in Hoa Binh Province, Vietnam

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Introduction

Production of indigenous breeds is an important livelihood activity for ethnic minorities in Vietnam, including Hoa Binh province with the *Tay* ethnic group accounting for the majority of pig raisers. Indigenous pigs in Hoa Binh have traditionally been kept under extensive management systems, including free rooming which may contribute to the occurrence of parasitic pork borne disease (PPBD) including Trichinellosis. Despite several studies of Trichinellosis among humans and pigs being documented (1 & 2) for Vietnam, no updated information is available on the present Trichinella sero-prevalence in indigenous pigs in Hoa Binh province, North Vietnam. The presented research aims to assess the occurrence of Trichinellosis in pigs and improve diagnostic capacity of butchers and lab staff on PPBD.

Material and Methods

Activities conducted since 2018 include a prevalence survey to access the occurrence of Trichinellosis in pigs and villagers. The sampling and results in villagers is part of another paper presented at this conference. The study was conducted in Da Bac district which has the highest pig population among all districts of Hoa Binh province. 352 indigenous pigs from six selected communes were sampled along with data collection of pig raising, age, consumption habits and gender (Figure 1). The communes were selected purposively based on high proportion of ethnic minorities and free rooming pigs. Sample size was calculated regarding an expected prevalence of 5% in pigs. All serum samples were tested for Trichinella antibodies using excretory/secretory antigen Ag-ELISA. Training of laboratory staff and butchers on diagnoses of PPBD was organised to address existing capacity gaps.

Results

In total, 13.6 percent (48/352) of pigs originated from 131 farms across the 6 communities of Da Bac district were serological positive for Trichinella. Pigs older than 6 months of age were more likely to be seropositive than pigs less or equal 6 months, with 19% (29/152) and 9.5% (19/200), respectively (OR = 2.24; 95% CI: 1.20 - 4.18; P = 0.011). Questionnaires concluded a very low knowledge of pig producers on PPBD with less than 2% having any knowledge. Risky consumption habits e.g. consumption of raw fermented pork were common in males and often related to village ceremonies. Capacity building efforts resulted in trained laboratory staff (6), veterinarians, public health officers and butchers (24) on diagnostic procedures for PPBD and/or hands-on meat inspection procedures. An observed challenge was the lack of feasible guidelines for meat inspection. It was noticed that the reporting system on slaughter check findings has limitations. Pigs in remote areas are mainly slaughter in home slaughter by “mobile” butchers without adequate meat inspection.

Discussion and Conclusions

The study provides first data on Trichinella sero-prevalence in indigenous pigs from ethnic minorities in Hoa Binh. Observed prevalence in pigs aligned with poor knowledge on PPBD and observed risky consumption habits may pose a considerable risk to consumers. More sensitive monitoring systems and further awareness raising is needed. The roaming/ semi-free roaming keeping systems indicate that the hygienic conditions of pig management is poor and can be a risk factors for the circulation of parasitic disease in indigenous pigs. The farmers must be encouraged to adopt adequate livestock-management practices. In addition, continued surveillance of Trichinella infection, including reinforcement of meat inspection is recommended. To address the lack of meat inspection in the study area the introduction of a pilot cell-phone based information system to record abnormal observations by butchers is currently explored. Apart from this simplified guidelines for meat inspection are planned to be introduced.

Acknowledgment

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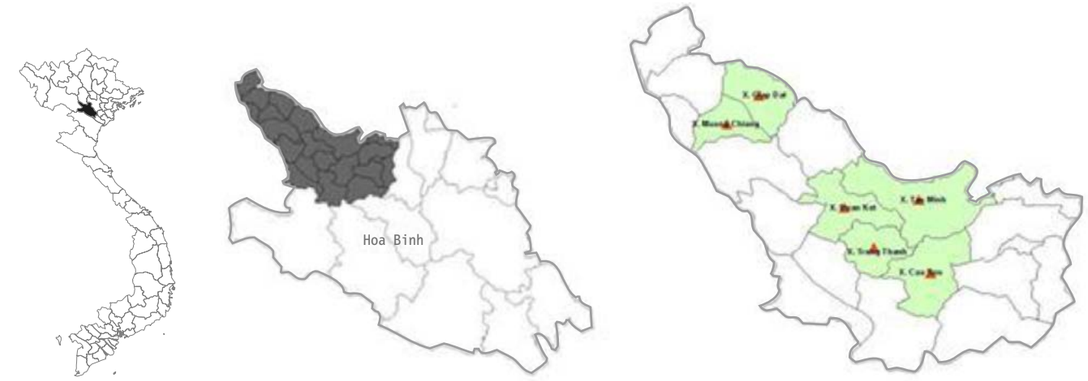

Figure 1: Sampled communes in Da Bac district of Hoa Binh province

References

(1) Van, D.N., Thi, N., V, Dorny, P., Vu, T.N., Ngoc, M.P., Trung, D.D., Pozio, E., 2015. Trichinellosis in Vietnam. Am. J. Trop. Med. Hyg. 92, 1265-1270.
(2) Unger F, Chau Thi Minh Long and Nguyen Viet Khong. Prevalence of trichinellosis and cysticercosis in indigenous pigs from ethnic minorities for selected communes in the Central Highlands (Dak Lak). Nairobi, Kenya: ILRI; 2016.

026

Reduction of sporulating and non-sporulating pathogens during anaerobic digestion of livestock manure in biogas plants

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Introduction

In the current context of developing renewable energies and recovering organic waste, on-farm anaerobic digestion (AD) represents a major challenge for the agricultural sector (energy and organic recovery of livestock manure and agricultural substrates). In France, most of biogas plants fed with manure operate at mesophilic conditions converting organic matter to biogas and by-product degradation, i.e. digestate. This digestate is usually spread as fertilizer on land after transformation or storage. Farm animals like pig, bovine and poultry are known to be reservoirs of various pathogenic microorganisms responsible of animal or human infections (Denis et al., 2011; Boscher et al., 2012, Souillard et al., 2014 and 2015, Moono et al., 2016; Gosling et al., 2018; Thépault et al., 2018). Because these pathogens can survive in manure, their fate during mesophilic AD appears to be a matter of public health concern.
In this study, we investigated the effect of mesophilic AD on the level of sporulating pathogens (*Clostridioides difficile* and *Clostridium botulinum*) and non-sporulating pathogens (*Salmonella* spp, *Listeria monocytogenes* and *Campylobacter* spp.).

Material and Methods

Our study was carried out on three on-farm biogas plants (BGP1, BGP2 and BGP3), two filled with pig manure (BGP1 and BGP3) and one with bovine manure (BGP2). Over one-year, they were visited eight times each. At each visit, three replicates of both inputs (manure) and digestates were collected for detection and enumeration (MPN/g) of *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp., *Clostridioides difficile* and *Clostridium botulinum*. A total of 144 samples (72 inputs, 72 digestates) were analyzed.

Results

All the pathogens were detected in manure at a frequency of 33.3% (*C. botulinum*), 88% (*C. difficile*), 92% (*Campylobacter* spp.), and 95.8% (*Salmonella* and *Listeria monocytogenes*) and in all three BGP, except *C. botulinum* which was not detected in manures of BGP1 and BGP2.
The pathogens were also detected in digestate at a frequency of 37.5% (*Campylobacter* spp.), 79.2% (*C. botulinum*), 83.3% (*L. monocytogenes*), 87.5% (*Salmonella* spp.) and 100% (*C. difficile*). However, no *Campylobacter* spp. could be isolated from digestates of BGP2.
In manure, the level in MPN/g varied in mean from 249 to 368 for *Campylobacter*, from 1.1 to 359.1 for *Salmonella*, from 3.1 to 145.9 for *L. monocytogenes*, from 0.5 to 234.5 for *C. difficile* and from 0 to 3.5 for *C. botulinum* (Fig. 1).

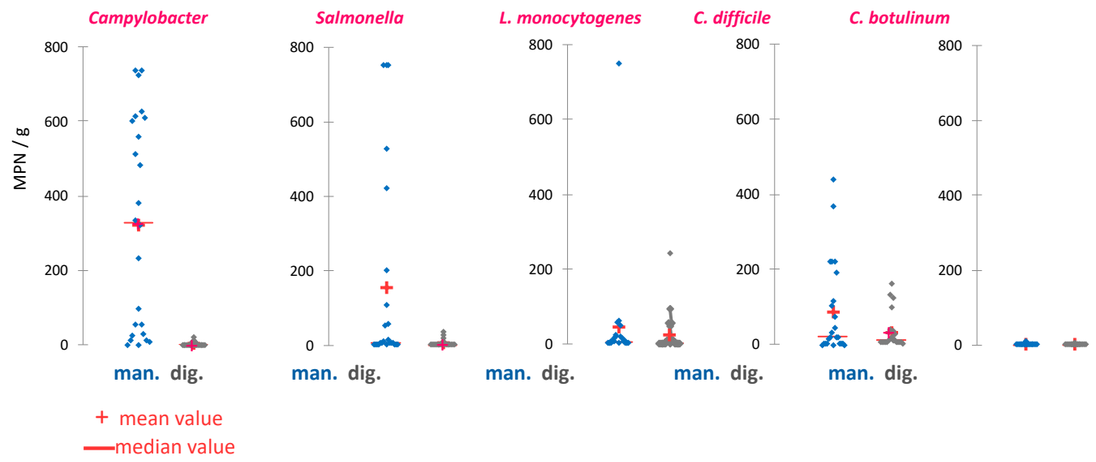

Figure 1: Concentrations of the pathogens in manures and digestates

In raw digestate, the level in MPN/g varied in mean from 0 to 6.3 for *Campylobacter*, from 1.1 to 6.9 for *Salmonella*, from 3 to 45.7 for *L. monocytogenes*, from 8.2 to 80.1 for *C. difficile* and from 0.3 to 2.4 for *C. botulinum* (Fig. 1). Concentration of *C. botulinum* was therefore very low in both samples, manure and raw digestate, with a maximum of 13 MPN/g.
During AD, the average level of pathogens decreased between manure and digestate by 2 Log_{10} (*Salmonella* spp.), 0.3 Log_{10} (*L. monocytogenes*), 2.1 Log_{10} (*Campylobacter* spp.), 0.4 Log_{10} (*C. difficile*) and 0.1 Log_{10} (*C. botulinum*).

Discussion and Conclusion

Our study showed that non-sporulating pathogens like *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp. can be detected in digestate after anaerobic digestion like in previous studies (Kearney et al., 1993; Bonetta et al., 2011; Orzi et al., 2015) suggesting that these pathogens can survive this process, even if their concentrations are reduced during the process. *C. botulinum* concentration was very low, whether in manures or in digestates, which confirms study of Froschle et al, (2015). In this study, *C. difficile* was also frequently detected in digestate with similar levels of *C. difficile* concentration.
With this one-year survey, we demonstrated that mesophilic AD does not lead to bacterial growth and even reduced concentration of sporulating and non-sporulating pathogens. Thus, such treatment of livestock manure can be effective in reducing the presence of these pathogens, and reduce consequent spreading in the environment after post-treatment (eg. storage or post-digestion) of digestates.

Acknowledgements

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References

Bonetta, S., Ferretti, E., Bonetta, S., Fezia, G., Carraro, E. 2011. Microbiological contamination of digested products from anaerobic co-digestion of bovine manure and agricultural by-products. Lett Appl Microbiol, 53, 552-7.
Boscher E., Houard E., Denis M. (2012) Prevalence and distribution of *Listeria monocytogenes* serotypes and pulsotypes in sows and fattening pigs in farrow-to-finish farms (France, 2008). Journal of Food Protection 75 (5):889-895
Denis M, Henrique E, Chidaine B, Tircot A, et al. (2011) *Campylobacter* from sows in farrow-to-finish pig farms : risk indicators and genetic diversity. Veterinary Microbiology 154: 163-170.
Froschle, B., Messelhausser, U., Holler, C., Lebuhn, M. (2015). Fate of *Clostridium botulinum* and incidence of pathogenic clostridia in biogas processes. J Appl Microbiol, 119, 936-47.
Gosling RJ, Mueller-Doblies D, Martelli F, Nunez-Garcia J, et al., (2018) Observations on the distribution and persistence of monophasic *Salmonella Typhimurium* on infected pig and cattle farms. Vet Microbiol.227:90-96.
Kearney, T. E., Larkin, M. J., Frost, J. P., Levett, P. N. (1993). Survival of pathogenic bacteria during mesophilic anaerobic digestion of animal waste. J Appl Bacteriol, 75, 215-9.
Moono P, Foster NF, Hampson DJ, Knight DR, et al. (2016) *Clostridium difficile* infection in production animals and avian species: a review. Foodborne Pathog Dis. 2016 Dec;13(12):647-655.
Orzi, V., Scaglia, B., Lonati, S., Riva, C., et al. (2015). The role of biological processes in reducing both odor impact and pathogen content during mesophilic anaerobic digestion. Sci Total Environ, 526, 116-26.
Souillard R, Woudstra C, Le Maréchal C, Dia M, et al. (2014) Investigation of *Clostridium botulinum* in commercial poultry farms in France between 2011 and 2013. Avian Pathol. 2014;43(5):458-64.
Souillard R, Le Maréchal C, Hollebecque F., Rouxel S. et al., (2015) Occurrence of *C. botulinum* in healthy cattle and their environment following poultry botulism outbreaks in mixed farms. Vet Microbiol Volume 180: 142-145.
Thépault A, Poezevara T, Quesne S, Rose V, et al. (2018) Prevalence of thermophilic *Campylobacter* in cattle production at slaughterhouse level in France and link between *C. jejuni* bovine strains and campylobacteriosis. Front Microbiol. 2018 Mar 19;9:471.

027

Hepatitis E - analyzing the occurrence in slaughter pigs for a risk assessment of raw meat products

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Introduction

The hepatitis E virus (HEV) of genotype 3 and 4 is known as a zoonotic agent. In this context, the pig was identified as the main animal reservoir. In Europe, the consumption of raw or undercooked pork products represent a potential risk for HEV infections in humans. In humans, HEV infections can cause acute hepatitis, which is usually self-limiting. Chronicity in immunocompromised patients and a high mortality rate of up to 28% in pregnant women have been reported (Meng 2011).
In Germany, according to § 7 of the German Infection Protection Act (IfsG, 2019), the direct or indirect detection of HEV in humans must be reported to official health services. In 2018, a total of 3,275 cases of hepatitis E was reported to the Robert Koch Institute (RKI 2019).
As pigs are a main reservoir of HEV several studies were performed identifying the antibody status of fattening pigs across EU member states. With a seroprevalence of up to 96% (Wutz et al. 2013), HEV shows a wide distribution among fattening pigs in Germany. Nevertheless, national studies examining the occurrence of HEV RNA in liver or muscle samples from pigs are rare.
The objective of this study was to estimate the risk of HEV entering the food chain via pork products based on serological tests and on the analysis of pork liver and muscle samples from the same animal used for the production of pork liver and pork meat products.

Materials and Methods

In 2018, a total of 250 fattening pigs from 25 farms (10 pigs per farm) were sampled in an abattoir in North-West Germany. One sample of ham muscle, one sample of liver tissue and one sample of the muscle of the diaphragm pillar were collected from each pig during the slaughter process. Each animal was tagged individually and samples were taken at different stages of the slaughter line. Livers were collected and stored in boxes during the slaughter process as usual until sampling. All samples were chilled and transported to the institute's laboratory. Muscle samples from the diaphragm pillar were stored at -30 °C and liver and ham muscle samples were stored at -80 °C until laboratory examination.
To determine the seroprevalence, meat juice from the diaphragm pillar samples was serologically tested for HEV antibodies using the Priocheck™ HEV Antibody porcine ELISA Kit (Thermo Fisher Scientific®, USA) according to the manufacturer's manual. The liver and muscle samples were analysed for the presence of HEV RNA by real-time RT-PCR according to Jothikumar et al. (2006) after RNA extraction with the RNeasy® Mini QIAcube Kit (QIAGEN®, Germany).
For each pig the antibody status will be gathered and herd status will be analysed, too. Afterwards, the presence of HEV antibodies for each animal will be compared with the presence of viral RNA in the liver and the muscle.

Results

In total, 62% (155/250) of the meat juice samples were positive for antibodies against HEV at a single animal basis. At herd level, 72% (18/25) of the herds were positive. Herds were considered to be positive, if at least one of the ten samples was positive.
For the herd seroprevalence four groups, according to the serological detection rate, were defined. The herds investigated were allocated to one of these groups using their antibody prevalence (Table 1).

Table 1: Allocation of herds according to the antibody status

serological detection rate	Proportion of herds (n/N)
0% (HEV seronegative)	28% (7/25)
10%-30% (low prevalence)	8% (2/25)
60%-90% (high to very high prevalence)	16% (4/25)
100% (all samples are HEV seropositive)	48% (12/25)

Table 2: Detection of HEV in liver and ham muscle from slaughter pigs

Number of analysed samples	Number of HEV positive tested samples	Viral Prevalence
liver: 126	18	14%
muscle: 133	0	0%

Analysed so far, HEV RNA was detected in 14% (18/126) of the liver samples (Table 2), which came from HEV seropositive pigs. Whereas in liver samples from HEV seronegative pigs, HEV RNA could not be detected, until now.
So far, all investigated muscle samples were negative (0/133) for HEV RNA (Table 2).

Conclusion
The serological results show that HEV antibody prevalence is relatively high in fattening pigs included in this study (62%). The sporadic presence of HEV in liver samples indicates that pig liver or pig liver products may represent a potential risk for HEV infection if consumed raw or undercooked or if the rules of kitchen hygiene are not observed.
In addition, HEV positive livers do not seem to be associated with HEV positive ham muscles.
Based on the results obtained so far, it appears possible to use serological tests to predict the presence of HEV RNA in the liver of fattening pigs.

References

IfsG (2001): Infection Protection Act in the version of January 1, 2019
Jothikumar, N., T. L. Cromeans, B. H. Robertson, X. J. Meng and V. R. Hill (2006): A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods, Vol. 131, 65-71
Meng, X. J. (2011): From barnyard to food table: the omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. Virus Res, Vol. 161, 23-30
Robert Koch-Institut, Epidemiological Bulletin (1/2019) January 3, 2019
Wutz, K., Meyer V. K., Wacheck S., Krol P., Gareis M., Nolting C., Struck F., Soutschek E., Bocher O., Niessner R. and Seidel M. (2013): New route for fast detection of antibodies against zoonotic pathogens in sera of slaughtered pigs by means of flow-through chemiluminescence immunochips. Anal Chem, Vol. 85, 5279-5285

028

Hepatitis E virus: an investigation of within-herd transmission and factors affecting risk of infection in slaughter age pigs

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Introduction
Human infection with Hepatitis E virus (HEV) is an increasing public health concern in Europe. The virus is endemic in parts of Asia and Africa, where genotypes 1 and 2 dominate and are transmitted between people via sewage-contaminated drinking water. HEV in Europe was previously associated with travel to endemic regions, but incidence of indigenously acquired infection has increased over the last decade due to the emergence of HEV genotype 3 (G3), which also infects pigs and is associated with zoonotic transmission (Adlhoch *et al.*, 2016).
Foodborne transmission of HEV G3 is believed to be an important route for human infection in Europe. HEV RNA has been detected in pork products (e.g. Berto *et al.*, 2012) and consuming pork products has been identified as a risk factor for infection (Said *et al.*, 2014). Efforts to reduce the risk of HEV contamination in the pork food chain have so far largely focused on developing methods for viral inactivation during processing. Measures to prevent HEV entering the food chain in the first place are also needed, but have received relatively little attention. Developing such measures requires an understanding of HEV transmission within the farm environment, which is currently lacking. Furthermore, on-farm practices that might mitigate the risk of actively infected pigs going to slaughter must be identified and investigated. Here we present the results of an on-farm pilot study that begins to address these knowledge gaps.

Methods
The HEV infection status of a cohort of pigs was followed from farrowing to pre-slaughter on an indoor English farrow-to-finish farm. The cohort comprised 153 piglets born to 11 sows. Five sampling visits took place from May-October 2018 to coincide with key management events for the cohort as follows: pre-farrowing, pre-weaning, prior to movement into grower accommodation, prior to movement into finisher accommodation, and one week prior to slaughter.
Throughout production, pigs were housed as several groups in multiple pens. Observational data collected from a UK abattoir study suggested that late mixing of finisher pigs could be a risk factor for active infection at slaughter. We therefore used coloured ear tags to identify pigs from different litters and track group mixing throughout production. At each visit, fresh faecal droppings were collected from each group and tested for HEV RNA using a qPCR. Viral shedding in faeces was used as a proxy for infection status. HEV presence was determined per group and HEV prevalence was estimated across the entire study cohort on each sampling occasion. In addition, HEV prevalence in all growers and finishers present on the farm was estimated at each visit to investigate general trends within the herd. Environmental samples (including wildlife faeces, standing water, and swabs of farm equipment) were also tested for HEV RNA to identify potential sources of contamination in the farm environment.

Results
Prevalence across all growers was consistently high at all visits (75-87%; Figure 1a) and always higher than in finishers (10-38%; Figure 1b). HEV RNA was detected in 43/67 environmental samples and was found in all production areas (farrowing, weaner, grower, and finisher accommodation), including a cleaned, unoccupied pen.
HEV prevalence in the study cohort fluctuated over time (Figure 1c). HEV was not detected in any sow sampled pre- or post-farrowing, nor in any litter sampled just prior to weaning. After weaning, the cohort was sorted into seven groups of ~30 pigs and placed into weaner accommodation. Seven weeks later, HEV prevalence in the cohort was 26% but HEV was only present in 2/7 groups.
The cohort was subsequently sorted into two larger groups of approximately 60 and 100 pigs and housed in grower accommodation. After six weeks, HEV was present in both groups and prevalence across the cohort was 100% (Figure 1c). The larger group subsequently retained a stable composition for the remainder of the fattening period, and prevalence fell to 23% when sampled one week prior to slaughter. Pigs in the smaller group were sent to slaughter before they could be sampled as finishers, therefore a comparison of HEV presence between study cohort finisher groups was not possible. However, prevalence in the remaining cohort group was generally lower than prevalence in the non-cohort finisher buildings, where pigs had experienced a greater degree of late-stage mixing.

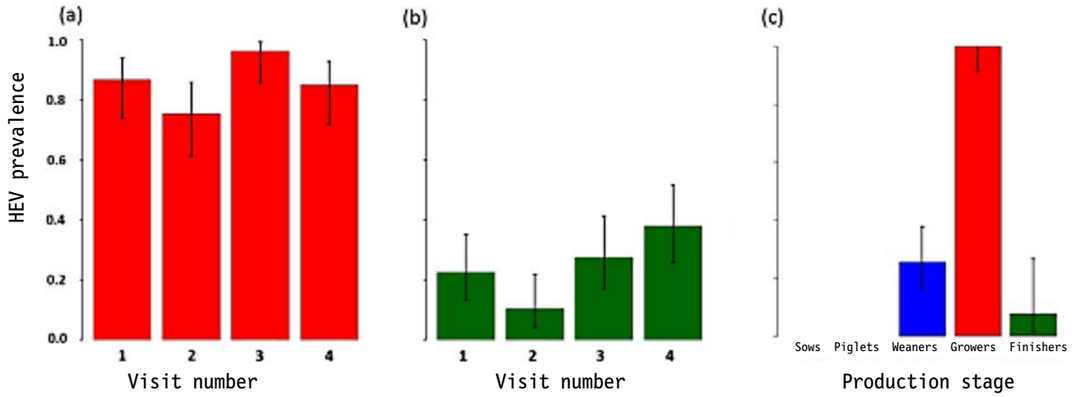

Figure 1: Prevalence of HEV RNA in faeces from (a) growers (b) finishers and (c) the study batch

Conclusion

The results suggest that HEV infection was persistent in this pig herd, and that contamination of the farm environment was widespread. Preventing infection in the herd completely is therefore unlikely to be a viable option for control.

HEV RNA was not detected in any sow faeces, either because they are not infected or they are infected but not shedding detectable levels of virus. Future studies will incorporate sampling of blood and other tissues to disentangle these mechanisms.

HEV RNA was first detected in the study cohort at the weaner stage. Infection may have therefore first entered the cohort after weaning, possibly after maternal antibodies had waned. However, latent infection in younger pigs is also possible, if maternal antibodies suppress viral shedding in faeces. HEV was not detected in all weaner groups. This variation may be linked to age at weaning or degree of mixing when weaned.

HEV appeared to rapidly spread through the cohort once introduced. All samples were positive by the end of grower stage. Only one group was available for sampling as finishers. This group had not experienced further mixing since leaving the weaner unit and HEV prevalence fell considerably by one week prior to slaughter. In contrast, other (non-cohort) finishers on the farm had experienced a greater degree of mixing as growers/finishers and prevalence in these pigs tended to be higher. This suggests that minimising group mixing between weaning and slaughter, especially in the latter part of the finishing period, may reduce the risk of active HEV infection and viraemia at slaughter.

Our study highlights that several factors are likely to contribute to the overall risk of active HEV infection in slaughter pigs, including biological processes that mediate within-pig infection dynamics (e.g. presence of maternal antibodies) alongside management practices on farm that might influence exposure to infection during primary production (e.g. timing and degree of group mixing). The results from this study will inform further multi-farm investigations of HEV epidemiology in pig herds and the use of herd management strategies for limiting entry of swine-associated HEV to the human food chain.

References

Adlhoch, C. *et al*. (2016). Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. J Clin Virology, 82, 9-16.

Berto, A. *et al*. (2012). Hepatitis E virus in pork food chain, United Kingdom, 2009-2010. Emerg Inf Dis, 18, 1358-1360.

Said, B. *et al*. (2014). Hepatitis E virus in England and Wales: indigenous infection is associated with the consumption of processed pork products. Epid & Inf, 142, 1467-1475.

029

The entry of Listeria monocytogenes into the food chain via slaughter pigs

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Introduction

Listeria monocytogenes is an important human foodborne pathogen and the causative agent of the rare but severe human listeriosis, which has a high mortality rate. Pregnant women, elderly and immunocompromised persons and newborns are particularly at risk. Listeria species are ubiquitous in the environment and frequently found in raw foods such as meat, vegetables, dairy products and delicatessen products intended for consumption without further heating (Allerberger and Huhulescu 2015; Allerberger 2007). During processing food can be cross-contaminated by the pathogen present in the processing environment. Many animals, including pigs, and humans can carry the bacterium without showing clinical symptoms. Particularly in the light of the antibiotic minimization concept of the 16th Amendment to the German Medicines Act (AMG), the question arises whether the significant reduction in the use of antibiotics in pig farming since 2011 (Wallmann et al. 2018) has led to an increase in the incidence of Listeria spp. in the pig population.

The aim of this project is to investigate whether the Listeria monocytogenes strains responsible for human disease can be isolated from fattening pigs or the slaughtering environment. Various studies concerning this issue brought contradictive results. Among others, Borch et al. (1996) came to the conclusion that the contamination of pork with L. monocytogenes originated mainly from the processing environment and not primarily from the animals themselves while other studies identified slaughter pigs as a possible primary source of the pathogen in the food chain (Fredriksson-Ahomaa et al. 2009; Hellström et al. 2010).

Material and Methods

To examine the way of transmission of L. monocytogenes, this study investigates the occurrence of this bacterium in slaughter pigs in northwestern Germany as well as in the slaughtering and processing environment. Fecal and tonsillar samples from 200 fattening pigs from 20 herds (10 animals per herd) immediately after slaughter and environmental samples from the slaughterhouse were qualitatively tested for Listeria spp. The samples were processed according to a modified ISO 11290-1:2017 protocol. The species were confirmed by MALDI-TOF MS. Furthermore, the isolates will be subtyped using the method of Whole Genome Sequencing (WGS).

Results

We found a very low detection frequency of L. monocytogenes in tonsils (1%, 2/200 tonsil samples) and could not isolate any L. monocytogenes in fecal samples (0%, 0/200 fecal samples). Positive results of L. monocytogenes were found in environmental samples (8%, 6/77 environmental samples) taken from the slaughterhouse (2/39), the cutting plant (2/11), rubber boots (0/15) as well as the processing plant (2/12). Other Listeria species, especially L. innocua und L. welshimeri, were found in the animal samples as well as in the environmental samples more often.

Discussion and Conclusion

Due to these results, we consider tonsils of slaughter pigs as a reservoir for L. monocytogenes and as a low, but existent risk for contamination of meat products. In order to determine the zoonotic potential of the isolates and to compare the isolates found in pig tonsils with those found in the slaughtering and processing environment, the ANSES institute, EU reference laboratory for L. monocytogenes, will perform Whole Genome Sequencing on the identified Listeria spp. isolates.

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References

Allerberger, F.: Listeria. In: PhD Shabbir Simjee (Hg.): Foodborne Diseases, 27-39.

Allerberger, F.; Huhulescu, S. (2015): Pregnancy related listeriosis: treatment and control. In: Expert review of anti-infective therapy 13 (3), 395-403. DOI: 10.1586/14787210.2015.1003809.

Borch, E.; Nesbakken, T.; Christensen, H. (1996): Hazard identification in swine slaughter with respect to foodborne bacteria. In: International Journal of Food Microbiology 30 (1-2), 9-25. DOI: 10.1016/0168-1605(96)00988-9.

Fredriksson-Ahomaa, M.; Gerhardt, M.; Stolle, A. (2009): High bacterial contamination of pig tonsils at slaughter. In: Meat science 83 (2), 334-336. DOI: 10.1016/j.meatsci.2009.06.004.

Hellström, S.; Laukkanen, R.; Siekkinen, K.-M.; Ranta, J.; Maijala, R.; Korkeala, H. (2010): Listeria monocytogenes contamination in pork can originate from farms. In: Journal of Food Protection 73 (4), 641-648. DOI: 10.4315/0362-028X-73.4.641.

Wallmann, J.; Bode, C.; Bender, A.; Heberer, T. (2018): Abgabemengenerfassung von Antibiotika in Deutschland 2017. In: Deutsches Tierärzteblatt 66 (9), S. 1238-1247.

030

Population genetic structure of Listeria monocytogenes strains isolated from the pig and pork meat production chain in France

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Introduction

Listeria monocytogenes (*Lm*) is one of the main causative agents for foodborne infections in Europe in terms of severity of the illness and fatality rate (EFSA-ECDC, 2015). In France, listeriosis causes less than 0.1% of foodborne illnesses, but has the highest rate of mortality (20-30%) and hospitalizations (98.9%) among foodborne infections (Goulet et al., 2013; Van Cauteren et al., 2017). Meat products – and more specifically pork meat – are regularly reported as contaminated, with a prevalence of up to 12% in raw products (Roussel et al., 2010; Kerouanton et al., 2011). Understanding the origin of these contaminations remains an important public health issue.

Lm can survive for long periods of time in unfavorable environments that do not allow the strains to grow (Carpentier and Cerf, 2011). This factor makes its circulation difficult to trace. A better understanding of *Lm* genetic population structure may help to characterize the circulation routes.

In France, the major CCs responsible for clinical cases are present in food samples (Maury et al. 2016). In particular, CC1, CC2, CC4 and CC6 are strongly associated with a clinical origin and the most likely to cause disease, in particular human central nervous system infections or maternal-neonatal listeriosis (Maury et al., 2016). Other CCs, such as CC9 and CC121, are associated with food production sectors (Henri et al., 2016; Maury et al., 2016). The introduction sources of these CCs in the food supply chain are not well understood.

An overview of *Lm* genetic diversity, along the entire pig and pork production chain, is needed to improve food safety, identify the contamination routes and prevent human infections.

Here, we focused on 687 *Lm* strains isolated in France along the entire pig and pork production chain, from pig farming to finished food products. All strains were typed by pulsed-field gel electrophoresis (PFGE), and then assigned to an Multi-locus Sequence Typing Clonal complexes (CC), using a mapping method specifically developed for this study. The distribution and prevalence of CCs in the different pig and pork production chain compartments were compared. Then, the CCs obtained were compared with those obtained from 1106 strains isolated from the other main food production sectors in France.

Materials and Methods

Panel of 687 strains isolated from the pig and pork production chain

The strains were isolated, in 85 of the 101 French *départements*, from national studies (Roussel et al., 2010; Roussel et al., 2012, Roussel et al., 2014a) either research projects (Kerouanton et al., 2011) nor with corporate clients of IFIP or ANSES.

Strains from pig farming (PF), from the food processing environment (FPE) & from finished food products (FFP)

A total of 91 PF strains in majority isolated from the study of Boscher et al., 2012 in 2008 in the Brittany region (represented 58% of the French pig production in 2008). Eighty four FPE strains were isolated from surface sampling carried out in 37 food factories. Five hundred and eighteen FFP strains were isolated at the processing plant or at the point of sale. In this compartment, two groups were defined: (i) unprocessed meat (UM), including, fresh meat, minced meat and meat preparations (n=248); (ii) meat products (MP) including, non-heat-treated or heat-treated products (n=270).

Panel of 1106 strains isolated from other food production sectors

The strains came from five main food sectors: “Meat products” (excluding pork meat) (n=284), “Milk products” (n=287), “Fish and fishery products” (n=237), “Food products combining several food categories” (n=205) and “Fruit, vegetables, cereals and herbs” (n=67). Finally, 26 without assignment to a specific food sector.

PFGE typing & mapping MLST /PFGE

The PFGE and PFGE profile interpretation, PFGE MLST mapping & statistical analysis of distribution was performed according to Félix et al. 2018 methods.

Results

Strain genetic diversity from pig farming and pork production (687 strains)

Comparison of the MLST clonal complex distributions between the three compartments

CC121 was not observed among the strains identified in the PF compartment, but was one of the most prevalent CCs in the FPE (25%) and FFP (22.4%) compartments (Figure 3). The distribution of CC121 was comparable in these two compartments (p-value > 0.27). CC9 was associated with the FFP, but not with PF or FPE compartments (p-value < 0.001). CC37, CC77 and CC59 were associated with the PF, but not with the FPE or FFP compartments (p-value < 0.004). The distributions of CC8, CC1, CC5 (n > 30) and CC6, CC4-CC217 and CC7 (n < 30) were comparable in the three compartments (p-value > 0.038) (Figure 1).

Finished food products

The most prevalent CCs in the UM and MP groups were CC9 (29.0% and 17.4%), CC121 (20.4% and 24.4%) and CC8 (8.1% and 7.0%). The prevalence of CC9 significantly decreased between the UM and MP groups (p-value < 0.001).

Strain genetic diversity compared between the pork sector and the other food production sectors The distributions of CC5, CC6 and CC2 were comparable between the Pork sector and the five other food production sectors (p-value > 0.038).

The distribution of the CC121 was comparable in the Pork sector and the Meat products, Food products combining several food categories and Fruit, vegetables, cereals and herbs sectors (p-value > 0.118). Compared with the Pork sector, the prevalence of CC121 was 10 times lower in the Milk products sector (p-value < 0.001), but one-third higher in the Fish and fishery products sector (p-value < 0.001) (Figure 2).

The distribution of CC9 was comparable between the Pork sector and the following two sectors: Meat products and Food product combining several food categories (p-value > 0.028). In contrast, CC9 was rarely found in the three other food sectors (p-value < 0.001) (Figure 2).

The prevalence of CC1, CC6 and CC4-CC217 was comparable in the Pork sector and in the four other production sectors (p-value > 0.026), except the Milk products sector in which these CCs were more abundant (p-value < 0.006) (Figure 2).

Discussion

First, this study aimed to understand the genetic diversity of *Lm* strains isolated along the pig and pork production chain in France and to compare it between the three compartments: pig farming (PF), food processing environment (FPE) and finished food product (FFP). To our knowledge, this study represents the largest and the most representative study ever performed in France.

One of the main results obtained here is that the major CCs of pork strains were not equally distributed among the three compartments. Three CC (CC37, CC59 and CC77) strains were rarely found in the FPE and FFP compartments, but were prevalent and associated with the PF compartment. CC37, the most prevalent CC in the PF compartment in our study, was frequently isolated in other studies dealing with primary production or wild environment (Linke et al., 2014, Dreyer et al., 2016, Haase et al., 2014). CC37 is likely better adapted to pig farms than to the pork production environment.

Second, this study aimed to compare the genetic diversity between the Pork sector and the other food production sectors on a strain panel collected over 27 years of sampling, from hundreds of processing facilities and retail stores. Among the major CCs obtained, we distinguished three CCs (CC5, CC6 and CC2) considered ubiquitous, because they were found in comparable proportions in all sectors.

CC9 was predominantly isolated from meat products several Euroepan studies (Leong et al., 2017, Martin et al., 2014, Ebner et al., 2015 Nielsen et al., 2017, De Cesare et al., 2017). CC9 contamination was shown for mammalian meat production, regardless of meat type, suggesting that the contamination is likely not related to the primary contamination of livestock animals. Several studies report increased detection of CC9 strains at the slaughterhouse, after carcass dressing and prior to transfer to the ultraclean meat processing area (Fravalo et al., 2013; Lariviere-Gauthier et al., 2014; Neira et al., 2015). In contrast to CC9, CC121 was not associated with a given food sector. However, CC121 was the most prevalent in the Fish and fishery products, Pork and Meat products sectors, making this CC possibility related to the food processing after slaughtering that have in common ultra clean process.

Conclusion

The results obtained in this study led to a better understanding of the structure of the *Lm* population isolated from the pig and pork production sector. CC9 and CC121 are associated with food production, most likely because processing steps, such as slaughtering or stabilization treatments, favor their settlement and recontamination of the food produced. Both results indicate that processing steps are likely the source point of contamination.

Molecular serotype	85% PFGE cluster	MLST CC mapped from PFGE cluster	Pig farming compartment	Food production environement compartment	Finished food product compartment		
					Total	Unprocessed meat, including fresh meat, minced meat and meat preparations	Meat products including non-heat-treated and heat-treated products
IIa	A	CC121		25,0	22,4	20,2	24,4
IIc	B	CC9	1,2	10,7	23,0	29,0	17,4
IIa	C	CC8	10,6	10,7	7,5	8,1	7,0
IVb	D	CC1	9,4	3,6	4,8	3,6	5,9
IVb	E	CC6	7,1	1,2	3,3	3,6	3,0
IIb	F	CC5	2,4	7,1	4,2	3,6	4,8
IVb	G	CC2			4,1	2,4	5,6
IVb	H	CC4 - CC217	1,2	1,2	2,7	3,2	2,2
IIa	I	CC37	12,9	2,4	2,7	3,2	2,2
IIa	J	CC31		2,4	1,2	0,4	1,9
IIa	K	CC155		6,0	1,4	0,4	2,2
IIa	L	CC204		2,4	0,6	0,4	0,7
IIb	M	CC3		4,8	1,9	1,2	2,6
IIb	N	CC59	8,2		0,6	0,8	0,4
IIa	O	CC7	1,2	3,6	1,0	0,8	1,1
IIa	P	CC14	1,2		1,9	2,4	1,5
IIb	Q	CC77	11,8	1,2	1,2	0,8	1,5
IIb	R	CC224	8,2	2,4	0,8	1,2	0,4
IIa	S	CC11	3,5	4,8	0,8	0,8	0,7
IIa	T	CC18			0,4	0,8	
IIa	U	CC193		1,2	1,5		3,0
IIa	V	CC101 - CC90	1,2		0,4		0,7
IIa	W	Not assigned	1,2		0,6	0,8	0,4
IIa	X	CC21	2,4				
IIa	Y	CC91			0,8	1,2	0,4
IIa	Z	CC20			0,4	0,4	0,4
Other PFGE clusters			16,5	9,5	10,0	10,5	9,6
Total			85	84	518	248	270

Figure 1: Distribution of the 26 major mapped clonal complexes (CCs) within the pig and pork meat production chain

Molecular serotype	85% PFGE cluster	MLST CC mapped from PFGE cluster	Pork sector (excluding pig farming strains)	Meat products sector (excluding pork meat)	Milk products sector	Fish and fishery products sector	Food products combining several food categories sector	Fruit, vegetables, cereals and herbs sector	Without assignment to a specific food sector	Total	Total including pig farming
IIa	A	CC121	22,8	21,5	2,8	34,2	26,9	22,4	26,9	21,3	20,3
IIc	B	CC9	21,3	21,8	3,5	3,8	15,1	1,5	11,5	14,3	13,7
IIa	C	CC8	8,0	6,3	2,8	5,5	6,3	14,9	3,8	6,5	6,7
IVb	D	CC1	4,7	5,6	9,1	2,5	5,4	6,0		5,3	5,5
IVb	E	CC6	3,0	3,9	7,0	3,0	3,4	7,5	7,7	4,1	4,2
IIb	F	CC5	4,7	4,6	2,8	3,4	4,9	4,5		4,1	4,0
IVb	G	CC2	3,5	3,9	2,4	5,5	4,9	4,5	3,8	3,9	3,7
IVb	H	CC4 - CC217	2,5	3,2	9,1	0,8	1,5	3,0	7,7	3,5	3,3
IIa	I	CC37	2,7	1,4	4,5	0,4		6,0		2,2	2,7
IIa	J	CC31	1,3	4,6	1,0	3,4	3,9	6,0	7,7	2,7	2,6
IIa	K	CC155	2,0	3,2	1,7	4,6	2,0			2,4	2,3
IIa	L	CC204	0,8	1,4	0,7	6,3	4,9		3,8	2,2	2,1
IIb	M	CC3	2,3	1,8	3,8	0,4	2,0		3,8	2,1	2,0
IIb	N	CC59	0,5	1,1	2,8	4,2	0,5	1,5		1,5	1,8
IIa	O	CC7	1,3		3,8	2,5		3,0		1,7	1,7
IIa	P	CC14	1,7	2,1	1,0	2,5	2,0			1,7	1,7
IIb	Q	CC77	1,2	0,4	1,7		0,5		3,8	0,9	1,4
IIb	R	CC224	1,0	0,7	2,4		1,5			1,1	1,4
IIa	S	CC11	1,3		1,0	0,4	0,5	1,5	3,8	0,9	1,0
IIa	T	CC18	0,3	1,4	2,8	0,4	0,5			0,9	0,9
IIa	U	CC193	1,3	0,4	1,4	1,3				0,9	0,9
IIa	V	CC101 - CC90	0,5	0,4	2,1	1,3	0,5			0,8	0,8
IIa	W	Not assigned	0,5	0,4	3,5					0,8	0,8
IIa	X	CC21		0,4	2,8	0,4	0,5			0,6	0,7
IIa	Y	CC14	0,7	0,4	1,0	0,8		3,0		0,8	0,7
IIa	Z	CC20	0,3	0,4	1,4	0,8	1,0		3,8	0,7	0,7
	Other		30,0	9,2	20,9	11,4	10,2	14,9	11,5	12,1	12,3
	Total		602	284	287	237	205	67	26	1708	1793

Figure 2: Distribution of the 26 major mapped clonal complexes (CCs) in the pork production sector and in other food production sectors

MONITORING AND SURVEILLANCE

031

Salmonella in pigs from weaning to slaughter

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Introduction

Salmonella continues to be one of the most important causes of foodborne gastrointestinal illness in humans. Food producing animals are the main cause of human salmonellosis (1). *Salmonella* reduction at the farm level is important to mitigate *Salmonella* transmission from pigs to humans. Some pigs shed *Salmonella* in feces despite appearing healthy. The subclinical carriers can exacerbate levels of *Salmonella* in the barn and slaughterhouse and infect pigs with no previous exposure during transportation and lairage. The presence of intermittent shedders and the variable nature of *Salmonella* infection over time present limitations to point-prevalence studies (2). A clear understanding of the shedding patterns over the entire production stage on commercial pig farms is crucial for implementing effective monitoring and control measures. The objective of this study was to examine the *Salmonella* status in pigs from birth to slaughter.

Material and Methods

Pig selection. Fourteen groups of pigs from eight farrowing sources were studied; six farrowing sources contributing two cohorts each. Piglets in Cohort One were born between May and August, and piglets in Cohort Two between October and January. For each cohort, 4-8 piglets were selected from each of 8-10 sows within 96 hours of birth and identified with an ear tag.

Sample collection. Fecal or rectal swab samples were collected from piglets prior to 4 days of age (only in seven groups), and form all pigs at weaning, at the end of the nursery, grower, and finisher periods. At slaughter, palatine tonsils and submandibular lymph node samples were collected from a subset of pigs.

Salmonella isolation. Ten grams of fecal or tissue samples was transferred into a stomacher bag and homogenized in 50 mL of tetrathionate broth (TTB), and incubated for 24 h at 37 °C. Then, 0.1 mL of TTB culture was inoculated into 9.9 mL of Rappaport Vassiliadis (RV) broth and incubated at 42 °C for 24 h. Finally, a loopful of RV culture was streaked onto xylose-lysine-tergoitol 4 agar (XLT4) and incubated at 37 °C for 24 h to 72 h. *Salmonella* isolates were confirmed with *Salmonella* O Antiserum Poly A-I & Vi.

Data analysis. A multilevel mixed-effects logistic regression method was used to analyze *Salmonella* shedding in feces across the stages of production, as well as to analyze the associations between the presence of *Salmonella* in tissue samples and fecal shedding.

Results

Salmonella was cultured from 12.6% of 3339 fecal samples collected from 809 pigs; 35.1 and 12.1% of pigs shed *Salmonella* at least once or more, respectively. The proportion of pigs positive at each stage of production and at slaughter is shown in Figure. Overall, *Salmonella* was recovered from 4.9% of pigs at 1-4 days of age, 10.5% at weaning, 12.6% at the end of the nursery period, 12.3% in the grower period, and 20.2% of pigs at the finisher stage. *Salmonella* shedding increased over time with older pigs more likely to test positive (P=0.01). At slaughter, *Salmonella* could be isolated at least from one tissue sample in 23.1% of pigs. Out of the 100 pigs that shed *Salmonella* in feces at the finisher stage, only 50% of pigs tested positive in tissues at slaughter. Out of 463 pigs negative for *Salmonella* shedding at the finisher stage, 17.5% tested positive in tissues at slaughter. The presence of *Salmonella* in tissue samples collected at slaughter was not associated with fecal shedding at the finisher stage. However, the number of times a pig shed *Salmonella* on the farm was only borderline significant with presence of *Salmonella* in tonsils (P=0.06).

Discussion and Conclusion

In this study there was an increase in *Salmonella* shedding from early life until the finisher stage. This is similar to previous study reporting the proportion of pigs shedding *Salmonella* increased from the end of the nursery period until slaughter (3). It is possible that as time progressed, pigs have become infected to *Salmonella*, while previously infected pigs may have been infected by a new serotype (4). Further, pigs in the present study were shipped to an off-site weaning barn which might provide an opportunity for exposure to *Salmonella* during transportation to a second facility (5,6). Therefore, it may be prudent from a food safety perspective to evaluate risk factors and interventions that help mitigate *Salmonella* shedding at later stages of production. Presence of *Salmonella* in tissues at slaughter was not significantly associated with on-farm fecal shedding. It is likely that non-shedder pigs could have become infected with *Salmonella* during transportation and lairage. The presence of repeat shedders and the lack of association between

Salmonella shedding on farm and its presence in tissues at slaughter is a food safety concern that warrants attention to implement control measures at the slaughter level.

Acknowledgements
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References

1) Pires SM, Vieira AR, Hald T, Cole D. (2014): Source attribution of human salmonellosis: an overview of methods and estimates. Foodborne Pathog Dis. 11(9):667-76.
2) Pires AFA, Funk JA, Bolin CA. (2013): Longitudinal study of *Salmonella* shedding in naturally infected finishing pigs. Epidemiol Infect.141(9):1928-36.
3) Dorr PM, Tadesse DA, Zewde BM, Fry P, Thakur S, Gebreyes WA. (2009): Longitudinal study of *Salmonella* dispersion and the role of environmental contamination in commercial swine production systems. Appl Environ Microbiol. 75(6):1478-86.
4) Saranya N; Farzan A; O'Sullivan TL; Friendship RM. (2018): Time course of *Salmonella* shedding and antibody response in naturally-infected pigs during grower-finisher stage. Can J Vet Res.; 82(2):139-145.
5) Carlson SA, Barnhill A, Griffith R. (2012): Salmonellosis. In: Zimmerman J, editor. Diseases of Swine. 10th ed. ChichesterWest Sussex: Wiley-Blackwell publishing; p. 821-33.
6) Nollet N, Houf K, Dewulf J, et al (2005): Distribution of *Salmonella* strains in farrow-to-finish pig herds: a longitudinal study. J Food Prot. 68(10):2012-21.

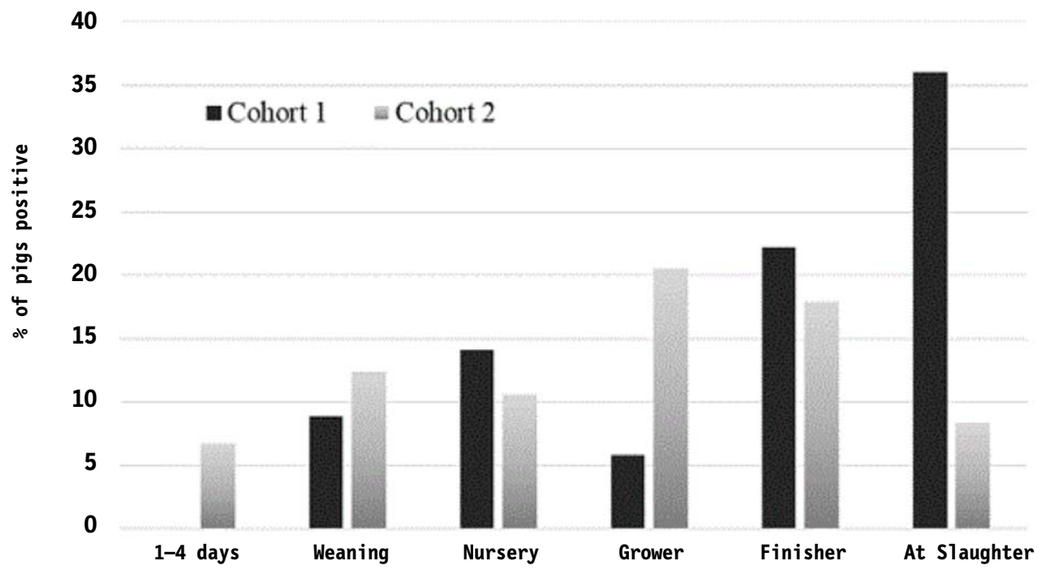

Figure 1: Proportion of pigs testing positive for Salmonella in feces on farm and in tissues at slaughter

032

Detection of Salmonella antibodies in oral fluid samples from pigs. A tool for easier monitoring of fattening Herds?

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Introduction
In Germany, all pig-fattening farms take part in a mandatory serological screening for Salmonella via meat juice or blood samples shortly before slaughtering. Depending on the results, farms are classified in 3 categories (cat. 1: 0-20% positive samples, cat. 2: 20-40% positive samples, cat.3 > 40% positive samples). Within the framework of the German-Dutch INTERREG V A-project Food Protects, we tested for antibodies against Salmonella spp. in oral fluids (OF) for classification of the herds.

Material and Methods
We chose 10 pig farms with a high and 10 with a low burden of Salmonella for the study. We took 2 x 5 blood (BS) samples, 2 x 1 OF and one pooled faecal sample at the same day in groups at beginning, in the middle and at the end of the fattening period. In one farm with a high burden, we followed one group and took samples from 2 pigs from the 10th to the 22th week of life. We took OF every week and BS every 4th week.
Individual serum samples were analysed by Swine Salmonella ELISA IDEXX and compared to the OF samples using another Swine Salmonella ELISA adapted to OF by using a special conjugate appropriate for testing OF samples. The dilution of BS was 1:100 and the dilution of OF was 1:2. For the OF samples, we prolonged the incubation time from 60 min (BS) to 120 min.
The cut-off value for Salmonella OF ELISA was determined by ROC analysis.

Results
For the OF Swine Salmonella-ELISA Kit, the cut-off values of 29 OD% (positive) and 10 OD% (negative) were determined at the specificity and sensitivity level of greater than 95%. Results achieved by the OF Swine Salmonella ELISA represented the approximate mean of the results of all individual BS samples of the same animal group. The 120 statistical mean values from BS results were compared to OF results of the same animal group; 94 (78.3%) of these results were identical, in less than 13.3% (10 and 16 animal groups) the results differed between the BS- and OF-ELISA.

Discussion and Conclusion
OF is a good tool for Salmonella herd monitoring. We could detect herds with a high burden of Salmonella comparable using BS-ELISA. It is easy to take the OF-samples and you can take them more often. In the BS we found more individual different and you have to take more samples.With OF an additional diagnostic tool is available to classify herds.

References

Kittawornrat A., Prickett J. and Wang C., (2011). Diagnostic performance of a commercial PRRS serum antibody ELISA adapted to oral fluid specimens: longitudinal response in experimentally-inoculated populations. AAVLD Annual Conference Proceedings, p. 100.
Strutzberg-Minder K., Boehmer J., Fischer S., Homuth M. et al. (2015). Monitoring influenza A virus infection in pigs by using a competitive enzyme-linked immunosorbent assay to detect virus antibodies in pen-based oral-fluid specimens". J Swine Health Prod.; 23 (3):126-131.
White D., Rotolo M. and Olsen C., (2013). Recommendations for pen-based oral-fluid collection in growing pigs. Journal of Swine Health and Production - Volume 22, Number 3.

033

A biomolecular DIVA-strategy for Salmonella spp. - diagnostics in Swine

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Introduction
Salmonellosis remains of utmost significance regarding all levels of food production, in particular for public farmers, practitioners, food industry and public health authorities.
Decades ago, Germany together with many other current member states of the European Union agreed on measures to reduce *Salmonella* spp. infections as the cause of foodborne diarrhoea.
Hence, for Swine Production in Germany a monitoring system based on antibody detection was implemented and used to categorize farms in three different categories (low, medium, high number of positive samples). Once a farm enters category 3 (high positive) a plan of action against *Salmonella* spp. infection has to be worked out including adjustments of biosecurity, disinfection, feeding and the vaccination program. In order to accompany these measures antigen-detection is mandatory to verify the success. An evaluation of Salmonella serovars in food samples divided into animal species revealed that in 2015 *Salmonella Typhimurium* (ST) was the dominant serovar related to pig meat stated by Dr. Istvan Szabo at the Symposium "Zoonosen and Lebensmittelsicherheit" in 2016. Next to traditional diagnostic tools for antigen detection like cultivation and typing of isolates, biomolecular techniques have broadened the diagnostic spectrum. This study reviews the traditional diagnostic tools and points towards a new approach regarding the Differentiation of Infected and Vaccinated Animals (DIVA) of *Salmonella Typhimurium* - field strains and *Salmonella Typhimurium* - vaccine strains.

Material and Methods
In total 511 samples were examined over a period of 6 months at the AniCon Labor GmbH. The sample material was divided in faecal and environmental swabs. Furthermore, pigs were sampled 1 week and 2 weeks post vaccination. All submitted samples were derived from pigs which were vaccinated with a commercial modified live vaccine (Salmoporc) produced by IDT Biologika in Dessau. The vaccine strain is a *Salmonella* Typhimurium - Mutant.
All samples were enriched with buffered peptone water (BPW) at 37 degrees for at least 16 hours according to DIN EN ISO 6579-1:2017. After that 1 ml of the BPW were pipetted into a sterile tube. The majority of the 1 ml were implemented in a modified Rappaport - Vassiliadis - Soy Broth and incubated at 42°C for another 24hours. Material of all samples, which indicated bacterial growth in form of a swarming area, was then transferred to a Xylose-Lysin-Desoxycholat-Agar. The next step was the cultivation of a *Salmonella* spp.-isolate on Columbia agar for 24hours at 37°C in order to perform serum-agglutination and further differentiation between field- and vaccine strain using a commercial test kit developed by IDT Biologika GmbH, Dessau; Germany. The commercial test kit is based on the evaluation of bacterial growth characteristic in two selective media and can be performed on cultivated *Salmonella Typhimurium* - strains only.
A smaller aliquot of the 1 ml peptone solution was used to perform biomolecular detection of *Salmonella* spp. with the Kylt® *Salmonella* spp. DNA Extraction and Real-Time PCR Detection Kit according to the manufacturers´ instructions.
Subsequently, all positive samples were further examined with the new Kylt® ST DIVA Real-Time PCR Detection Kit according to the manufacturers´ instructions.

Results
Out of 511 samples 375 samples were negative for *Salmonella* spp. in all detection methods. In 136 out of 511 samples Salmonella spp. was detected by *Salmonella* spp. - Screening - PCR.
I. A successful cultivation of *Salmonella* spp. was achieved in 62 out of 136 *Salmonella spp.* - Screening - PCR positive samples. The isolates were typed by serological agglutination in the following descending order: *Salmonella Typhimurium* (n=44), *Salmonella Ohio* (n=8), *Salmonella Infantis* (n=6), *Salmonella Derby* (n=1) and others (n=2).
Differentiation of the 44 *Salmonella Typhimurium* isolates was performed by using a DIVA-method developed by IDT Biologika, Dessau, Germany. 27 isolates were identified as vaccine strains, 11 isolates were identified as field strains and 8 isolates were not tested.
The biomolecular DIVA-method revealed the following results using the same buffered peptone enrichment as used for the cultivation of the isolates: ST vaccine strain positive (n=24), ST field strain positive (n=9), ST vacc & ST field strain (n=9) and ST not detectable (n=18).
II. Furthermore, a number of 64 positive samples, from which no successful cultivation was possible, remained. The biomolecular DIVA-method showed the following results: ST vaccine strain positive (n=46), ST field strain positive (n=3), ST vaccine strain & ST field strain positive (n=7) and not detectable (n=8).

Discussion and Conclusion
This study reveals the successful attempt to establish a biomolecular DIVA method which is able to differentiate between *Salmonella Typhimurium* - field strains and *Salmonella Typhimurium* - vaccine strains. It can be carried out from faecal and environmental swabs which were enriched in buffered peptone water at 37 degrees for at least 16 hours. Hence, the differentiation could be performed within one day after sample receipt. Furthermore, non-viable as well as viable genome sequences of *Salmonella Typhimurium* could be differentiated. This ST DIVA PCR-method is most successfully used in combination with a *Salmonella* spp. Screening - PCR. Utilising this diagnostic approach would not only decrease the examination costs and speed up the process of result reporting but also increase the sensitivity for the detection of *Salmonella* spp. in pig herds. In summary, the new biomolecular ST DIVA-strategy for *Salmonella* spp. has the potential to be used as a monitoring tool in *Salmonella Typhimurium* vaccinated pig herds.

034

The smartphone based PCR lab in a bag

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Introduction

Point-of-care (POC) PCR diagnostics has arrived in veterinary medicine. To meet the rising demand for rapid, reliable molecular diagnostic tools for use away from centralized laboratories in vet clinics and even on farms, INDICAL introduces a novel POC PCR platform that transforms your smartphone into a portable lab for the real-time PCR diagnosis of animal diseases. At the heart of it is a handy, ultra-portable qPCR thermocycler. This thermocycler enables multiplex real-time detection of up to 27 targets from a single sample or 9 samples to be tested for up to 3 targets each. It also comes with shelf-stable PCR reagents, meaning no cold chain is required.

The qPCR test results are analysed in real time on your smartphone. In this study, we compared INDICAL's new POC qPCR platform to commonly used real-time PCR thermocyclers to see whether it was just as good or even better at detecting infectious animal pathogens.

Methods

For this study, DNA and RNA samples from different viral pathogens such as African Swine Fever Virus and Influenza A Virus were tested. The purified nucleic acids were then analysed using INDICAL's certified virotype ASFV PCR Kit and the virotype Influenza A RT-PCR Kit on the portable qPCR thermocycler versus the standard protocol developed for central labs and tested on two widely used standard thermocyclers. Furthermore, new lyophilized PCR reagents especially developed for POC diagnostics were tested and first results compared to the performance of the certified lab assays on the different thermocyclers.

Results

Testing ASFV-positive DNA in real time qPCR showed better Ct value results on INDICAL's portable qPCR thermocycler than on the BioRad CFX96.

Influenza A virus-positive RNA was detected with better Ct values on the new portable qPCR thermocycler compared to the Agilent Mx3005P. First tests of the lyophilized PCR reagents show comparable results on the different thermocyclers.

Conclusions

INDICAL's qPCR platform for POC applications with its ultra-portable qPCR thermocycler and hand-held smartphone-based analysis achieved comparable or even better results when compared to the standard molecular lab equipment also used here.

The qPCR thermocycler can also be combined with a novel portable extraction solution using small cartridges to extract nucleic acids without any lab equipment. This ultra-fast POC extraction is currently in validation.

INDICAL's solution for veterinary POC diagnostics also includes the possibility of implementing new PCR assays using lyophilized reagents without the need for a cold chain.

035

Establishing a serum bank of confirmed cysticercosis positive and negative samples

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Introduction

Porcine cysticercosis is a neglected zoonotic disease caused by *Taenia solium*. Despite recent gains in the understanding of the nature and the prevalence of the disease, and successes in health interventions *T. solium* cysticercosis is still endemic and affects poor people in the resource-limited countries. The formal postmortem-inspection at slaughter commonly relies on visual inspection of predilection sites such as heart, diaphragm, masseters, tongue, neck, shoulder, intercostal and abdominal muscles (Gracey, 1986). Exploring other overlooked muscular regions or organs as predilection sites is essential to supplement the current post-mortem inspection procedures. Tongue test was reported to have 70% sensitivity and 100% specificity of in the detection of porcine cysticercosis (Gonzalez et al., 1990). Lightowlers et al. (2015) (Lightowlers, Assana, Jayashi, Gauci, & Donadeu, 2015) estimated that slicing of the heart, tongue and masticatory muscles at a thickness of approximately 3 mm had a diagnostic sensitivity of approximately 80% in lightly infected animals and recommended tissue dissection as a highly specific and relatively low-cost method for diagnosis of porcine cysticercosis. Compared to tongue examination, ultrasonography has been found to more sensitive (100% versus 91%) but less specific (90% versus 98%), although these differences were not statistically significant (Flecker et al., 2017). A recent study estimated prevalence at 37.6% in western Kenya (Thomas et al., 2016). The existing serological tests that detect circulating *T. solium* cyst antigens have poor specificity thus limiting their diagnostic capacity. Fine carcass dissection method is considered a gold standard for detecting porcine cysticercosis with lesions consisting of cysticerci in cyst measuring 5-8 mm by 3-5 mm, translucid and filled with brownish to pinkish liquid. Sometimes the head of the metacestodes can be seen as white spot. Cysts are in the following active muscles; heart, tongue, masseters and diaphragm, shoulder, intercostal and oesophagi. More rarely cysti are found in lymph nodes, liver, spleen, lungs and brain.

Objective

The aim of this project is to collect a bank of blood and serum samples from pigs confirmed to be cysticercosis positive and negative via fine dissection. These samples will then be used for future diagnostic test validation.

Materials and Methods

Twelve slaughterhouses have been recruited from two counties in western Kenya with the help of the County Veterinary officials. Pigs are sourced through local butchers and purchased at the market rate. Each pig is identified, and relevant meta-data such age, sex and area of origin recorded. Blood samples are collected from the jugular vein and lingual palpation performed peri-mortem. Following slaughter, the carcass is weighed and dressed following a specific protocol. The carcass and organs are transported to the field lab in Busia and refrigerated. Fine dissection is performed on the carcass and organs in slices approximately 3mm and checking for the presence of cysts. All relevant data is recorded electronically, while the serum and uncoagulated blood are frozen for future diagnostic work.

Results

We have so far processed twenty carcasses, and all were confirmed to be having no cysts. This work poses challenges especially with lack of supply in the market. Pigs are purchased at market rate although the pricing is usually not fixed thus making the process of bargaining difficult. The average dissection time is four hours. We project to dissect a total of 110 pigs in the next 6 months.

Conclusion

At the end of this project, a bank with confirmed cysticercosis positive and negative blood and serum samples will be established. These results will be made available via open access so as to expedite validation of diagnostics kits with higher specificity This, in turn, will aid quicker and more accurate diagnosis of the disease.

References

Flecker, R. H., Pray, I. W., Santivañez, S. J., Ayvar, V., Gamboa, R., Muro, C., … O'Neal, S. E. (2017). Assessing ultrasonography as a diagnostic tool for porcine cysticercosis. *PLoS Neglected Tropical Diseases*, *11*(1), e0005282.

Gonzalez, A. E., Cama, V., Gilman, R. H., Tsang, V. C. W., Pilcher, J. B., Chavera, A., … Miranda, E. (1990). Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. *The American Journal of Tropical Medicine and Hygiene*, *43*(2), 194-199.
Gracey, J. F. (1986). *Meat hygiene*. Baillière Tindall.
Lightowlers, M. W., Assana, E., Jayashi, C. M., Gauci, C. G., & Donadeu, M. (2015). Sensitivity of partial carcass dissection for assessment of porcine cysticercosis at necropsy. *International Journal for Parasitology*, *45*(13), 815-818.
Thomas, L. F., Jayne, L., Harrison, S., Toye, P., Glanville, W. A. De, Anne, E., … Fèvre, E. M. (2016). Prevalence of Taenia solium cysticercosis in pigs entering the food chain in western Kenya, 233-238. https://doi.org/10.1007/s11250-015-0949-6

036

Change of livestock trade networks during epidemic outbreaks

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Introduction
The trade of livestock is an essential risk factor in the spread of infectious animal diseases. In order to allow for efficient back tracing and forward tracing in case of a disease outbreak, all EU member states are obliged to report livestock movements to central databases. We focus on pig trade in Germany as an example of such a data set. Our aim is to investigate the impact of a disease outbreak on the network structure. In order to measure this impact, we make use of real outbreak data of classical swine fever.Being logistic hubs and responsible for large animal movements, traders play a key role in the trade network. In order to quantify network changes during a disease outbreak, it is hence strongly advisable to use information about the holding type in the pig production chain. However, in many datasets the types of the producing farms or whether the agent is a trader, is unknown.

Methods
We introduce two index numbers, that can be used to identify the position of a producing holding in the pig production chain. First, the balance of traded animals over a certain time span. This number is related to the role of the holding. Second, the trader index that counts the number of purchases that are sold directly after the purchase. Using these index numbers, we can resolve the flux of traded animals between different node types over time. This resolution is much higher than considering the total number of traded animals alone. In particular, the impact of a disease outbreak can be measured at different parts of the production chain.

Results
We resolve the number of traded animals between different holding types. After the outbreak, trade restrictions are implemented. These trade restrictions have a strong impact on the trading behavior of different farm types. In particular, we could observe behavioral changes towards higher biosecurity after the outbreak.

Conclusions
It should be noted that the technique introduced above can in particular be used to identify traders. Analyzing the pig trade network in Germany from 2005 to 2007, we demonstrate that our algorithm is very sensitive in detecting traders. Since the methodology can easily be applied to trade networks in other countries, we anticipate its use for augmenting the datasets in further network analyses and targeting control measures.

Reference

Köppel, L., T. Siems, M. Fischer, and H.H.K. Lentz. 2018. Automatic classification of farms and traders in the pig production chain. Prev Vet Med 150:86-92.

037

Decomposition of wild boar carcasses

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Introduction
Wild boar infected with African swine fever (ASF) usually die from infection, so their bodies become exposed to scavengers, including healthy conspecifics. In a previous study, wild boar were observed sniffing, poking, and chewing on bare bones from dead conspecifics even after skeletonization was complete (Probst et al. 2017). Since ASF-virus is extremely stable in the environment, this behaviour might be sufficient for ASF transmission, if for example the bone marrow still contains infectious ASF virus.
Against this background, when ASF is introduced into a wild boar population, it is crucial to estimate as precisely as possible the time of death of the first carcasses found in the field to estimate (i) the time point of disease introduction and (ii) the size of the already affected area. However, little is known about the decomposition process in wild boar.

Material and Methods
We describe the macroscopic stages observed in three decomposition trials with a total of eight carcasses. To prevent scavengers from gaining access, all carcasses were exposed in cages.
Trial 1 (domestic pig, wild boar), started in August 2017, was designed to test the hypothesis “Wild boar and domestic pigs are similar in terms of carcass persistence time as well as occurrence and sequence of carrion-related insects”.
Trial 2 (sun, shade, water, buried), started in September 2018, aimed to address the question: How long does it take for a wild boar piglet to decompose in different microenvironments? Carcasses (21-23 kg) were exposed to direct sunlight, in the shadow, in a mixture of soil and tap water or buried in a shallow grave, respectively.
Trial 3 (sun, shade), was started in October 2018 to investigate whether the differences observed in trial 2 are also true for adult wild boar.

Results
The opening of the abdomen occurred in the wild boar later than in the domestic pig. While only bones and pieces of desiccated skin were left over from the domestic pig after twelve months, one and a half year later a large proportion of a hard and crumbly substance (adipocere) remained from wild boar. In trial 2, the piglet in the sun decomposed more rapidly than the other piglets. In trial 3, the differences between sun and shade were not as large as expected.

Discussion and Conclusion
The decomposition process of wild boar carcasses may vary substantially as it depends on the influence of several factors including the weight of the dead animal, the season at the time of death and weather conditions. Especially in winter, it may take several months until a wild boar carcass is skeletonized and fully decomposed. We also found that the decomposition process of wild boar seems to be slower than in domestic pigs, probably owing to their hard and thick skin covered with bristles. This type of skin presumably retains moisture for a longer time and might slow down the rate, at which maggots assimilate carcass material. We also found that sunlight accelerates the decomposition process in piglets, while standing water may slow it down. However, in adult wild boar the difference between sunlight and shade is not so obvious, possibly because the skin protects the inner organs and soft tissues so effectively, that environmental factors including direct sunlight loose relative importance.

Reference

Probst C, Globig A, Knoll B, Conraths FJ and Depner K (2017): Behaviour of free ranging wild boar towards their dead fellows: potential implications for the transmission of African swine fever. Royal Society Open Science 4, doi:10.1098/rsos.170054

PRE-HARVEST FOOD SAFETY CONCEPTS

P1

Water pipe deposits in swine nursery units as a possible reservoir of Salmonella?

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Introduction

The quality of drinking water is crucial for the health, welfare and performance of swine. As a consequence of poor water quality, undesirable substances as well as microorganisms can be introduced into the food chain. The farmer himself is responsible for ensuring that water is suitable for animal nutrition in accordance with legislation and that technical installations are sufficient, so that the risk of water contamination is minimized. So far, there is neither a guidance for risk assessment according to inorganic and organic deposits nor biofilms in drinking water installations on farms. It is known, that components in water originating from deposits/ biofilms can cause a bad taste of drinking water. Hence, this might lead to a decreased uptake of water by the pigs. It is also discussed that biofilms might be a reservoir for pathogens.

Materials and Methods

Deposits in drinking water installations in 15 piglet rearing farms were sampled and analyzed for their physical, chemical and microbiological characteristics. Based onto results from analysis of deposits from the first five farms, a practical approach for a risk assessment on farms was elaborated and tested on ten farms. Deposits were classified with respect to their inorganic proportion and by microbiological culture methods. Different cleaning concepts were tested under laboratory conditions on the respective pipes containing farm-specific deposits.

Results

In four farms *Escherichia coli* and *Salmonella enterica* (predominantly S. Typhimurium var. Copenhagen) were isolated in a number of biofilms from water pipes. The antibiotic resistance patterns of respeceive isolates were compared with those from isolates originating from routine samples or from those reported in literature. Cleaning concepts based onto alternating applications of basic and acid cleaning substances combined with mechanical flow impulses were successful to remove most of the deposits.

Discussion and Conclusion

Inorganic deposits and biofilms are farm-specific with a high variation between farms depending on water origin, pipe installation, dosage of substances by water, technical devices and operation. The results of the study suggest, that water pipes might be a reservoir for zoonotic *Salmonella* strains and that pigs consuming faecally contaminated drinking water are at risk to be infected. Furthermore Salmonella detection may be of importance for the prevalence of seroreagents in the context of salmonella monitoring. The fact, that pathogens were most frequently detected in the periphery of the pipeline system near to the drinkers, suggested that predominantly a retrograde bacterial contamination from drinkers takes place on farm. In addition the resistancey patterns and the minimal inhibitory concentrations of antimicrobial substances of the potentially pathogenic microorganisms did not differ from those reported in other studies or routinely tested. If a high load of *E. coli* or *Salmonella* is detectable in water pipes of nursery systems, water origin, pipe installation and drinker technique should be checked and a pipe cleaning procedure might be recommendable.

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P2

Evaluation of the efficiency of novel orally administered subunit vaccine to reduce the prevalence of Salmonella in swine under field conditions

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Introduction

Control of *Salmonella* sp. in swine production undergoes a systemic vision of the problem, and an integrated program focused on the main stages of production. Control measures at the stage of primary animal production are required for a reduction in the number of carrier and shedders animals that reach slaughter. Among the various tools available, vaccination is a traditional and consolidated concept in preventive veterinary medicine.

Salmonella sp. has on its surface large antigenic molecules (immunodominant molecules), membrane LPS, which are easily recognized by the immune system, and are the target of most line vaccines. These molecules tend to be specific to a particular serovar and / or serogroup (Arguello et al., 2012), and vaccines offering limited protection against heterologous serovars (Bearson et al., 2016).

To contribute to the solution of this problem, the aim of this research was to evaluate a subunit vaccine, based on secondary antigens, where a common genetic sequence for all *Salmonella* sp. was cloned into an expression plasmid, and inserted into *Bacillus subtilis*, which produced subunits (peptides) that were incorporated by microparticles, composing the mucosal vaccine. In order to be effective in controlling any serovar of *Salmonella* enterica (broad spectrum).

Material and Methods

The field trial was carried out on 20 swine fattening unit (pens held 10-20 pigs), belonging to the same agroindustrial integration system. The experimental unit was the swine batch, of which 10 were vaccinated (vaccinated group-VG) and 10 controls (control group-CG).

Two mL of the vaccine were orally administered at four ages. After the second dose of the vaccine, blood was collected with anticoagulant (n=32/group). Blood samples were collected during the first week of fattening (n=30/batch) and slaughter (n=30/batch). Mesenteric lymph nodes-MLN (n=30/batch) and faeces (n=20/batch) were collected at slaughter. Serological analysis was performed using a commercial-ELISA (Herd Check Swine *Salmonella*®IDEXX Laboratories, ME, USA), tested in three cut-off points (S/P relation, 10%, 20%, and 40% of optical density-OD).

The MLN and faeces were submitted to *Salmonella* isolation (ISO 6579: 2002), and the quantification, by most probable number technique- mNMP, following the ISO/TS6579-2:2012. The vaccine ability to induce phagocytic cells was evaluated. All statistical analyses were performed using commercial software SAS® 9.3: 2012.

Results

The group effect was not significant (p> 0.05) in any collection period for the two variables, the seroconversion at different cut-off points and the mean optical density. At slaughter, the isolation of *Salmonella* sp. from MLN in VG (115/300; 38.33%; IC 95%) presented a higher percentage than CG (90/300; 30%; IC 95%). The excretion of the agent in the faeces also had a significant group effect on the isolation of *Salmonella* sp. lower in CG (47/199; 23,62%; IC 95%) than in VG (66/200; 33%; IC 95%).

The quantitative method, mNMP was used to estimate the amount of *Salmonella* sp. positive isolates of faeces. There was statistical difference between the groups, VG presented a greater percentage of isolation. The CG was 0.07 (± 0.04) log NMP/g, while the VG ranged from> 0.16 to 0.06 log NMP/g. The F test of the analysis of variance detected a significant effect ($p < 0.05$) for the group in the faeces NMP.

Through the flow cytometry results it was possible to demonstrate that the activity of the phagocytic monocytes was altered by vaccination (p=0,067).

Discussion and Conclusions

The VG showed higher frequency of detection of *Salmonella* sp. than the CG, with a difference of 8.33% of carriers of *Salmonella* sp. in the MLN, 9.38% of shedders swine and 0.09 log in the faeces colony forming unit NMP at slaughter.

In addition to the effect of vaccination under carriers and shedders of *Salmonella* sp. was performed the immunological evaluation of the swine front of vaccine. It is known that the destruction of microorganisms phagocytosed by macrophages is due to the production of nitric oxide (NO) and other intermediates, which are produced due to the classic (Th1) activation of the macrophages through IFN-γ or LPS (Classen, Lloberas, and Celada, 2009). However, for intracellular bacteria, such as *Salmonella* sp., the ingestion of these by macrophages can provide a safe haven, protecting the bacteria from complement-mediated extracellular death. Eze et al. (2000) demonstrated that the virulent strain 16M of *Brucella melitensis* was efficiently phagocytosed by mouse peritoneal macrophages in the presence of

hyperimmune anti-LPS serum of *B. melintenis*. Once internalized, the bacterium multiplied efficiently in non-activated macrophages, and its elimination occurred only when the activation of macrophages by IFN-γ was induced. In this study, when evaluating all farms together, an increase in the phagocytic activity of peripheral monocytes was found in VG. Despite this, the data do not allow to infer if this increase of the phagocytic activity resulted in the effective direction of field strains by macrophages, or whether these cells have potentiated the multiplication of the pathogen serving as a replication site. The results of isolation in the faeces, MLN and mNMP point to the second hypothesis, once percentage of detection of *Salmonella* sp. was higher in the vaccinated group than in the control group.The vaccine tested had no effect on the seroprevalence of batches at the time of slaughter. It was concluded that the vaccination program with the oral subunit vaccine did not confer a reduction in the spread and amplification of infection on the farms that had an impact on the prevalence of swine carriers and shedders of *Salmonella* sp. at slaughter. These results allow us to state that the form of presentation of the antigen in the vaccine has not yet been sufficient to stimulate immunity that could withstand the field challenge.

References

Arguello H, Rubio P and Carvajal A (2012): *Salmonella*Control Measures at Farm in Swine Production. In: BASSAM, A.A., GUTLER, J.B. (Eds). *Salmonella*- Distribution, Adaptation, Control Measures and Molecular Technologies. In tech. p.99-122.

Bearson BL, Bearson SMD and Kich JD (2016): A DIVA vaccine for cross-protection against *Salmonella*. Vaccine.34, 1241-1246.

Classe A, Lloberas J and Celada A (2009): Macrophage Activation: Classical Vs. Alternative. In: Reiner NE. (Ed). Macrophages and Dendritic Cells.p. 29-43.

Eze MO, Yuan L, Crawford RM et al (2000): Effects of Opsonization and Gamma Interferon on Growth of *Brucella melitensis*16M in Mouse Peritoneal Macrophages In Vitro. Infection and Immunity. 68, 257-263.

P3

Lipid-caused antagonism of the bactericidal activity of thymol and thymol-β-D-glucopyranoside is not overcome by emulsifiers

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Introduction

Strategies are sought to reduce the carriage and dissemination of zoonotic pathogens and antimicrobial resistant microbes within food-producing animals and their production environment. Thymol is an essential oil shown to be a potent bactericide *in vitro* but demonstration of its efficacy when fed to animals has been inconsistent, due largely to its lipophilicity which limits its passage and subsequent availability in the distal gastrointestinal tract. Conjugation of thymol to glucose to form thymol-β-D-glucopyranoside can decrease absorption of the conjugate, thereby promoting passage to more distal intestinal sites where pathogens primarily reside, yet *in vivo* efficacy of the conjugate remains suboptimal. It is possible that hydrolysis and absorption of thymol-β-D-glucopyranoside and free thymol may still have been rapid enough within the proximal small intestine to preclude their delivery to the cecum and large intestine. Considering that modern swine diets often contain 5% or more fat, we hypothesized that even at 60 to 80% apparent digestibility there may be passage of enough residual undigested lipid to the distal intestinal tract to sequester free or conjugated thymol within lipidic microenvironments, thereby limiting the availability and subsequent effectiveness of these biocides.

Material and Methods

Freshly voided feces collected from 25 kg conventionally-reared pigs maintained on unmedicated feed were mixed (0.5% wt/vol) with ½-strength Mueller Hinton broth prepared under 100% N_2 gas. Fecal suspensions were then inoculated with novobiocin- and nalidixic-acid resistant (NN-resistant) challenge strains of *Salmonella enterica* serovar Typhimurium (NVSL 95-1776) or *Escherichia coli* K88 to achieve initial concentrations of approximately 10^6 colony forming units (CFU)/mL. The ½-strength broth was used to avoid excessive acid production within the fecal suspensions and the NN-resistant inocula, grown overnight at 37°C in tryptic soy broth supplemented with 25 µg of novobiocin/mL and 20 µg/nalidixic acid mL, were used to facilitate recovery and differentiation of the challenge strains from indigenous fecal microbes. The resultant suspensions were distributed (5 mL/tube) under a constant flow of 100% N_2 gas to 18 x 150 mm crimp top tubes that had been preloaded with or without 0.3 mL of vegetable oil and with or without small volumes (≤ 0.5 mL) of a 600 mM stock solution of thymol-β-D-glucopyranoside or thymol, prepared in ethanol, to achieve 6 mM upon addition of fecal suspensions. Control tubes were preloaded with 0.2 mL ethanol. In another experiment, fecal suspensions preloaded as above with oil and thymol-β-D-glucopyranoside were tested without or with additions of bile salts or taurine (0.6 or 8 mg/mL, respectively) added to assess the impact of bile acid-based micelles or their de-conjugation on pathogen survivability. The emulsifying agents Tween 20 or Tween 80 (each at 1% vol/vol) or polyoxyethylene (40) stearate (at 0.2% vol./vol) were also tested to assess their potential impact on the bactericidal activity of thymol-β-D-glucopyranoside. Tubes were closed with stoppers and incubated at 39°C for 24 h. The NN-resistant *S.* Typhimurium and *E. coli* K88 were enumerated via viable cell count on Brilliant Green or MacConkey agars supplemented with 25 µg novobiocin/mL and 20 µg naladixic acid/mL. Log_{10} CFU of NN-resistant *S.* Typhimurium and *E. coli* K88 were tested for treatment effects using a general analysis of variance and LSD separation of means. All incubations were conducted with n = 3 experimental units per treatment condition.

Results

The bactericidal effect of 6 mM free or conjugated thymol against *S.* Typhimurium and *E. coli* K88 are presented in Figure 1A and B. When expressed as log_{10}-fold reductions of CFU/mL, the addition of 3% added vegetable oil decreased (P < 0.05) the anti-*Salmonella* effects of thymol and thymol-β-D-glucopyranoside by 90 and 58%, respectively, compared to CFU reductions achieved during cultures without added oil (6.1 log_{10} CFU/mL). Addition of vegetable oil decreased (P < 0.05) the anti-*E. coli* activity of free and conjugated thymol by 86 and 84%, respectively, compared to reductions achieved in cultures incubated without added vegetable oil (5.7 log_{10} CFU/mL). Inclusion of taurine (8 mg/mL) or bile acids (0.6 mg/mL) had no effect on the antagonist-effect of vegetable oil on the bactericidal activity of thymol-β-D-glucopyranoside (not shown) and this antagonist effect was not overcome by further addition of the emulsifiers polyoxyethylene (40) stearate (0.2%), tween 20 or tween 80 (each at 1%) (Figures 2).

Discussion and Conclusion

Results from the present study are consistent with previous findings indicating that thymol-β-*D*-thymol glucopyranoside and free thymol exhibit potent bactericidal activity against *S.* Typhimurium and *E. coli* K88 when incubated with mixed populations of porcine gut bacteria. As hypothesized, the bactericidal activity of these compounds was decreased when the mixed populations were incubated with 3% added vegetable oil. Based on these results, it seems reasonable to suspect undigested lipid in the distal gut may be one of potentially several factors limiting the efficacy of free or conjugated thymol. Accordingly, additional research is warranted to learn how to overcome obstacles diminishing bactericidal activity of free and conjugated thymol in the lower gastrointestinal tract of food-producing animals.

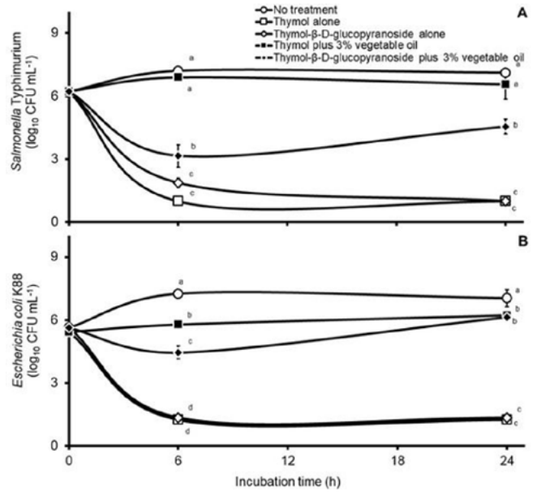

Figure 1: Concentrations of S. Typhimurium (A) or E. coli K88 (B) during incubation with mixed populations of porcine fecal bacteria treated without or with either thymol or thymol-β-D-glucopyranoside (each at 6 mM) in the absence or presence of 3% added vegetable oil. Values at each time point with unlike letters differ (P < 0.05)

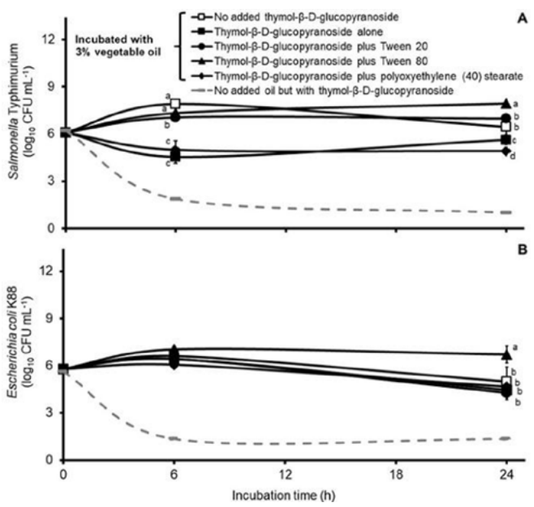

Figure 2: Concentrations of S. Typhimurium (A) and E. coli K88 (B) during culture with mixed populations of porcine fecal microbes with 3% vegetable oil, 8 mg taurine/mL and 6 mM thymol-β-D-thymol without or with added 1% tween® 20, 1% tween® 80 or 0.2% polyoxyethylene (40) stearate. Values at each time point with unlike letters differ (P < 0.05). Concentrations of populations cultured similarly except without added fat and emulsifiers are shown as shaded dashed-line for comparison

P4

Assessing the role of private haulage companies in the spread of swine infectious diseases in Great Britain (GB)

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Introduction

Understanding the complexity of any live animal trade network is critical for predicting the spread of infectious diseases in livestock industries, assessing the benefit for prevention and control measures, and designing cost-efficient surveillance programmes. However, attention has mainly focused on the direct movements of live animals between premises, whereas the role of haulage vehicles used to transport these animals, an indirect route for disease transmission, has largely been ignored. Here we aimed to both assess the impact of sharing haulage vehicles from livestock transport service providers on the connectivity between farms and the risk posed by such behaviour on the spread of swine infectious diseases in GB.

Methods

Using movement records from Scotland, England and Wales from April 2012 to March 2014, we built a series of directed and weighted networks consisting in two layers of identical nodes, linking nodes (farms) through (1) the direct movement of pigs and (2) the shared use of individual haulage vehicles. Haulage contact definition integrates the date of the move and the contamination period (the duration in which lorries are left contaminated by pathogens and act as fomites). In these networks, all contacts were aggregated over the period of either 7 days or 28 days, which were chosen to be similar to farm-level infectious period for key swine viruses, such as African and classical swine fevers and foot-and-mouth disease. We first performed descriptive network analyses to assess the role of haulage on network connectivity. The reproduction number *R* was then computed to explore how viruses may spread throughout the GB pig sector.

Results

Our results showed that sharing livestock haulage vehicles increases the number of indirect contacts between farms and may be a more important driver than the direct movement of animals, when considering disease transmission during an outbreak in the pig sector in GB. In particular, sharing haulage vehicles, even if lorries´ contamination period is < 1 day, will limit the benefit of the standstill regulation, increasing the number of premises that could potentially be infected in an outbreak and more easily rising *R* above 1.

Conclusions

This work confirms that sharing haulage vehicles has significant potential for spreading infectious diseases within the pig sector. The cleansing and disinfection process of haulage vehicles is a critical control point for risk mitigation in an outbreak.

ZOONOTIC PATHOGENS IN THE PORK CHAIN

P5

Porcine blood as sporadic source of foodborne hepatitis E virus for pork meat products: preliminary results

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Introduction

Hepatitis E virus (HEV) is recognised as a zoonotic pathogen transmitted via foodstuff. The aim of the present study was an assessment of the occurrence of HEV in porcine blood, liver and raw minced meat used for production of pork meat products.

Material and Methods

An incoming raw material (IRM) encompassing porcine blood (56 samples), liver (47 samples) and minced meat (56 samples) were analyzed for the presence of HEV and porcine adenovirus (pAdV) as an index virus of faecal contamination. IRM was collected from the local slaughterhouse and meat retailers. Virus extraction from pig liver and minced meat was performed using TRIzol (TRI Reagent®) followed by isolation of viral RNA using a NucliSens kit (BioMérieux) (Szabo et al., 2015). A QIAamp® Viral RNA Mini Kit (Qiagen) was used for processing of blood samples. A detection of HEV and pAdV was conducted using the virus specific duplex real-time (RT) PCR protocols with subsequent quantification of HEV genome copy numbers (Maunula et al., 2013). Molecular typing of detected HEV strain was carried out based on the virus ORF2 PCR amplicons (Huang et al., 2002). The correct operation of the detection methods was monitored using a sample process control virus added to each sample before the analysis (Rzeżutka et al., 2008).

Results

In total, 159 samples were tested for the presence of enteric viruses. HEV was solely detected in one sample of porcine blood which contained 1.4 x 10^4 HEV genome copy/ml. None of the tested samples of pork liver (0/47) and minced meat (0/56) was positive for HEV RNA. A sequence analysis of the virus ORF 2 genome fragment identified HEV 3e subtype. PAdV was present in six samples of pig's blood (6/56).

Discussion and Conclusion

Sporadic detection of HEV in porcine blood suggests that blood could be a virus source for pork meat products when used for their production. Likewise these results may also indicate at low prevalence of HEV infections in pigs raised in Poland. Additionally, the sporadic finding of pAdV in IRM confirms maintaining of good sanitary conditions during animal slaughter and subsequent processing of meat and blood.

References

Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE, Meng XJ (2002): Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. Journal of Clinical Microbiology, 40, 1326-1332

Maunula L, Kaupke A, Vasickova P, Söderberg K, Kozyra I, Lazic S, van der Poel WH, Bouwknegt M, Rutjes S, Willems KA, Moloney R, D´Agostino M, de Roda Husman AM, von Bonsdorff CH, Rzeżutka A, Pavlik I, Petrovic T, Cook N (2013): Tracing enteric viruses in the European berry fruit supply chain. International Journal of Food Microbiology, 15, 177-185

Rzeżutka A, Chrobocińska M, Kaupke A, Mizak B (2008): Application of an ultracentrifugation-based method for detection of feline calicivirus (a norovirus surrogate) in experimentally contaminated delicatessen meat samples. Food Analytical Methods, 1, 56-60

Szabo K, Trojnar E, Anheyer-Behmenburg H, Binder A, Schotte U, Ellerbroek L, Klein G, Johne R (2015): Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. International Journal of Food Microbiology, 215, 149-156

P6

Salmonella risk categorization of finnish fattening pig farms

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Introduction

Salmonella spp. prevalence in pigs is very low in Finland, Sweden and Norway compared to other European countries (EFSA and ECDC, 2018). The Finnish *Salmonella* Control Program for pigs includes bacteriological monitoring at slaughterhouses, and the prevalence of *Salmonella* culture-positive lymph node samples at slaughter has been < 0.1% and no *Salmonella* spp. have been found in carcass swabs or pork during the 2010s (Anon., 2017; https://www.ruokavirasto.fi/globalassets/teemat/zoonoosikeskus/zoonoosit/bakteerien-aiheuttamat-taudit/salmovalvontaohj_siat2016paivheinakuu2017.pdf, visited January 13, 2019). EFSA (2011) stated that incoming pig batches should be risk-ranked based on the herds' status of *Salmonella* spp. and suggested that this ranking could be based on historical serological testing of meat juice. This is in use in some European countries. We piloted serological *Salmonella* monitoring in Finnish context.

Material and Methods

Meat samples of ca. 10 g of muscle from the diaphragm were collected at slaughter from 1353 fattening pigs originating from 259 farms (mean 5 samples/farm). Blood samples at the end of the fattening period were collected from 1116 fattening pigs at 57 farms (mean 20 samples/farm). The *Salmonella* antibodies were analyzed using commercial ELISA tests: the SALMOTYPE Pig Screen test for meat juice (Labor Diagnostik GmbH, Leipzig, Germany) and the Pigtype® Salmonella Ab (Qiagen, Leipzig, Germany) for serum samples. A cut-off value OD20% was used. Farms were allocated into risk categories according to the within-farm seroprevalence using the Danish and German schemes (Alban et al., 2012; QS Qualität und Sicherheit GmbH, 2018) and our modified scheme (Table 3).

Results

Salmonella antibodies were detected in 3.1% of the meat juice samples and in 17.6% of the blood samples, using a cut-off value of OD20%. The OD values were low. Only 0.1% of meat juice samples and 1.9% of blood samples had OD values >40%.

All farms were in German category 1 (Table 1). Most (98%) farms were in Danish category 1 and only 2% of farms were in Danish category 2 (Table 2).

In our modified categorization, majority of the farms were allocated to the risk category 1 (within-farm seroprevalence < 20%), and only few (< 2%) farms had within-farm seroprevalences >40% (Table 3).

Table 1: Serological results from Finnish fattening pig farms allocated according to the German Salmonella control programme using a cut-off value OD40

Risk category	Meat juice samples (259 farms)	Serum samples (57 farms)	Corrective actions in German QS
Category 1, Low, within-farm seroprevalence ≤20%	100% of farms	100% of farms	None
Category 2, Medium, within-farm seroprevalence >20-40%	0% of farms	0% of farms	Check and document the hygiene status
Category 3, High, within-farm seroprevalence >40%	0% of farms	0% of farms	Bacteriological sampling, epidemiological investigation, corrective actions at farm

Table 2: Serological results from Finnish fattening pig farms allocated according to the Danish Salmonella control programme using cut-off value OD20%

Risk category	Meat juice samples (259 farms)	Serum samples (57 farms)	Corrective actions in Danish programme
Category 1, Low, within-farm seroprevalence <40%	98.1% of farms	98.2% of farms	None
Category 2, Medium, within-farm seroprevalence 40-65%	1.9% of farms	1.8% of farms	Penalty fee
Category 3, High, within-farm seroprevalence >65%b	0% of farms	0% of farms	Penalty fee, slaughtered separately

Table 3: Serological results from Finnish fattening pig farms allocated according to modified categories using a cut-off value of OD20%

Risk category	Meat juice samples (259 farms)	Serum samples (57 farms)
Category 1, Negligible, within-farm seroprevalence <20%	88.4% of farms	75.4% of farms
Category 2, Low, within-farm seroprevalence 20-40%	9.7% of farms	22.8% of farms
Category 3, Medium/High, within-farm seroprevalence >40%	1.9% of farms	1.8% of farms

Discussion and Conclusion

Within-farm *Salmonella* seroprevalences were generally low in Finnish fattening pig farms. This reflects the favorable *Salmonella* situation of pig farms in Finland and is consistent with results from the Finnish National *Salmonella* Control Program. However, differences between farms were found, so serological monitoring could be used to direct preventive measures at the farms at risk, and to target microbiological sampling.

When allocating farms to risk categories, the targets of the programme and corrective actions must be considered. The German and Danish serological sampling programmes are part of their reduction strategies, while Finland is applying an eradication policy. Consequently, the German and Danish categorizations are not directly applicable in the Finnish context. We piloted a modified allocation of farms (Table 3). In category 2, the farmer could be recommended to self-check the biosecurity measures using a specific checklist. If meat juice samples were used, approximately 10% of the farms would fall within this category in the current Finnish situation. Category 3 would indicate an elevated food safety risk, which could result in bacteriological sampling and a biosecurity check at the farm in question. Approximately 2% of farms would fall into this Category 3 in the current Finnish situation. The eradication decision cannot be based only on highly sensitive serological monitoring, because the cost of *Salmonella* eradication is very high on pig farms (Finnish Food Safety Authority Evira, 2018). In the Finnish context, subsequent procedures for eradicating the pathogen from a farm would follow whenever *Salmonella* spp. is isolated from animals at the farm. This modified categorization system is only an example, and it would need to be adjusted and optimized after additional data collection.

Serological *Salmonella* monitoring would provide us with large-scale farm-level data which would enable us to follow farm-level trends and detect changes readily and sensitively. However, in Finland this would have only a limited positive impact on food safety, because the current situation is already excellent. Therefore, a cost-benefit analysis should be conducted before applying the method in practice.

References

Anonymous (2017): Finland's report on trends and sources of zoonoses and zoonotic agents in foodstuffs, animals and feedingstuffs in 2016.

Alban, L., Baptista, F. M., Mogelmose, V., Sorensen, L. L., Christensen, H., Aabo, S. and Dahl, J. (2012): *Salmonella* surveillance and control for finisher pigs and pork in Denmark - A case study. Food Res. Int., 45, 656-665.

EFSA (2011): Scientific opinion on the public health hazards to be covered by inspection of meat (swine). The EFSA J., 9, 2351.

EFSA and ECDC (2018): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J., 16, e5500.

P7

Ecology of Salmonella and antimicrobial resistance in a pig slaughterhouse

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Introduction

Salmonella is responsible for a large number of food associated infections. To guarantee food safety, a better understanding of *Salmonella* ecology and adaptation strategies on the food production chain constitutes a prerequisite. In a *One Health* perspective, data on *Salmonella* antibiotic resistance in food environments are also crucial to decipher transmission routes of resistant foodborne pathogens as well as resistance genetic determinants involved, and the role of process and selection pressures underwent in food industries (as cleaning and disinfection) in bacterial adaptation and antimicrobial resistance emergence.

Methods

Occurrence of *Salmonella* was investigated at six different areas along a pig slaughter chain and through 4 sampling campaigns, each time before and after cleaning and disinfection (C&D) procedures. A total of 48 surface samples were collected. *Salmonella* strains were characterized using serotyping and pulsotyping to trace persistent strains in the slaughterhouse. Minimal inhibiting concentrations (MIC) were also determined for various relevant antibiotics and for biocides used in the slaughterhouse. In addition, associated indigenous bacterial communities were characterized using 16S rRNA amplicon sequencing.

Results

Salmonella was present at nearly all sampling areas but was not isolated from the neck clipper. Thirty eight strains were isolated and five serotypes were identified: S.4,5,12:i:- (50%), Rissen (16%), Typhimurium (16%), Infantis (10%) and Derby (8%). We observed a high prevalence of the monophasic variant of the serotype Typhimurium in the slaughterhouse. Sixteen PFGE types were identified among the 38 strains (Table 1). Some strains were found at different dates and potentially at the same sampling area suggesting that they persisted in the slaughterhouse despite of C&D procédures (data not shown).

Approximately 70% of isolated *Salmonella* strains exhibited resistance to ampicillin and sulfamethoxazole, 80% to tetracycline and 10% to chloramphenicol. There was statistically no significant evolution of CMI comparing strains before and after C&D procedures concerning both biocides and antibiotics (Figure 1).

Bacterial diversity analyses showed that populations in the slaughterhouse were highly dominated by γ-proteobacteria and especially by the Moraxellaceae family (genus *Psychrobacter*, *Moraxella*, *Enhydrobacter* and *Acinetobacter*) at the different sampling areas (data not shown).

Population compositions were overall stable in time at a given sampling area suggesting that the surface populations were resident populations within the slaughterhouse, rather than populations introduced each week by the new swine bands. C&D procedures tended to reduce bacterial diversity by eliminating the minority species but did not greatly impact the composition of dominant species.

Conclusions

Cleaning and disinfection procedures applied in this slaughterhouse did not appear to affect the biocides and antibiotics resistance of isolated *Salmonella* strains. Microbial flora diversity analyses showed that populations were resident with persistent *Salmonella* strains isolated at the same sites over time.

Together, such data participate to the construction of a comprehensive view of *Salmonella* ecology in food environments integrating associated resident microbial flora and the distribution of antimicrobial resistance in relation to processing conditions.

Table 1: Serotype and PFGE-types diversity among the 38 isolated Salmonella strains

Serotype (%)	4,5,12:i:- (58%)	Typhimurium (13%)	Rissen (10,5%)	Infantis (10,5%)	Derby (8%)
PFGE-type	B01, B02, B03, B04, B05, B06, B09, B15, B16	B09	B10, B11	B12	B08, B13, B14

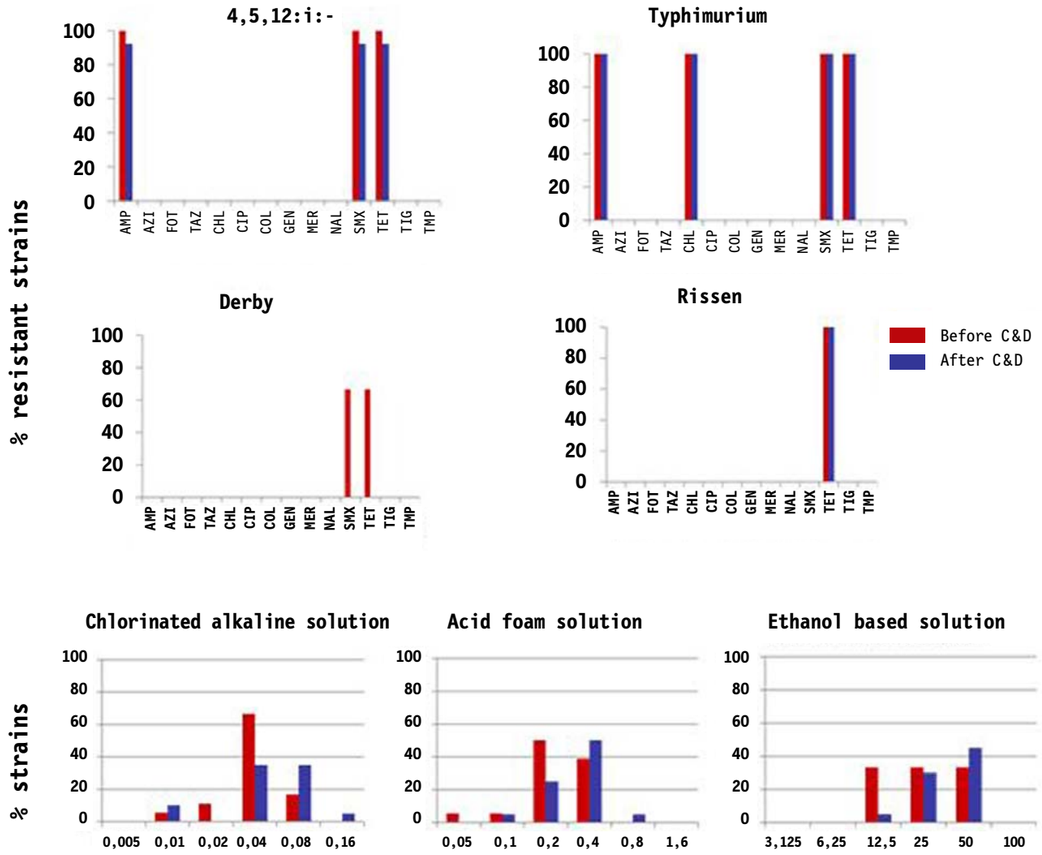

Figure 1: Salmonella resistance to biocides and antibiotics before and after C&D

P8

Zoonoses Monitoring programme results about Salmonella in pigs

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The Zoonoses Monitoring is a joint programme run by the Federal Government and the *Laender* (Germany's states) to raise and assess representative data on the occurrence of zoonotic agents and related antimicrobials resistance in food, feed, and live animals. Programme results are published annually by the Federal Office of Consumer Protection and Food Safety (BVL) in its Food Safety Reports. Zoonoses Monitoring is legally founded on the "General Administrative Provisions concerning zoonoses in the food chain" (*AVV Zoonosen Lebensmittelkette*), which in turn are based on Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents.

This contribution presents the major findings of tests for *Salmonella* spp. carried out along the pork food chain in the framework of the Zoonoses Monitoring programmes of the years 2009 to 2017. The test results show that fattening pigs frequently carry *Salmonella* spp. - about 8% to 9% of the faecal samples were *Salmonella*-positive - but detection rates continuously decline along the food chain. Pig carcasses were contaminated with *Salmonella* in about 3% to 4% of samples, while the contamination rate in fresh pork meat was 0.4% to 1.4%. Contamination rates in minced pork ranged between 0.7% and 5%. A trend analysis shows that *Salmonella* prevalence in pigs has remained roughly the same over the past few years, while it has declined in pig carcasses, fresh meat, and in particular in minced meat. This indicates that slaughter hygiene has improved, given the fact that the input by *Salmonella*-positive pigs has been the same. The Salmonella detection rate in pigs from farms categorised as category-I(one) (best serological *Salmonella* status) under the *Regulations to control the spread of Salmonella through slaughter pigs* ("Schweine-Salmonellen-Verordnung") was much lower than in pigs from category-III farms (worst serological *Salmonella* status) (5-7% in category-I-farm pigs versus 20 to 30% in category-III-farm pigs). So, the monitoring findings support the fact that the serological categorisation of fattening farms pursuant to the above *Salmonella* control regulations has a correlation with the bacteriological findings in pigs from these farms. At the same time they show that pigs from category-I farms, too, bring about a risk of contamination of the meat during the slaughter process. The findings in breeding sows and young pigs show that colonisation of the animals with *Salmonella* starts at the level of piglet farms (5.6% positive faecal samples in breeding sows, 10.3% positive faecal samples in young pigs) and highlight the importance of *Salmonella* control in breeding farms, to the end of preventing introduction of *Salmonella* in fattening farms through infected piglets. The monitoring programme results show that there are clear differences in the prevalence of *Salmonella* at the various levels of the pork food chain. Tests at the different stages of production allow tracing the paths of transmission of pathogens along the food chain. Continuous testing over years allow recognising trends and developments in the prevalence of pathogens in live animals and foodstuffs.

P9

VIVALDI - veterinary validation of point-of-care detection instrument

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In the VIVALDI project the consortium will validate new equipment (the VETPOD platform) for rapid on-site detection of zoonotic pathogens in industrial food and animal production chains.
The coordinator Technical University of Denmark (DTU) has developed the VETPOD platform based on Loop mediated isothermal amplification (LAMP) technology and optical read-out to a user interface, with disposable plastic cartridges (Lab-on-Chip, LOC) that can be adapted to an infinite number of assays for almost all pathogens.
We have a portable LOC system with optical detection: a system with polymeric chip made by injection moulded with multiple (8-32) chambers suitable for rapid online or on site detection of pathogens. The polymer chip with multiple chambers is able to perform LAMP to detect different pathogens at species level from multiple (8-30) samples within 30-60 min.
We want to validate the VETPOD platform for three important zoonotic pathogens: Avian Influenza Virus (AIV) or Highly Pathogenic Avian Influenza Virus (HPAIV), Salmonellaspp. and Campylobacter spp. For HPAIV this will include identification of H types (H5 and H7), for Salmonella the identification of the most important serovars S. Enteritidis, S. Typhimurium and S. Dublin, and for Campylobacter species identification of C. jejuni and C.coli.
The validation includes two stages:1) Validation by national reference laboratories in DK, SE, IT and FR. Each NRL will involve 10 external labs for ring trials.2) End-user validation at private labs (SMEs) in DE and IT. The equipment provider will prepare a business plan for sale of the VETPOD system. The private labs will prepare business plans for using the VETPOD platform for at site animal health detection as well as for online detection of zoonotic pathogens in food and animal production chains.
Project No: 773422
Duration: 36 months (January 2018 - January 2021)

P10

Dietary diformates and monolaurate - support for a healthy gut in sows during lactation - a short review

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Introduction
It is generally agreed that good gut health is effective against intestinal pathogens, a strategy that has only been made possible through the removal of antibiotic growth promoters in feed. Creating and maintaining a healthy intestinal environment has become essential to productivity and food safety programmes alike. Maintaining a healthy gut requires up to 25% of the daily protein and 20% of the dietary energy supplied with the feed. This strategy should be carefully planned into the dietary programme, in order to not waste resources (Hittel and Lückstädt, 2017).
The application of organic acids and their salts to diets for pigs has been studied extensively for more than 50 years. They have proved especially effective in maintaining growth performance since the ban on antibiotic growth promoters came into effect in Europe. Numerous trials have demonstrated their mode and magnitude of action and established effective doses for piglets, fattening pigs and sows. The use of formic acid and its double potassium salt in particular has been the subject of intense investigation, with the result that we now understand its dose-dependent effect on growth performance and feed conversion in pigs under a range of different environmental conditions and feed formulations (Lückstädt and Mellor, 2011). The main mode of action is its antimicrobial effect, which makes it comparable with antibiotic growth promoters; but organic acids also reduce pH in the stomach, which optimises conditions for pepsin activity; and increases the digestibility of nitrogen, phosphorus and a number of minerals. This is not only beneficial in sparing nutrients, but it also prevents losses that might otherwise contribute to environmental pollution. A similar impact in swine production was noted recently with sodium diformate (double salt of sodium formate and formic acid), which is produced similarly to potassium diformate with a patented production technology (Lückstädt and Petrovic, 2019).
However, while the antimicrobial impact of organic acids and their salts, including potassium or sodium diformate, is mainly directed against Gram-negative bacteria, medium chain fatty acids (C-6 to C-12) have also been shown to have an antibacterial impact against various Gram-positive bacteria (Preuss et al., 2005). This is especially true for lauric acid (C-12) and its monoglyceride ester, monolaurate. Lauric acid has the greatest antibacterial activity of all medium chain fatty acids. This effect is magnified if monolaurate is used (Batovska et al., 2009), making it a promising candidate as an additive or as an alternative to antibiotics for treatment of different diseases (Rouse et al., 2005).
Despite the well documented impacts of both additives, data on the combined impact of these additives on gut health in sows under commercial conditions are scarce. The current study reviews the impact of a combination of dietary diformates and monolaurate on its decontamination impact on Gram-negative and Gram-positive bacteria in sow faeces.

Material and Methods
Multiparous sows on commercial farms in Germany were fed either a commercial lactation diet as control - or a test diet, which contained additionally 1% of a diformate-monolaurate mixture (traded as Formi GML, ADDCON). On the 21st day of lactation, freshly excreted faecal matter was collected from all sows and analysed for *E. coli*, Enterococci, Streptococci and the total aerobic bacteria count. Data were analysed using the t-test and a significance level of 0.05 was used in all tests.

Results
Results of the microbial analysis revealed a strong significant impact of the product on the bacterial population in the faecal matter of sows. This holds true for *E.coli* and Streptococci / Enterococci counts, as well as the total aerobic bacteria count (Table 1).
Table 1: Bacterial count reduction rates (%) in sow faeces after feeding with 1.0% diformate-monolaurate (Formi GML) in the lactation diet
The significant reduction rates in the *E. coli* counts in the faeces were well above 90% and varied in the trials between 90% and 98%. Furthermore, the reduction in the Streptococci/Enterococci counts within the various trial periods were significant and varied from 75% to 99%. Finally, the count of total aerobic bacteria, among them the group of spoilage indicating bacteria, tended to be reduced (-94%).

Discussion and Conclusion
The addition of this combination of sodium diformate and monolaurate caused a significant improvement of the health status of sows. The impact against the Gram-positive Streptococci is especially noteworthy. This is particularly important since the EU-funded Focus Group is calling for actions to reduce the use

Table 1:

Trial	Bacteria	Reduction rate (%)	P-level
I	*E.coli*	-90.3	0.06
	Total Streptococci + Enterococci	-97.2	<0.01
II	*E.coli*	-98.3	<0.01
	Enterococci	-98.9	<0.05
	Streptococci	-75.1	<0.05
III	Total aerobic bacteria	-94.1	0.09

of antibiotic treatments on swine farms (eip-agri, 2014). In these three separate trials (Hittel and Lückstädt, 2017; Lückstädt and Hutter, 2018), the combined inclusion of diformate and monolaurate may therefore not only provide a healthy gut in sows, but might furthermore support a pork production chain with reduced zoonotic pathogen pressure. This will additionally help the EU-antibiotic reduction initiatives.

References

Batovska DI, Todorova IT, Tsvetkova IV and Najdenski HM (2009): Antibacterial study of the medium chain fatty acids and their 1-monoglycerides: Individual effects and synergistic relationships. Polish Journal of Microbiology 58 (1), 43-47.

EIP-Agri (2014): Focus Group - Reducing antibiotic use in pig farming - Final report, p.40 (accessed online: http://ec.europa.eu/eip/agriculture/sites/agri-eip/files/eip-agri_fg3_pig_antibiotics_final_report_2014_en_0.pdf)

Hittel J and Lückstädt C (2017): Dietary potassium diformate and medium chain fatty acids - support for a healthy gut in sows during late gestation. Tagungsband 16. BOKU-Symposium Tierernährung 2017, 91-94.

Lückstädt C and Hutter C (2018): Dietary sodium diformate and monolaurate affect faecal pathogen load in lactating sows. Book of Abstract, 14th International Symposium Digestive Physiology of Pigs.

Lückstädt C and Mellor S (2011): The use of organic acids in animal nutrition, with special focus on dietary potassium diformate under European and Austral-Asian conditions. Recent Advances in Animal Nutrition - Australia, Vol 18, 123-130.

Lückstädt C and Petrovic S (2019): Dietary sodium diformate supplementation in sows during late gestation and lactation can improve performance of suckling piglets. Proceedings of BSAS - Advances in Animal Biosciences.

Preuss HG, Echard B, Enig M, Brook I and Elliott TB (2005): Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Molecular and cellular biochemistry 272 (1-2), 29-34.

Rouse M, Rotger M, Piper K, Steckelberg J, Scholz M, Andrews J and Patel R (2005): *In vitro* and *in vivo* evaluations of the activities of lauric acid monoester formulations against *Staphylococcus aureus*. Antimicrobial Agents Chemother. 49, 3187-3191.

P11

Development of a quantitative PCR method coupled with PMA to quantify viable Salmonella spp. cells in the pork supply chain

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In 2017, Salmonella spp. was implied in 30% of foodborne diseases in France (SPF, 2019). Few data on the contamination levels of *Salmonella* spp. are available along the pork supply chain. The protocol of the standard method (ISO/TS 6579-2:2012) is time-consuming and culture-based methods using chromogenic media are less efficient for matrices with high levels of background flora, and for recovering stressed cells. Along the food chain, the cells may be impacted by various stresses (e.g. chemical or thermal), which may lead to physiological changes and the emergence of viable but non-culturable cells (VBNCs).

This study aims to develop a protocol for the quantification of viable *Salmonella spp.* cells from pork carcasses and faeces based on a quantitative TaqMan® PCR (qPCR) method combined with propidium monoazide (PMA) treatment to exclude DNA from dead cells. Performances of the PMA-qPCR method were assayed using different ratios of viable (including heat-stressed cells) and non-viable (heat-inactivated) *Salmonella* cells from pure culture in nutrient broth, and artificially contaminated samples of faeces and pork back fat. Different PMA concentrations and light exposure conditions were tested. For each sample analysis, the concentrations of total, viable and cultivable fractions of *Salmonella* cells were determined by using qPCR, PMA-qPCR and culture-dependent approaches (the standard miniaturized MPN for faeces or chromogenic *Salmonella* plating media for pork back fat), respectively.

The PMA-qPCR reaction developed in the present study exhibited a 100% inclusivity and exclusivity for *Salmonella spp*. For both matrices, the PMA-qPCR allows a quantification of VBNC cells of *Salmonella spp.* even in the presence of dead cells. The limits of quantification of the PMA-PCRq set from artificially contaminated pork back fat and faeces were $1{,}6.10^2$ genome copies/cm² and 5.10^2 genome copies/g, respectively. The PMA-qPCR method was effective to determine the impact of thermal stresses on the behaviour of *Salmonella spp.* cells in artificially contaminated samples of faeces (4°C) and pork back fat (60°C and 100°C). This method will be useful to identify farming practices related to high/low level of *Salmonella* contamination in pigs. Quantitative data on carcasses and pork cuts are also of interest to qualify slaughtering procedures and their impact on the contamination of meat with *Salmonella spp.*. A development of the method on pork cuts is planned.

P12

Optimization of the detection of Clostridium botulinum in pig and cattle manures and in digestates from on-farm biogas plants

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Introduction

Anaerobic digestion (AD) is a sustainable technology for converting livestock manure into biogas. A raise in the number of agricultural biogas plants (BGP) has been observed recently in several European countries. The fate of pathogens, in particular *Clostridium botulinum* (Cb) during AD and the sanitary risks through spreading on land appears to be a matter of public health concern. Asymptomatic carriage of Cb has in fact been demonstrated in fecal contents of cattle and pigs [1, 2]. Clostridia being spore-forming anaerobic bacteria, their ability to form spores confers them a high resistance to environmental conditions, while their ability to grow under mesophilic anaerobic conditions raises the question of their future and the potential for their multiplication during AD. Proliferation and environmental contamination through digestate spreading on lands when using manures that may contain Cb for AD have been hypothesized [3, 4]. A first study conducted to address this topic using laboratory-scale digester invalidated this hypothesis [5]. Further studies including field investigations are now required.

Manure and digestate are complex matrices with rich microflora. The detection and enumeration of pathogens in such matrices can be challenging, especially in the absence of selective media and when the level of the pathogen is low or close to the limit of detection of the method. No prescriptive or consensual method is available for the detection of Cb in such matrices. It is thus necessary to adapt protocols developed for food or clinical samples to maximize the detection of target pathogens.

The objective was to optimize the detection of Cb in manure and digestate samples using naturally contaminated samples collected in agricultural BGP.

Material and Methods

Samples

Manure and digestate were collected from five biogas plants (BGP1 to BGP5) located in France. The livestock effluents to be treated through AD were either pig manure (BGP1, 3 and 4), cattle manure (BGP2) or both (BGP5). Each BGP was visited once. The manure and digestate of each BGP were collected in three replicates, transported at room temperature for less than one hour, and analyzed on the same day.

Detection of *C. botulinum*

For detection, two methods (M1 and M2) and six protocols (P1 to P6) were used (Fig. 1) regardless of the form (vegetative or spore cells). For the first method (M1), 25 g of each sample were 10-fold diluted in pre-reduced Trypticase Peptone Glucose Yeast broth (TPGY) and homogenized using a Pulsifier (Microgen, Surrey, UK) for 15 seconds. For the second method (M2), 10 g of each sample were 10-fold diluted in pre-reduced TPGY, incubated for 10 minutes at 70°C in a water bath, and cooled for one minute in cold water.

The samples were then incubated at 37°C in an anaerobic chamber (A35, Don Whitley) filled with anaerobic gas (10% H_2, 10% CO_2, 80% N_2). After 24 hours (P1 and P4), four days (P2 and P5) and 10 days (P3 and P6) of incubation, 1 ml was collected for DNA extraction.

DNA extraction was performed using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel) according to the manufacturer's instructions.

Detection of the encoding genes for BoNT types A, B, E and F and a group III target was performed using real-time PCR with a Bio-Rad CFX96 thermal cycler as previously described [6, 7]. A sample was considered positive when a characteristic amplification was detected.

Results

Six protocols were compared for the detection of Cb (Fig. 1), with or without thermal treatment at 70°C, and with different incubation periods (24 hours, 4 days and 10 days). The highest detection level (16 positive samples out of 30) was obtained using the P1 protocol (Fig. 1) by analyzing 25 g samples 10-fold diluted in TPGY without thermal treatment, with 24 hours of incubation at 37°C in an anaerobic chamber. Cb was detected in all BGP except in BP1. The most common gene (present in 100% of the positive samples) was that encoding BoNT type B.

Discussion and Conclusion

Several protocols were tested here on naturally contaminated manure and digestate samples to select the most suitable one to be able to detect Cb. A short incubation period was selected for the detection of Cb in manure and digestate on the contrary to some previous studies studying environmental samples [8, 9]. Optimal incubation period of only 18 hours for the detection of Cb in pig fecal samples was already observed [1]. This protocol is now available to evaluate the fate of Cb during AD.

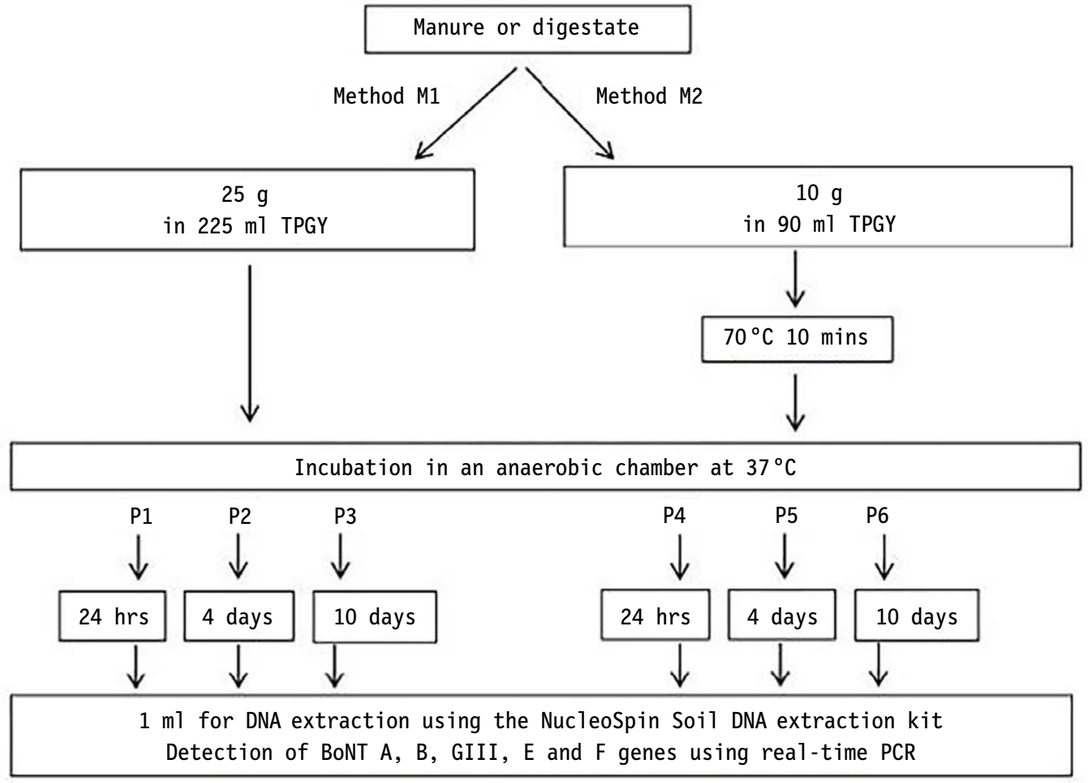

Figure 1: Sample analysis workflow for the detection of C. botulinum for manure and digestate

References

1. Dahlenborg M, et al.: Development of a Combined Selection and Enrichment PCR Procedure for *Clostridium botulinum* Types B, E, and F and Its Use to Determine Prevalence in Fecal Samples from Slaughtered Pigs. Applied and Environmental Microbiology 2001, 67(10):4781-4788.

2. Dahlenborg M, et al.: Prevalence of *Clostridium botulinum* types B, E and F in faecal samples from Swedish cattle. International Journal of Food Microbiology 2003, 82(2):105-110.

3. Botulinumtoxikosen - Infektionsrisiken für Mensch und Tier (unter besonderer Berücksichtigung von Schwein bzw. Rind). Nutztierpraxis aktuell 33, 14-18.http://www.botulismus.org/index_htm_files/botulinumtoxikosen%2003032010.pdf (accessed 19 April 2019).

4. Boll C: Chronischer Botulismus - Tod aus der Biogasanlage. Wild und Hund 2011(10):14-19.

5. Froschle B, et al.: Fate of *Clostridium botulinum* and incidence of pathogenic clostridia in biogas processes. J Appl Microbiol 2015, 119(4):936-947.

6. Fach P, et al.: Development of real-time PCR tests for detecting botulinum neurotoxins A, B, E, F producing *Clostridium botulinum*, *Clostridium baratii* and *Clostridium butyricum*. J Appl Microbiol 2009, 107(2):465-473.

7. Woudstra C, et al.: Molecular gene profiling of *Clostridium botulinum* group III and their detection in naturally contaminated samples originating from various European countries. Appl Environ Microbiol 2015.

8. Neuhaus J, et al.: Detection of *Clostridium botulinum* in liquid manure and biogas plant wastes. Folia microbiologica 2015.

9. Vidal D, et al.: Real-time polymerase chain reaction for the detection of toxigenic *Clostridium botulinum* type C1 in waterbird and sediment samples: Comparison with other PCR techniques. Journal of Veterinary Diagnostic Investigation 2011, 23(5):942-946.

P13

Occurrence of Brachyspira hyodysenteriae and Brachyspira pilosicoli in Polish pig herds

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Introduction

Pathogenic intestinal spirochetes of pigs include *Brachyspira hyodysenteriae*, the cause of swine dysentery, and *Brachyspira pilosicoli*, the cause of porcine colonic spirochetosis. Most *Brachyspira* species have a restricted host range, whereas *B. pilosicoli* colonizes a wide range of hosts including humans and has natural potential to be transmitted between species (Hampson and Burrough 2019). There is potential for zoonotic transmission, especially in places where animals and humans live in close proximity, or for people working with intensively farmed pigs or chickens due to increased risk of exposure. Some species of the genus *Brachyspira* including *B. pilosicoli* can cause disease in human. There are few reports about *B. pilosicoli*-associated human intestinal spirochetosis (HIS). Most of these studies have involved observation of colorectal biopsy specimens that show spirochetes attached to the epithelial surface, to form a “false brush border” (Hampson 2018).

Subclinical colonization of pigs with *B. pilosicoli* occurs commonly on some farms (Biksi et al. 2007) On other farms, the spirochete may be isolated from diseased pigs alone or as part of a mixed infection with other enteric pathogens (Stege et al. 2000; Reiner et al. 2011). Recent changes in the management of pig farms and movement of pigs within the EU have resulted in shift in the relative prevalence of pathogenic *Brachyspira* species. Very few studies report the prevalence of *B. hyodysenteriae* in pig in Poland but only one concerning *B. pilosicoli.* The aim of the study was to preliminary assess current occurrence of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in Polish pig herds.

Material and Methods

Between 2017 and 2019, a total of 247 samples of pig feces were submitted to The National Veterinary Research Institute (NVRI). These samples were obtained form 60 different Polish pig herds form pigs older than 7 weeks. All these samples were submitted to NVRI to be evaluated for swine dysentery and/or porcine proliferative enteritis. Some of them were obtained from pigs subjected to routine monitoring and other came from pigs with clinical sings of diarrhea. Total genomic DNA was extracted from the fecal samples using commercial isolation kit (Genomic Mini, A&A Biotechnology, Gdynia, Poland), according to the manufacturer’s recommendations.

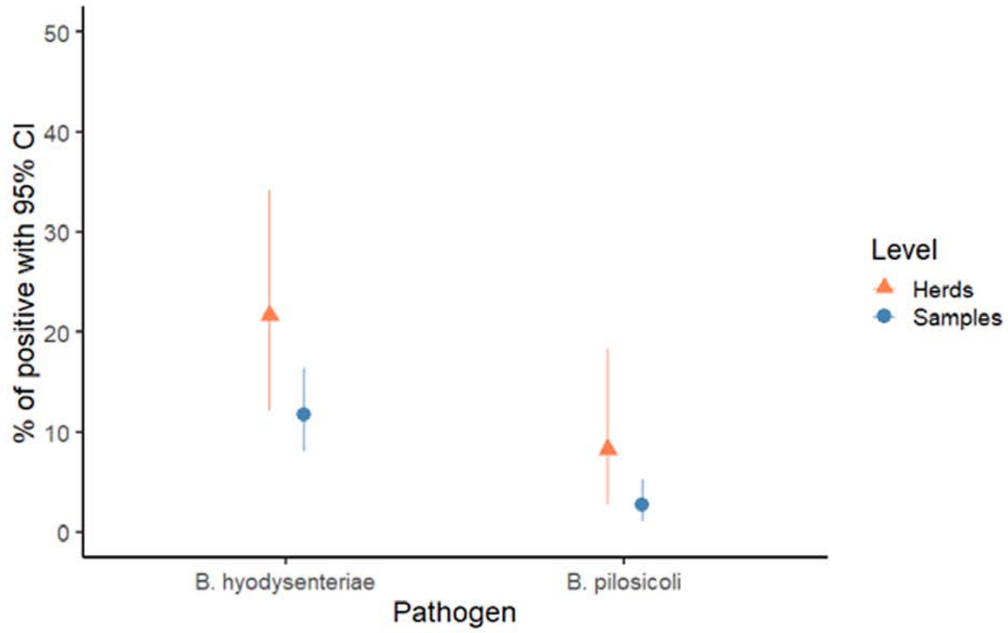

Figure 1: Occurrence of B. hyodysenteriae and B. pilosicoli in 247 samples from 60 Polish pig herds

Extracted DNA samples were stored at -20˚C until examination. All samples were tested by separated real time PCR assays for *B. hyodysenteriae* and *B. pilosicoli* according to the methods described previously (Zmudzki et al. 2012; Ståhl et al. 2011). A herd was defined as positive when at least one fecal sample taken from the herd had a positive PCR result. Percentages of positive samples/herds with a 95% two-sides exact binominal confidence interval (CI) were reported.

Results

Overall occurrence of *B. hyodysenteriae* and *B. pilosicoli* in pig herds in Poland is presented at Figure 1. Among total amount of 247 samples 138 were submitted to laboratory of NVRI for routine monitoring of pig herds. The remaining 109 samples originated from pigs with clinical problems such as diarrhea or enterocolitis. The real time PCR detected *B. pilosicoli* DNA in seven samples from pigs in 5 different herds. Which means that 2,8% (95% CI, 1,1% - 5,3%) of samples and 8,3% (95% CI, 2,8% - 18,4%) of herds were positive for *B. pilosicoli.* In terms of *B. hyodysenteriae* 11,7% of samples (95% CI, 8,0% - 16,4%) from 21,7% herds (95% CI, 12,1% - 34,2%) were positive in real time PCR. Samples in which *B. hyodysenteriae* were detected originated form pigs with clinical problems, all samples from routine monitoring programs were negative for this pathogen. In case of *B. pilosicoli* all positive samples were collected from apparently heathy pigs.

Discussion and Conclusion

The results of the study confirm that *B. pilosicoli* infections occur in Polish pig herds. Previous study reported only one positive sample among 127 tested from 23 pig farms was not fully reliable, especially if we taking into account lack of clinical signs (Pławińska et al. 2004). Our results show that *B. pilosicoli* is present in Polish pig herds but it seems that prevalence is rather low - 8,3% of positive herds. But it is interesting taking into account significantly higher prevalence of *B. pilosicoli* in other countries such as Germany - 31.6% (Reiner et al. 2011) or Denmark - 19% (Stege et al. 2000). Therefore, active sampling from Polish pig herds is necessary to assess true prevalence of *B. pilosicoli*. There is also a need for further investigation of the association between presence of *B. pilosicoli* in feces and the clinical signs or pig performance. The risk associated with zoonotic potential of this pathogen is difficult to assess, but it seems to be low based on obtained results - 2,8% of positive samples.

Another finding highlight that swine dysentery is still common cause of diarrhea among pigs from Polish herds despite of improving biosecurity, hygiene and management.

Acknowledgment

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References

Biksi I, Lőrincz M, Molnár B, Kecskés T, Takács N, Mirt D, Cizek A, Pejsak Z, Martineau G, Sevin J, Szenci O (2007): Prevalence of selected enteropathogenic bacteria in Hungarian finishing pigs. Acta Veterinaria Hungarica, 55, 219-227.

Hampson DJ (2018) The spirochete Brachyspira pilosicoli, enteric pathogen of animals and humans. Clinical Microbiology Reviews, 31, pp.e00087-17

Hampson DJ, Burrough ER (2019): Swine dysentery and Brachyspiral colitis. Diseases of Swine, 11th ed., John Wiley & Sons, 951-970.

Pławińska J, Jakubowski T., Rzewuska M, Binek M (2004): Occurrence of Lawsonia Intracellularis and Brachyspira spp. infection in swine suffering from diarrhoea. Polish Journal of Veterinary Sciences, 7, 203-206.

Reiner G, Hillen S, Kixmöller M, Willems H (2011): Analysis of bacterial load and prevalence of mixed infections with Lawsonia intracellularis, Brachyspira hyodysenteriae and/or Brachyspira pilosicoli in German pigs with diarrhoea. Berliner und Munchener tierarztliche Wochenschrift, 124, 236-241.

Ståhl M, Kokotovic B, Hjulsager CK, Breum SØ, Angen Ø (2011): The use of quantitative PCR for identification and quantification of Brachyspira pilosicoli, Lawsonia intracellularis and Escherichia coli fimbrial types F4 and F18 in pig feces. Veterinary Microbiology, 151, 307-314.

Stege H, Jensen TK. Møller K, Baekbo P, Jorsal SE (2000) Prevalence of intestinal pathogens in Danish finishing pig herds. Preventive Veterinary Medicine, 46, 279-292.

Zmudzki J, Szczotka A, Podgórska K, Nowak A, Grzesiak A, Dors A, Pejsak Z (2012): Application of real-time PCR for detection of Lawsonia intracellularis and Brachyspira hyodysenteriae in fecal samples from pigs. Polish Journal of Veterinary Sciences, 15,267-273.

P14

MRSA in carcass abscesses of slaughtered piglets

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Roasted piglets (about 2 months old and 6 to 8 kg live weight) constitute an important gastronomic dish in Portugal, being the production and slaughter of these animals of increasing economic importance. In 2015, 1 148 025 piglets were slaughtered in Portugal. From those, 956 carcasses (0.08%) were totally condemned due to the presence of multiple abscesses, representing the third main cause of piglets´ carcass condemnation at post mortem inspection. One of the pathogens enrolled in the etiology of abscesses it is Staphylococcus aureus and, within this specie, MRSA may represent and additional threat, if present. The main objective of this study was to evaluate the involvement of MRSA in abscesses in piglet carcasses at slaughter. During 12 weeks in the spring 2016, 48 samples of abscesses purulent content were aseptically collected from piglets carcasses condemned at post mortem inspection. Briefly, at laboratory, samples were inoculated in Brain Heart Infusion Broth and after plated in Manitol Salt Agar (OXOID™) agar. Suspicious colonies were identified by Gram staining and catalase test. Those positive to both testes, were plated in ORSAB agar (Oxacillin Resistance Screening Agar Base, OXOID™) and suspicious isolates of MRSA were confirmed by using a multiplex PCR assay targeting the 16S rDNA, nuc and mecA. In this study, MRSA was identified in 23 samples (23/48, 48%), being the first report of MRSA identified in carcass abscesses of piglets in Portugal. Since all analysed samples were from carcasses declared unfit for human consumption, the presence of MRSA can´t be considered a direct food safety issue. Nevertheless, although it is known that asymptomatic slaughtered pigs may be a source of MRSA into the abattoir, the high prevalence (48%) found in carcass abscesses must be take into consideration by FBO as an important and additional source of contamination, requiring provision of adequate decontamination measures to avoid cross contamination. Also, personnel must be aware of the potential risk of exposure during manipulation of these carcasses. More studies should be undertaken, at primary production level, to understand the reason and level of this problem, under One Health perspective.

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P15

Granulomatous lymphadenitis in swine: validation of national data based on identification by the service of federal inspection (SFI)

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Introduction

The granulomatous lymphadenitis (GL) in pigs is mainly caused by *Mycobacterium avium hominissuis* (*MAH*), who belongs to the *Mycobacterium avium* Complex (MAC), considered non-tuberculous mycobacteria (NTM). Although GL does not affect swine zootecnical performance, economic losses occur during the slaughter line by condemning viscera and carcasses. The lesion is characterized by one or more foci of granuloma, which most frequently affect the organs of the digestive tract and peripheral lymph nodes. The main differential diagnosis encompasses *Mycobacterium bovis* (*M. bovis)* and *Mycobacterium tuberculosis* (*M. tuberculosis*), who belongs to the *Mycobacterium tuberculosis* Complex (MTbC), of relevant zoonotic potential. Nevertheless macroscopic examinations and histopathology are insufficient to determine the etiologic agent involved. Federal Meat Inspection had registered the frequency of 0,81% of lymphadenitis in Brazil from 2012 to 2014. The aim of this study was to investigate the etiology of the granulomatous lesions for validation of the national data of inspection and build a database to further risk analysis.

Material and Methods

In 2017 the federal veterinary inspectors collected mesenteric lymph nodes with granulomatous lesions of 399 swine production farms located in eight states (Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais, Mato Grosso, Mato Grosso do Sul e Goiás), representing 158 brazilian municipalities. If available, mesenteric lymph nodes with GL of three swine on each farm were sampled, totaling 257 lymph nodes from finisher pigs and 142 from sows/boars. The tissues were submitted to histological examination and bacterial analysis. Mycobacterial isolation and identification were performed according to OIE (2018). Briefly, the isolates from Lowenstein Jensen and/or Stonebrink media, positive to acid-fast bacteria in Ziehl-Neelsen (ZN), were typified by Polymerase Chain Reaction (PCR) protocols (Table 1) for genus and species, supporting the distinction between *MAH*, M*ycobacterium avium avium/silvaticum*, *M. bovis* and *M. tuberculosis.*

Results

Comparing histological findings with macroscopic examination, the Service of Federal Inspection (SFI) correctly identified 85% of granulomatous lesions in individual basis. The rate isolation of mycobacteria was 32,08% (128/399), of which 76,56% (98/128) were positive for *MAH*, 1,56% (02/128) for *M. bovis*, and 21,87% (28/128) only for *Mycobacterium* spp. The identification of *Mycobacterium* species by state is shown in table 2.

Discussion and Conclusion

Overall, the results had shown a good assurance between the evaluation performed in the slaughter line by the veterinarians inspectors and the histopathologic exam. The positive predictive value is higher than 80% when we compare the macroscopic examination

Table 1: Description of the primers used in the Polymerase Chain Reaction (PCR)

Primers	Sequence	Lenght of PCR product	Reference
DNAJ	5´- GGG TGA CGC GAC ATG GCC CA -3´ 3´- CGG GTT TCG TCG TAC TCC TT -5´	236bp	(TAKEWAKI et al., 1993)
IS1245	5´- GCC GCC GAA ACG ATC TAC- 3´ 3´- AGG TGG CGT CGA GGA AGAC -5´	427bp	(GUERRERO et al., 1995)
IS901	M IS901F 5‘- GGATTGCTAACCACGTGGTG -3‘ M IS901R 3‘- GCGAGTAGCTTGATGAGCG -5‘	577bp	(MORAVKOVA et al., 2008)
INS	INS1 5´- CGTGAGGGCATCGAGGTGGC - 3´ INS2 3´- GCG TAGGCGTCGGTGACAAA -5´	245bp	(VAN EMBDEN et al., 1993)
RD4	5‘ AACGCGACGACCTCATATTC 3‘ 3‘ AAGGCGAACAGATTCAGCAT 5‘	400bp	(SALES et al., 2014)

Table 2: Identification of Mycobacterium species by swine production farms as a function of the state of origin

State of origin	MAH	Mycobacterium spp.	M. bovis	Negative	Number of samples
Goiás	01(10.00)	0 (0.00)	0 (0.00)	09 (90.00)	10
Minas Gerais	13 (18.06)	11 (15.28)	0 (0.00)	48 (66.67)	72
Mato Grosso do Sul	0 (0.00)	0 (0.00)	0 (0.00)	03 (100.00)	03
Mato Grosso	0 (0.00)	0 (0.00)	0 (0.00)	4 (100.00)	04
Paraná	17 (31.48)	06 (11.11)	0 (0.00)	31 (57.41)	54
Rio Grande do Sul	15 (28.30)	06 (11.32)	02 (3.77)	30 (56.60)	53
Santa Catarina	42 (24.85)	05 (2.96)	0 (0.00)	122 (72.19)	169
São Paulo	09 (27.27)	0 (0.00)	0 (0.00)	24 (72.73)	33

and the lymph nodes that showed histopathological lesions of granulomatous lymphadenitis. The present study shows a high prevalence of *MAH* causing GL in Brazilian farms, confirming this subspecies as the most prevalent in the swine population as it has been described in other countries. The hypothesis of fecal-oral transmission between animals may justify the permanence and prevalence of *MAH* subspecies in pig farms. The two samples that were positive for *M. bovis* were collected at the same slaughterhouse, but they were from different farms, located in different towns. Both of farms raise pigs and dairy cattle. Anyway the source of the infection was not defined. Nevertheless, due to the disease prevalence in pigs and differences in zoonotic potential between the etiological agents, lesions of porcine granulomatous lymphadenitis should be considered in the definitions of the exams performed by the SFI.

References

Agdestein A. *et al.* (2014) Novel insights into transmission routes of *Mycobacterium avium* in pigs and possible implications for human health. Veterinary Research, vol. 45.

Coldebella A. *et al.* (2017) Reports of Brazilian Federal Meat Inspection System in swine slaughterhouses. In: 12th International Symposium on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork - Proceedings Book, Foz do Iguaçu, august 21-24, 2017, p.251-254.

Guerrero C. *et al.* (1995) A novel insertion element from *Mycobacterium avium*, IS1245, is a specific target for analysis of strain relatedness. Journal of Clinical Microbiology, vol. 33, p. 304-7.

Moravkova M. *et al.* (2008) Strategy for the detection and differentiation of *Mycobacterium avium* species in isolates and havily infected tissues. Research in Veterinary Science, vol. 85, p. 257-264.

Sales M.L. *et al.* (2014) Evaluation of molecular markers for the diagnosis of *Mycobacterium bovis*. Folia Microbiologica, vol. 59, p. 433-438.

Takewaki S. *et al.* (1993) Genus-specific polymerase chain reaction for the mycobacterial dnaJ gene and species-specific oligonucleotide probes. Journal of Clinical Microbiology, vol. 31, p.446-50.

Van Embden J.D. *et al.* (1993) Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. Journal of clinical microbiology, vol. 31, p. 406-9.

World Organization For Animal Health (OIE). (2018). - Terrestrial Animal Health Code, Online version, OIE, Paris. Available in: < http://www.oie.int/standard-setting/terrestrial-manual/access-online/>.

P16

Pathologic diagnosis of zoonotic parasitosis in slaughter pigs in Brazil

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Introduction

Brazil is the fourth largest swine producer and pork exporter in the world, the slaughter under the federal meat inspection service achieved 37 million pigs in 2017 (https://sidra.ibge.gov.br). Brazilian meat inspection system is under a modernization process and new procedures are just standardized and regulated for pigs reared in farms submitted to health animal service rules. In order to supply the risk analysis for meat inspection modernization, several studies on zoonotic hazards were conducted in Brazil last years. This one was focused on zoonotic parasitosis once that in sanitary *post mortem* examination, the inspectors can identify lesions compatible with cysticercosis (Satyaprakash et al., 2018), hydatidosis (De La Rue, 2008) and sarcosporidiosis (Zainalabidin, et al., 2017).

Cysticercosis is caused by metacestodes of *Taenia solium*. Primarily, cysticercosis is an infection of pigs that act as an intermediate host of *T. solium*. Pigs are infected by ingestion of contaminated water, soil or feed with the eggs of *Taenia solium* expelled from tapeworm carriers. The eggs develop into cysticerci in various organs and musculature causing porcine cysticercosis characterized by small round whitish viscous cyst (7 to 15 mm), located mainly in the lingual muscles, masseters, heart and diaphragm (Satyaprakash et al. 2018).

Cystic echinococcosis is a zoonotic disease caused by the genus *Echinococcus* (*Cestoda: Taeniidae*). Pigs are considered important intermediate hosts of the larval stage by eggs ingestion from contaminated environment with feces of definitive host. The intermediated host develops hydatid cysts in the liver and the parasite cycle can be complete if a definitive host ingest this organ without a heat treatment (De La Rue, 2008).

Sarcosporidiosis is a disease caused by cyst forming coccidian, namely, *Sarcocystis* spp. Pigs can be infected when consuming food contaminated with fecal material of carnivores containing the sporocysts of *Sarcocystis* spp. The whitish filamentous, spindle-shaped, rice-grain-like, macrocyst-forming sarcocyst has been observed in the muscles of pigs, mainly in the heart, tongue and diaphragm (Zainalabidin, et al., 2017). The aim of this study was to validate the macroscopic diagnosis of these lesions detected by veterinary inspection service using histopathology analysis.

Material and Methods

From May 2017 to May 2018 was performed a prospective study with the collaboration of federal meat inspectors, which were asked to collect all lesions suspected of cysticercosis, hydatidosis and sarcosporidiosis found during routine of meat inspection. These samples were sent to animal pathology laboratory of Embrapa Swine and Poultry Research Center. It was analyzed a total of 361 samples, 296 were muscle samples suspected of sarcosporidiosis, 64 cystic livers suspected of hydatidosis and 1 heart sample suspected of cysticercosis. The tissue samples were collected in 10% buffered formalin and sent to the laboratory for processing by the routine histopathology technique.

Results

In 34 (53.1%) liver samples, *Cysticercus tenuicollis*, the larval form of *Taenia hydatigena*, was identified. The macroscopic characteristics of these lesions were single or multiple cysts, colorless fluid, thin membrane and a cephalic invagination corresponding to the scolex (Figure 1A). In the histopathology analysis it was observed that the cysts have a membrane that invaginates in only one scolex (Figure 1B), which has suckers and many hooks. In the other 30 liver samples, there were no parasites inside the cysts and it was not possible to identify the cause of the lesions. *Echinococcus spp.* was not identified. No sarcosporidiosis suspect lesion was found in the finishing pigs. All muscle samples analyzed were from culling sows. In 163 (55%) of these samples, granulomatous myositis (Figure 1C) compatible with *Sarcosporidium* spp. infection was observed. Intact sarcocysts were also observed in some of these samples (Figure 1D). No parasitic lesion was identified in the remaining 45% of the samples. Histopathology was not conclusive in the heart sample suspected of *Cysticerccus* spp. infection. The histologic lesion consisted of a circumscribed area of granulomatous inflammation on the surface of the myocardium.

Discussion and Conclusion

The reports of Brazilian Federal Meat Inspection System in swine slaughterhouses (Coldebella et al., 2017), have shown results of carcass condemnation and trimming data on more than 97 million pigs slaughtered between 2012 and 2014.The zoonosis injuries condemnations/trimming were reported in very low frequency. Among the total of organs and carcass inspected cysticercosis was registered in 0.00092%, sarcosporidiosis in 0.00051% of the cases.

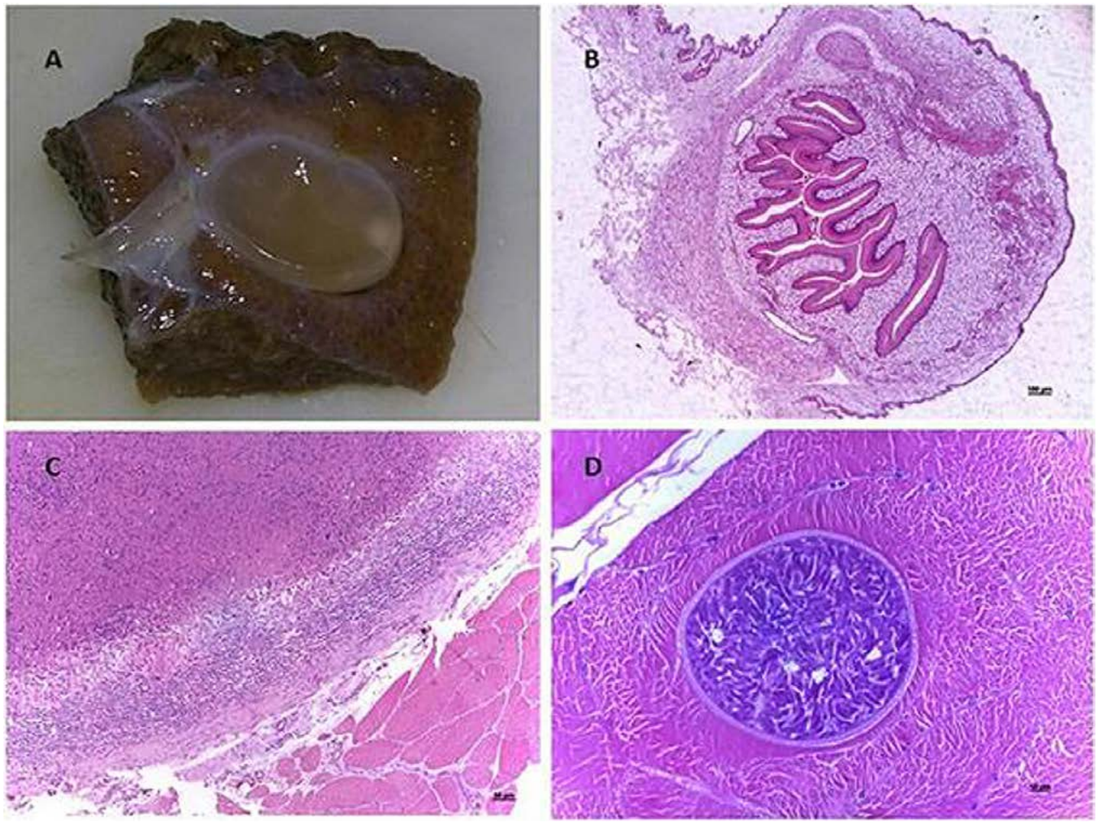

Photo 1: A- C. tenuicollis/liver; B- C. tenuicollis scolex; C- myositis/Sarcocystis; D- sarcocyst/tongue

The hydatidosis frequency was not noteworthy. The results show that most of cystic livers found in finisher pigs was related with *Cysticercus tenuicollis* infection, a non-zoonotic parasite. Pigs can be intermediate hosts of *Taenia hydatigena* (Monteiro et al., 2015). Pigs can be infected by coming in contact with feces of canids or felines contaminated with the infecting eggs (Rojas et al, 2018). Even though this parasite is not a threat for consumers, it is a critical indicator of biosecurity failure in pig farms. This information should be provided to field professionals to improve farm biosecurity procedures.

Sarcosporidiosis was not identified in finishing pigs, but was a prevalent infection in culling sows, probably due to the longer life cycle of these animals. The results show the importance of the carcasses inspection in culling sows, owing to the zoonotic potential of the disease.

Cysticercosis seems not to be a problem in Brazilian swine industry, since just one suspect lesion was detected in about 37 million slaughtered pigs. All these results are useful for meat inspection modernization based on risk analysis.

References

Coldebella, A. et al. (2017): Reports of Brazilian Federal Meat Inspection System in Swine Slaughterhouses. 12th Intern. Symp. on the Epidem. and Cont. of Biol., Chem. and Phys. Hazards in Pigs and Pork Foz do Iguaçu - Brazil August 21-24.

De la Rue M.L. (2008): Cystic echinococcosis in southern Brazil. Rev Inst Med Trop Sao Paulo, 50(1), 53-56.

Monteiro, D. U. et al. (2015): *Echinococcus granulosus sensu lato* and *Taenia hydatigena* in pig in Southern Brazil, Braz. J. Vet. Parasitol., 24 (2), 227 - 229.

Rojas, G. et al. (2018): *Taenia hydatigena* en cerdo procedente de uma zona rural del Estado Yaracuy, Venezuela, Saber, Universidad de Oriente, Venezuela, 30, 636-641.

Satyaprakash, K, et al. (2018): Pathological and molecular identification of porcine cysticercosis in Maharashtra, India, Acta Parasitologica, 63(4), 784 - 790.

Zainalabidin, F. A., et al. (2017): Prevalence of Muscular Sarcosporidiosis in Slaughtered Domestic Pigs in Perak, Peninsular Malaysia, Tropical Life Sciences Research, 28(1), 161-166.

P17

Colostrum supply of suckling piglets and Salmonella seroprevalence in piglet rearing - Is there an relationship?

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Introduction

Salmonella are still a problem in pork production. Increasing litter sizes and more newborn piglets with low birth weights at the same time make an adequate colostrum supply more difficult. This study investigated the hypothesis, that modern piglet producing farms with a high farrowing rate and an increased *Salmonella* prevalence in piglet rearing show a more unfavourable colostrum supply in suckling piglets.

Methods

An association of 250 northern German piglet producing farms has been organizing a voluntary biannual health-status-monitoring on piglets (25 kg BW) since years. The monitoring includes an ELISA for *Salmonella* antibodies. On basis of these data 12 *Salmonella*-conspicuous and 12 *Salmonella*-inconspicuous farms were selected. These were similar in terms of hygiene, herd size and performance. Each farm was visited once 24-48 hours after the main farrowing day. On each farm 4 litters were sampled and 2 light-weight, 2 medium-weight and 2 heavy-weight piglets per litter were weighed and a blood sample was taken. The blood samples were tested for the colostrum supply by means of the Ig-Immunocrit-method. Furthermore, *Salmonella* optical density (OD)-values were tested by Herdcheck® *Salmonella* ELISA (IDEXX Laboratories, Hoofddorp, The Netherlands). Differences between both groups depending on body weight were statistically analysed by using the t-test (level of significance: $p < 0.05$).

Results

This study provides preliminary evidence that when comparing *Salmonella*-conspicuous farms and *Salmonella*-inconspicuous farms, colostrum supply could be a critical factor to be considered. The fact that there was no difference in the body weight of piglets in both groups suggests that there may be differences in colostrum management. Further studies have to investigate the reasons for the differences in the colostrum supply of light weigh piglets and the impact on the *Salmonella* seroprevalence at the time of slaughter.

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Table 1:

		body weight [kg]		immunocrit		*Salmonella* - OD	
		Salmonella-inconspicuous farms	*Salmonella*-conspicuous farms	*Salmonella*-inconspicuous farms	*Salmonella*-conspicuous farms	*Salmonella*-inconspicuous farms	*Salmonella*-conspicuous farms
BW category	n	88	96	88	96	88	96
light		1.05 (±0.25)	1.05 (±0.29)	0.100[a] (±0.04)	0.087[b] (±0.04)	35.85 (± 38.66)	36.18 (± 39.31)
medium		1.38 (±0.25)	1.36 (±0.27)	0.107 (±0.03)	0.098 (±0.03)	38.71 (± 40.12)	37.59 (± 37.51)
heavy		1.69 (±0.27)	1.78 (±0.31)	0.114 (±0.03)	0.111 (±0.03)	43.65 (± 41.88)	41.77 (± 38.55)

[a, b] averages differ significantly within a row ($p < 0.05$)

P18

Microarray based genetic profiling of Staphylococcus aureus isolated from abattoir byproducts of pork origin

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Introduction

Roughly 23 million tons of pork meat are processed in the European Union annually with a rising tendency. A significant proportion of this meat is wasted during processing either due to shortcomings in the handling of sidestreams or due to low consumer acceptance and therefore limited marketability of products. In other parts of the world, especially various Asian regions, pig ear or pig tongue and other byproducts are considered a delicacy of great value. Also, in Europe, the movement of "nose to tail" eating has gained recognition in gastronomy and among the general public in recent years. It aims at utilizing all parts of an animal, giving special attention to the culinary potential of offal. Currently, information on the safety of such products is limited, and information on the occurrence of *Staphylococcus aureus* is missing. *S. aureus* is a common skin colonizing organism responsible for staphylococcal food poisoning (SFP). In 2015, EFSA reported 434 food-borne outbreaks due to staphylococcal enterotoxins (SE). Of these, 85 outbreaks were associated with meat or meat products. Generally, pork meat production has raised concern due to the transmission of livestock associated- methicillin-resistant *S. aureus* (LA-MRSA) from animals to humans. The most prevalent MRSA lineage in Europe is CC398, while in Asia CC9 is more frequent. The genetic profiles of *S. aureus* isolated from neck, belly, back, and ham of pig carcasses in Switzerland have been reported, but little is known about the occurrence of *S. aureus* on slaughtering byproducts.

In this study, ear, forefoot, heart, intestine, liver, rib bone, sternum, bladder, stomach, hind foot and tongue of porcine origin were screened for *S. aureus* and the detected isolates were further characterized. In order to unravel the genomic population structure of *S. aureus* isolates, *spa* typing and DNA microarray analysis were used.

The objectives of this study were to determine the prevalence of *S. aureus* found on abattoir byproducts of pork origin and to characterize their virulence gene and antibiotic susceptibility profiles.

Material and Methods

Overall, 524 items of abattoir byproducts of pork origin such as ear, forefoot, hind foot, heart, intestine, liver, rib bone, sternum, bladder, stomach and tongue from different abattoirs were screened for *S. aureus*. DNA microarray was performed using Staphytype genotyping kit 2.0 (Alere). In addition, the sequence of the polymorphic X region of the *spa* gene of each *S. aureus* isolate was determined (*spa* typing).

Results

Overall, 40 (0.08%) of the 524 sampled byproducts were positive for *S. aureus*. Parts with the highest prevalence were tongue (0.29%) and ear (0.24%), followed by rib bone (0.13%), sternum (0.09%), heart (0.07%) forefoot (0.02%) and liver (0.02%).

Of the 40 isolates obtained from pork byproducts, 39 could be assigned to a total of six clonal complexes (CC). The most prevalent CCs were CC9 (27.5%), CC1 (22.5%) and CC7 (22.5%). An attribution of CCs to the respective source of isolation (body part) showed no difference between CCs present at outer body parts and those on inner organs. It could be hypothesized that inner organs were contaminated during meat processing. This is supported by the fact that not all CCs were found in all abattoirs.

Twelve *spa* types were associated with the samples. The most frequent *spa* types were t091 (n = 9), t1491 (n = 8), t899 (n = 6) and t034 (n = 5).

Among the tested antibiotic resistance genes, *blaZ/I/R*, *qacC*, *fosB*, *vgaA*, *tetK/M*, and *aacA-aphD* were found. CC398 appeared to exhibit the most heterogeneous resistance profile compared to other complexes. For CC398 isolates, resistance genes *blaZ/I/R*, *vgaA* and *tetK* as well as *tetM* were detected.

No MRSA strains were detected among the *S. aureus* strains investigated in this study.

The studied set of isolates displayed a variety of enterotoxin genes, which were heterogeneously distributed within clonal complexes and *spa* types. *Sea* (N315) was present in CC1, CC7, CC49, and CC398. The gene coding for enterotoxin B (*seb*) was found in CC1, CC9, CC30, and CC398. The *seh* gene was distributed across CC1, CC7, CC9, CC30, and CC398. The most prevalent toxin genes were the *egc* encoded genes *seg*, *sei*, *sem*, *sen*, *seo* and *seu*, which were detected in 11 strains belonging to CC1, CC7, CC9, CC49, and CC398. Interestingly, only one strain isolated from a heart harbored the *sec* and *sel* genes. Only two strains (*spa* types t015 & t7439) did not harbor any of the tested enterotoxin genes.

SplitsTree analysis revealed an association of certain CCs and abattoirs. No association of CCs with particular body parts or outer/inner organs was observed. However, *S. aureus* from certain body parts were associated with certain abattoirs, e. g. *S. aureus* were only detected in sternum and rib bone samples originating from one abattoir. It could be hypothesized that such isolates stem from post mortem contamination during the slaughtering and meat handling process, rather than from the animal source.

Discussion and Conclusion

Sampling of pork byproducts in Switzerland demonstrated low prevalence of *S. aureus*. Microarray based genetic profiling of 40 *S. aureus* strains revealed a diverse population structure. No MRSA were detected. A variety of enterotoxin genes was found distributed over almost all clonal complexes. Overall, the isolates did not differ significantly from those found in previous studies in pork meat. Therefore, the investigated pork byproducts do not pose a greater health threat to consumers than conventional pork products with regard to *S. aureus*. Our findings suggest that occurrence of *S. aureus* on byproducts was linked to contamination during the slaughtering process in some abattoirs. Adequate handling of these processing sidestreams should ensure proper quality and therefore minimize product loss.

P19

Short duration acidified feed use as a pre-slaughter Salmonella intervention

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Introduction

Salmonella carriage and shedding in finisher pigs is a risk to carcase contamination at slaughter. The main impacts of Salmonella are for human health, thus, on-farm control strategies must be cost-effective to be implemented by farmers. Organic acids have been demonstrated to have an inhibitory effect on pathogens such as Salmonella, proving particularly successful in the poultry sector.

Recent studies indicate that existing on-farm controls can be undermined during transport or at slaughter. Stress, due to transportation, has been demonstrated to increase the shedding of Salmonella in faeces of pigs. Other common practices such as feed withdrawal and lairaging have also been associated with increased faecal shedding of Salmonella.

This study aims to determine the effectiveness of short-interval acidified feed intervention in pigs, in the period prior to slaughter, for reducing Salmonella carriage, faecal shedding and carcase contamination at slaughter. This study also assesses the impact of pig transport and use of lairage facilities on the occurrence of Salmonella.

Methods

Each of the five recruited pig farms supplied two similarly managed groups of finisher pigs. One group of pigs received their normal ration for the duration of the trial (control group). A second group were fed their normal ration with added Fysal MP©, an organic acid feed additive, at 5kg/tonne for four weeks prior to slaughter.

Each farm was visited before the beginning of the intervention to establish the baseline presence of Salmonella in each trial group. The farms were visited again after four weeks of intervention. At both visits, individual and pooled faecal samples were collected to calculate Salmonella presence. Environmental swab samples from the vicinity of each group of pigs, were also collected to evaluate the environmental contamination of Salmonella.

Study groups were then followed to slaughter. Samples of the lairage were collected prior to the entry of the trial pigs. On the slaughter line, whole guts were collected, from which caecal content samples were harvested. In three trials, ileo-caecal lymph nodes were also harvested from the whole guts, in addition to caecal contents, to determine Salmonella carriage.

To investigate carcase contamination, carcases were swabbed at the end of the line, prior to blast chilling. The lorry used to transport the pigs was also sampled before and after transport of the pigs to abattoir.

Results

Preliminary results suggest that the effectiveness of a short-duration organic acid intervention on Salmonella presence may be context-specific. Results of the first four trials indicate that abattoir lairages were highly contaminated with Salmonella before the entry of pigs (mean sample prevalence 80.0%). In addition, two transporters used in the first four trials were contaminated with Salmonella prior to the entry of trial pigs (mean sample prevalence 17.5%).

Discussion

Both abattoir lairages and transporters were contaminated with Salmonella before the entry of pigs. Studies have indicated that it is possible for pigs exposed to an environment contaminated with Salmonella to become infected rapidly, in as little as two hours. These environments could therefore pose a threat to contamination of pigs prior to slaughter. More effective cleaning and disinfection is required to minimise this risk.

Although initial results of the intervention were encouraging, reductions were not observed on all of the trial farms. The results of each trial will be presented and discussed in full at the conference.

P20

Diagnostic results of samplings before intervention for Salmonella started in pig herds in the Benelux

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Introduction

Salmonella is a well-known zoonosis and therefore, slaughterhouses put pressure on suppliers of slaughter pigs to deliver pigs with a low *Salmonella*-status. Aim of this paper is to give an overview of the diagnostic results from 56 herds in the Benelux that were sampled before interventions started, to see which *Salmonella* types are present in which locations and animal categories.

Material and Methods

Faecal, dust or sock samples were collected either by IDT Biologika staff or the herd veterinarian after instruction. Faecal samples are pools of faeces from the floor; dust was collected using a small cloth (Swiffer®) or socks which were worn over a boot. Sock and dust samples (figure 1 and 2 respectively) were collected in the compartments or in hallways connecting compartments of a certain animal category.

Photo 1: Collecting a sock sample of faeces for bacteriological investigation

Salmonella isolation was done based on ISO standards. Most isolates were serotyped to serogroup level with additional typing for S. Typhimurium and S. Derby. Results were collected in an Excel database (version: 14.0.7232.5000 (32 bit)) and pivot tables were used to create descriptive statistics.

Photo 2: Collecting a dust sample for bacteriological investigation

Results

934 Samples (faeces N=66, dust N=241, sock N=627) were collected from a total of 56 herds in the years 2015 (58 from 5 herds), 2016 (255 from 11 herds), 2017 (334 from 21 herds) and 2018 (287 from 19 herds). 39% of dust, 37% of socks and 29% of faecal samples were positive, respectively (table 1).

Table 1: Total number, number of Salmonella positive samples and negative samples for dust, sock and faecal samples collected in swine herds respectively.

sample	negative	positive	total	% positive
dust	146	95	241	39.4%
fecal	47	19	66	28.8%
sox	396	231	627	36.8%
total	589	345	934	36.9%

For 634 samples the sampling location or animal category was registered. Of the breeding gilts / grow/finishers 38% of 195 samples were positive, for weaned piglets 49% out of 209, for sow in the insemination (AI) centre 36% out of 49, for gestating sows 35% out of 60, and for farrowing sows 21% out of 87 samples were positive. 12 out of 24 samples from central corridors were positive. For breeding gilts / grow/finish pigs 35% (N=55) of the samples from the corridors were positive and 40% (N=140) from the actual compartments. For weaned piglets 42% (N=57) and 51% (N=152) were positive respectively (table 2).

A total of 250 Salmonella isolates were typed (Table 3). 73% of all *Salmonella* in weaned piglets were S. Typhimurium. In breeding gilts / grow/finish pigs this was 55%, in gestating sows 33% and in sows in the AI centre 22% respectively. Other *Salmonella* types found were S. Derby (9%), serogroup B

(not S. Typhimurium nor S. Derby) (10%), serogroup C (10%) or E (1%) or rough (N=1). 34 isolates were found to be Salmonella but could not be typed any further for various reasons.

Discussion

Weaned piglets are often positive for S. Typhimurium and therefore an important source of infection of breeding gilts or grow / finish pigs. Corridors are often contaminated and can be a source of infection. Both should therefore be included in a comprehensive intervention plan including all-in/all-out management, proper cleaning and disinfection of compartments and corridors, strict internal and external biosecurity, proper pest control, the application of organic acids in feed and/or drinking water and, if applicable, vaccination of sows, replacement stock and piglets against *Salmonella* Typhimurium.

Table 2: Breakdown of number negative, Salmonella positive and total number of samples by animal type and location

Location	Corridor *				pens				outside			Grand	Total
Animal category	neg.	pos.	total	% pos	neg.	pos.	total	% pos	neg.	pos.	total	total	% pos
breeding gilt / grower_finisher	36	19	55	35%	84	56	140	40%				195	38%
central corridor	12	12	24	50%								24	50%
farrowing sow	6	2	8	25%	63	16	79	20%				87	21%
gestating sow	26	15	41	37%	13	6	19	32%				60	35%
Dressing room	6	1	7	14%								7	14%
insemination sow	1		1		29	18	47	38%				48	64%
insemination sow / gestating sow					1		1					1	0%
Outside#									1	1	2	2	50%
quarantaine					1		1					1	0%
weaned piglet	33	24	57	42%	74	78	152	51%				209	49%
Grand Total	120	73	193	38%	265	174	439	40%	1	1	2	634	39%

* Corridor: corridor connecting several compartments for this animal type. # Outside in the yard.

Table 3: Breakdown of type of Salmonella found by animal category or location

Salmonella type / Animal category	Derby	Enterica subspefcies (rauhform)	Group B	Group C	Group E	pos no type	Typhimurium	Typhimurium+Derby	Grand Total
breeding gilt/grower_finisher	10		14	8	1	2	42		77
central corridor	1		1			3	6	1	12
farrowing sow	2		3	4		5	4		18
gestating sow	2	1	1	4	1	3	7	2	21
Dressing room								1	1
insemination sow	5		1	2		6	4		18
Outside in the yard							1		1
weaned piglet	2		4	7		15	74		102
Grand Total	22	1	24	25	2	34	138	4	250

P21

Identification of potential risk factors for Toxoplasma gondii in fattening pigs in the Netherlands using a Bayesian approach

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Introduction

Toxoplasma gondii is a relevant foodborne pathogen, it is estimated that up to one third of the world population has been exposed to the parasite (Tenter et al. 2000). In the Netherlands toxoplasmosis ranks second on a list of prioritized emerging zoonosis (Havelaar et al. 2010) and also second in disease burden among 14 foodborne diseases (Mangen et al. 2017). Data suggest that ingesting improperly cooked meat containing *T. gondii* is one of the major sources of infection in Europe and North America (Crotta et al. 2017; Guo et al. 2015). The contribution of pork to meatborne *T. gondii* infections is estimated to be 11% in the Netherlands (Opsteegh 2011) and is seen as an important possible source of human *T. gondii* infections (Foroutan et al. 2019). The European Food Safety Authority (EFSA) advised to perform serological testing of pigs and on farm audits on risk factors (EFSA 2011). To that end, a serological monitoring program was developed in a slaughterhouse in the Netherlands. In this study, the objective is to determine the association between within-herd seroprevalence, corrected for misclassification of samples through Bayesian analyses, and risk factors for *T. gondii* on fattening pig farms in The Netherlands.

Materials and Methods

From 2015 to 2018, HACCP based audits were performed on 75 fattening pig farms in The Netherlands to identify the presence of potential *T. gondii* risk factors. All farms were conventional pig farms, with 15 farms being farrow to finish. As overall seroprevalence of *T. gondii* in pigs in the Netherlands is low, estimated at 5% (1-12% 95% CI) by Foroutan et al. 2019, approached farms were chosen with the knowledge of previous serology data. In this way there would be farms with positive serum samples and farms without them included in the study. The audits were based on an updated version of the questionnaire from Mul et al. (2015) and covered the following topics: outdoor access, farm biosecurity, rodent control, presence of cats, feed and water supply. In addition, serum samples (n=6272) from fattening pigs were obtained at slaughter throughout the year before the audit on the farm was performed. These samples were used for antibody testing by a PrioCHECK™ Toxoplasma Antibody ELISA. Data were analysed using Bayesian statistics, with the within-farm *T. gondii* prevalence as dependent variable and potential risk factors as independent variables. As always with serology, misclassification due to false-positive or false-negative results can occur. Statistical methods have been developed to account for such misclassification, based on frequentistic as well as Bayesian approaches (Hui & Walter 1980; Joseph et al. 1995). First, all independent variables were analysed in a univariate logistic model, and variables with a probability ≤0.25 that zero is included in the 95% interval were analysed in a multivariable model. The multivariate logistic model was fitted using backward elimination until all remaining variables showed a probability ≤0.05 that zero is included in the 95% interval. Two-way interaction terms were evaluated similarly to the main variables regarding statistical significance.

Results

Descriptive results showed that 50 out of the 75 farms had 1 or more positive serum sample in the year before the audit was performed. In total 438 samples were positive out of the 6272 samples. Final Bayesian analyses are currently being conducted. However, preliminary results from data analysis using frequentistic logistic multivariate regression identified two significant risk factors: the accessibility of pig feed for cats and the provision of well water as drinking water for the pigs (Table 1).

Discussion and Conclusions

The use of serological testing seems to be a valuable guide and monitoring tool for the control of *T. gondii* in pork production. In a preliminary analysis, a higher within-herd *T. gondii* seroprevalence on fattening pig farms in the Netherlands was associated with the accessibility of pig feed for cats and the provision of well water as drinking water for the pigs. Improvements in farm management on fattening pig farms will likely contribute to reduction of the human disease burden and is presently studied.

References

Crotta M, Limon G, Blake DP, Guitian J. Knowledge gaps in host-parasite interaction preclude accurate assessment of meat-borne exposure to *Toxoplasma gondii*. International Journal of Food Microbiology 261 (2017) 95–101.
European Food Safety Authority; Scientific Report on Technical specifications on harmonized epidemiological indicators for public health hazards to be covered by meat inspection of swine. EFSA Journal 2011; 9(10):2371.
Foroutan M, Fakhric Y, Riahid SM, Ebrahimpoure S, Namroodif S, Taghipourb A, Spotingh A, Gamblei HR, Rostamie A. The global seroprevalence of *Toxoplasma gondii* in pigs: A systematic review and meta-analysis. Veterinary Parasitology 269 (2019) 42–52.
Guo, M, Dubey, JP, Hill, D, Buchanan, RL, Gamble, HR, Jones, JL, Pradhan, AK. Prevalence and risk factors for *Toxoplasma gondii* infection in meat animals and meat products destined for human consumption. J. Food Prot. 78 (2015), 457–476.
Havelaar, AH, van Rosse, F, Bucura, C, Toetenel, MA, Haagsma, JA, Kurowicka, D, Heesterbeek, JAP, Speybroeck, N, Langelaar, MFM, van der Giessen, JWB, Cooke, RM, Braks, MAB. Prioritizing emerging zoonoses in the Netherlands. 2010. PLoS ONE 5, e13965
Hui SL, Walter SD. Estimating the error rates of diagnostic tests. Biometrics 1980; 36: 167–171.
Joseph L, Gyorkos TW, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. American Journal of Epidemiology (1995); 141: 263–272.
Mangen, MJ, Friesema, IHM, Pijnacker, R, Mughini Gras, L, Van Pelt, W (2018). Disease burden of food-related pathogens in the Netherlands 2017; Bilthoven; National Institute for Public Health and the Environment.
Opsteegh M. *Toxoplasma gondii* in animal reservoirs and the environment. Dissertation Utrecht University 2011, Faculty of Veterinary Medicine
Tenter, AMAR, Heckeroth, and LM Weiss. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitology 2000; 30: 1217–1258.

Table 1: Variables analysed multivariably by backward elimination for association with the presence of T. gondii on 75 Dutch pig farms between 2015 and 2018 (univariable P ≤ 0.25)

Risk Factor	N Farms	Odds Ratio (95% CI)	P-Value
Goats			
Absent	67	Not applicable	0.176
Present	8		
Boots in stable			
Only inside	28	Not applicable	0.524
Also outside	47		
Professional pest control			
Yes	33	Not applicable	0.283
No	42		
Own cats at barnyard			
Absent	42	Not applicable	0.850
Present	33		
Pigfeed accessible for cats			
Absent	49	15.4 (3.0 – 79.4)	0.001
Present	26		
Pig drinking water			
Tap water	34	3.4 (1.1 – 10.7)	0.035
Well	41		
Pigfeed contains whey			
Absent	52	Not applicable	0.429
Present	23		
Pigfeed			
Dry feed	37	Not applicable	0.069
Wet/liquid feed	38		

P22

Pigs infected experimentally with the same dose of monophasic variant of Salmonella Typhimurium exhibit different shedding levels

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Introduction

Salmonellosis remains the most frequent foodborne zoonosis after campylobacteriosis (EFSA and ECDC 2017). The most frequent sources of human infection are food products of animal origin. Pork meat has been considered as one of the major sources (Bonardi 2017). Pigs colonized with *Salmonella* are usually asymptomatic healthy carriers (Rostagno and Callaway 2012) with varied levels and durations of fecal shedding (Ivanek *et al.*, 2012). Thus, understand the mechanisms that result in more or less shedding may provide tools for control. Indeed, it has been demonstrated in other species that a minority of the infected individuals (super-shedders) are responsible of most of the transmission (Gopinath *et al.*, 2014). In the frame of MoMIRPPC (EJP One Health), we wanted to evaluate the apparition of different shedding patterns among a pig population. Then, immune and microbiota analyses will be performed in order to identify markers link to the shedding status.

Material and Methods

An experimental trial was conducted with a total of 45 piglets divided into five groups: one group with five piglets as control and four groups each with 10 piglets (n=40) as inoculated pigs. The piglets came from 5 sows and were distributed in such a way as to avoid a maternal effect between the groups. At 7 weeks of age, the inoculated piglets received orally 10 ml of suspension of 10^8 CFU/ml of a monophasic variant of *Salmonella* Typhimurium strain. Pigs were followed during 3 weeks after inoculation before being necropsied. Twice a week, individual feces were sampled in order to quantify the level of *Salmonella* excretion during the trial. At necropsies, level of *Salmonella* was determined in tonsils, mesenteric lymph nodes (MLN), as well as in ileum and caecum contents from each pig. To facilitate the numeration, the strain inoculated was transformed to be resistant to rifampicine. Samples analyzed were diluted and directly plating on XLD agar plate supplemented with rifampicine.

All statistical analysis have been performed using R software version 3.5.2. The total level of excretion of each pig during the 3 weeks was determined by calculating, with a specific R script, the AULC (Air Under the Log Curve). Feces and intestinal contents have been frozen for future microbiota analyses. Blood was also sampled twice a week, to realize later on total blood count (TBC), serological and transcriptomic analyses.

Results

All control pigs remained negative for *Salmonella* throughout the course of the study while all the inoculated pigs were quantitatively positive for *Salmonella* shedding during all the study. *Salmonella* shedding varied according pigs and days between 1.48 to 9.09 Log_{10}CFU/g of feces. The excretion pic was observed at Day 2 post inoculation, with 6.77 ± 1.79 Log_{10}CFU/g in mean. The AULC calculation allowed us to identify three significantly different classes ($p< 0,01$). The three classes gathered 13, 16 and 11, high, intermediate and low shedders pigs respectively (Fig 1).
No difference were observed for the AULC value according mother ($p = 0.42$). Indeed, for each sow, among the 9 piglets of a same sow, piglets were distributed in the 3 class, low, intermediate and high shedders. However, AULC values according pens were significantly different ($p< 0,05$). The presence of a high shedder pig in a pen would maintain a high contamination pressure in the pen, and therefore a high excretion of several pigs in the pen all along the assay.
After necropsies, for all the pigs, tonsils, caecum and ileum contents were highly contaminated (in mean, 5.6, 3.7 and 3.5 Log_{10}UFC/g, respectively) unlike MLN (in mean, 0.85 Log_{10}UFC/g). We observed that for the group of high shedders, levels of contamination was significantly higher for MLN, ileum and caecum contents than for the group of low shedders ($p< 0.01$) (Table 1).

Discussion and Conclusion

Pigs infected experimentally with a same dose of monophasic variant of *Salmonella* Typhimurium exhibited different shedding levels. This was also described after S. Typhimurium infection (Knetter *et al.*, 2015). We also demonstrate that, in experimental conditions, these different shedding patterns are not linked to the mother. Indeed, high and low shedders pigs can originate from a same mother. In addition, in this study, when pigs are high shedders they also contain a significantly higher level of *Salmonella* in mesenteric lymph nodes, ileum and caecum.
However, the presence of a high shedder pig could be responsible of a high global excretion in a pen,

causing a high contamination pressure for other pigs. This result confirm the importance to focus intervention strategies specifically on animals able to shed high level of *Salmonella*. To lead these interventions, we need to improve our knowledge on markers in microbiota (Kim and Isaacson 2017) and/or in immune response (Huang *et al.*, 2011; Knetter *et al.*, 2015; Uthe *et al.*, 2009) that could promote the high excretion in pigs.

References

Bonardi S. *Salmonella* in the pork production chain and its impact on human health in the European Union. Epidemiology & Infection 2017; 145:1513-1526.

EFSA and ECDC. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2016. EFSA Journal 2017; 15(12):228 pp.

Gopinath S., Lichtman J.S., Bouley D.M., Elias J.E. and Monack D.M. Role of disease-associated tolerance in infectious superspreaders. Proc Natl Acad Sci U S A 2014; 111:15780-5.

Huang T.H., Uthe J.J., Bearson S.M., et al. . Distinct peripheral blood RNA responses to *Salmonella* in pigs differing in *Salmonella* shedding levels: intersection of IFNG, TLR and miRNA pathways. PLoS One 2011; 6:e28768.

Ivanek R., Osterberg J., Gautam R. and Sternberg Lewerin S. Salmonella fecal shedding and immune responses are dose- and serotype- dependent in pigs. PLoS One 2012; 7:e34660.

Kim H.B. and Isaacson R.E. Salmonella in Swine: Microbiota Interactions. Annu Rev Anim Biosci 2017; 5:43-63.

Knetter S.M., Bearson S.M., Huang T.H., et al. . *Salmonella enterica* serovar Typhimurium-infected pigs with different shedding levels exhibit distinct clinical, peripheral cytokine and transcriptomic immune response phenotypes. Innate Immun 2015; 21:227-241.

Rostagno M.H. and Callaway T.R. Pre-harvest risk factors for *Salmonella* enterica in pork production. Food Research International 2012; 45:634-640.

Uthe J.J., Wang Y., Qu L., Nettleton D., Tuggle C.K. and Bearson S.M. Correlating blood immune parameters and a CCT7 genetic variant with the shedding of *Salmonella enterica* serovar Typhimurium in swine. Vet Microbiol 2009; 135:384-388.

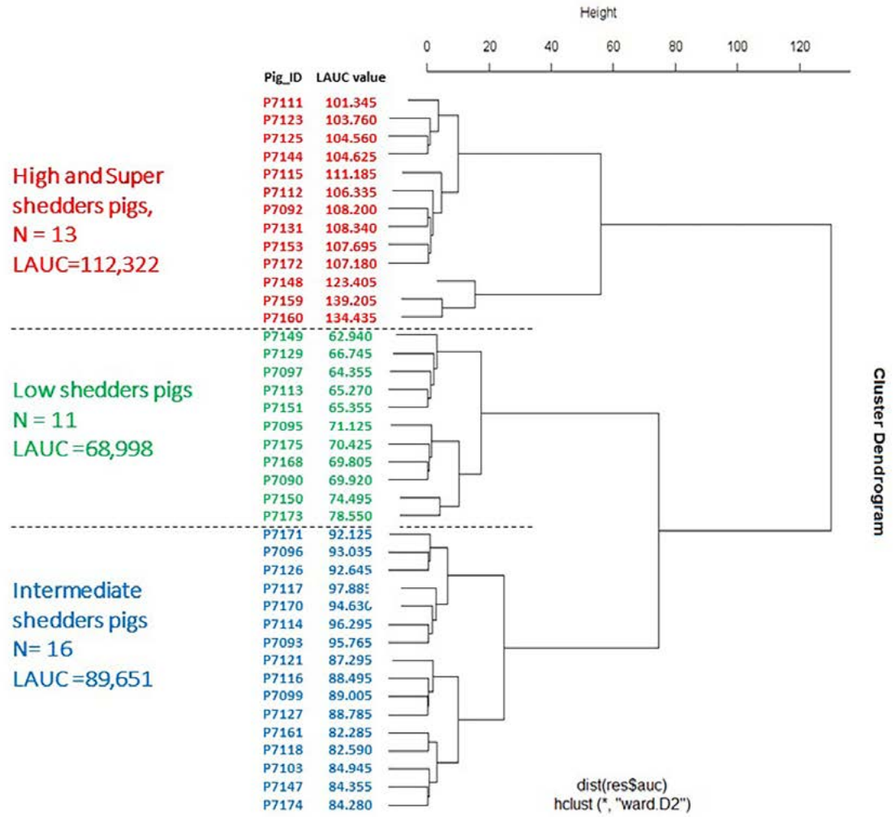

Figure 1: Hierarchical classification of pigs according the AULC calculated from the numeration values

Table 1: Salmonella positive samples and contamination levels in samples at necropsy, in log10 CFU/g

Samples	Tonsil		Mesenteric lymph nodes		Ileum content		Caecum content	
Shedding classes	N° of positives (%)	Mean ± SD	N° of positives (%)	Mean ± SD	N° of positives (%)	Mean ± SD	N° of positives (%)	Mean ± SD
High	13 (100%)	5.56 ± 0.28	12 (92,3%)	1.26 ± 0.60	13 (100%)	4.60 ± 0.96	13 (100%)	4.56 ± 1.47
Intermediate	16 (100%)	5.71 ± 0.42	15 (93,7%)	0.89 ± 0.69	16 (100%)	3.40 ± 0.84	16 (100%)	3.20 ± 1.35
Low	11 (100%)	5.49 ± 0.38	9 (81.8%)	0.35 ± 0.39	11 (100%)	3.10 ± 0.77	11 (100%)	2.45 ± 1.66
Total	40 (100%)	5.60 ± 0.37	36 (90%)	0.8 ± 0.68	40 (100%)	3.71 ± 1.06	40 (100%)	3.46 ± 1.66

SD: Standard deviation

P23

Prevalence of Salmonella spp. in piglet producing and rearing systems in North-Rhine-Westphalia

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Introduction

In Germany as well as in Europe, Salmonellosis is still the second most commonly recorded zoonosis (EFSA & ECDC 2017, RKI 2018). Fattening farms are committed to do frequent monitoring to reduce entry of Salmonella spp. in the food chain. This is regulated by law (Schweine-Salmonellen-Verordnung) since 2007. However, one problem, especially in farms with good hygienic management, is the housing of Salmonella-infected piglets. The purpose of our study was to investigate the prevalence of Salmonella spp. in piglet producing and rearing systems in North Rhine-Westphalia. The project was financially supported with resources of animal diseases fund (Tierseuchenkasse) NRW. The immediate objective was to reduce Salmonella burden of each farm by using individually adapted measures. The long-term objective was to evaluate general measures, which are able to permanently reduce Salmonella load in pig farms.

Methods

All piglet producers of North Rhine-Westphalia could volunteer for an initial survey of their Salmonella burden between 2016 and 2018. Each farm was analysed with regard to hygiene and biosecurity and sampling was done. Blood samples were collected from 20 sows, 10 pigs (weighted around 28kg), 10 gilts and tested for Salmonella antibodies using commercial ELISA test (Swine Salmonella Antibody Testkit; IDEXX Laboratories). Furthermore, faecal samples of each rearing unit and environmental swabs of a disinfected compartment were analysed. Each Salmonella isolate was serotyped and resistance test was carried out. All data were collected in a database (Microsoft Access 2016). Descriptive statistics were summarized using a commercial software program (Microsoft Excel, 2016).

Results

Overall 102 farms were visited for initial survey, 87 of them were sampled two times or more. There were different production types: sow breeders, piglet producers, farms only with piglet rearing units, grow-to-finish farms and farrow-to-finish farms. The number of sows varied from 40 to 2000 (mean 333, median 260). Rearing units had a mean size of 1595 piglets (median 1175; min. 100, max. 9000) and fattening units had a mean size of 1055 pigs (median 780); the smallest fattening unit of 20 pigs and the biggest farm with 5000 pigs. Status survey contained the question of measures already taken against Salmonella. In five farms, gilts were vaccinated against S.Typhimurium. One farmer vaccinated only sows and five farmers vaccinated sows and gilts against S.Typhimurium. In three farms gilts, sows and piglets were vaccinated against S.Typhimurium. Two of these farms used a stock-specific vaccination; all others used a commercial live vaccine. Approximately 9100 blood serum samples were collected. Excluding samples of Salmonella vaccinated animals, 4596 serum samples of sows were analysed and nearly 31% of them showed an optical density (OD%) over 40%. According to „QS-Salmonella-Monitoring-System“ in fattening pigs, those samples are Salmonella-antibody-positive. The proportion of positive samples (OD%=40) of gilts lies about 14% and about 12% in pigs with an average weight of 28kg. In total 2630 faecal samples were collected. On 81 out of 102 farms at least one positive faecal sample was found. In total 611 samples (23%) were Salmonella-positive by culture. Of 751 environmental swabs, 102 contained Salmonella spp. Three farms had only positive environmental swabs but in 25 farms, Salmonella was detected in faecal as well as in environmental samples. Serotyping resulted in 12 different Salmonella-Serovars. Most frequently S.Typhimurium was detected (80.79%), followed by S. Derby (4.91%) and S. Subspec. I. Rauform (1.26%). All other serovars were only a few cases (< 1% of isolates).

Conclusion

This study shows a seroprevalence of Salmonella in sows in North Rhine-Westphalia on a medium level. Hence, the seroprevalence in post-weaning pigs was on a low level, the detection rate of Salmonella spp. by culture was quite high. All results point to the fact that reducing the risk of Salmonella infection by pork has to start at the basis of the production pyramid.

P24

Is the porcine intestinal microvasculature not only permeable to nutrients but also to pathogens?

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Introduction

Salmonella Typhimurium penetrates the gut-vascular barrier in mice, gaining access to the bloodstream and liver (Spadoni *et al*, 2015). Despite it is still unknown how *Salmonella* Typhimurium disrupts the endothelial barrier, it is known that Plasmalemma Vesicle Associated Protein-1 (PV1), a measure of the “leakiness” of the endothelial barrier, is upregulated in blood capillaries upon *Salmonella* Typhimurium infection (Spadoni *et al*, 2016). PV1 is a component of the diaphragms found in endothelial fenestrae, transendothelial channels and caveolae (Stan *et al*, 2012). As only sparse data is available on porcine intestinal endothelium, the aim of the present study was to evaluate its ultrastructure with a focus on fenestration.

Material and Methods

Samples of small intestine of 4 pigs (before and after weaning) were available from our tissue bank. They were collected upon euthanasia and routinely processed for transmission electron microscopy. Capillaries from villus lamina propria were evaluated for morphology and size, distribution and density of endothelial fenestrae in semithin and ultrathin sections using light and transmission electron microscopy.

Results

Endothelial cells of subepithelial capillaries were characterized by marked attenuation and extensive fenestration in regions adjacent to the intestinal epithelium. These attenuated areas were void of organelles, caveolae, vesicles, inclusions and filopodia. The diameter of the fenestrae was 66.4nm (standard deviation 9.3nm). The fenestral density was found to be 3.1 fenestrae per µm (standard deviation of 0.9). Thicker lateral and distal faces contained numerous organelles and filopodia and the nucleus was almost always positioned toward these sites.

Discussion and Conclusion

General morphology of the normal intestinal capillary endothelia and distribution as well as morphology of fenestrae was found to be comparable to literature reports (mouse intestine (Milici *et al*, 1985); pig uterine mucosa (Keys & King, 1988)). In further studies, the influence of stress and pathogens, such as *Salmonella* Typhimurium, on ultrastructure and fenestrae of the gut-vascular barrier should be examined.

Experimental procedures approved by local state office of health and social affairs ‘Landesamt für Gesundheit und Soziales, Berlin’ (LaGeSo Reg. Nr. G0348/09).

References

Keys JL and King GJ (1988) Morphological evidence for increased uterine vascular permeability at the time of embryonic attachment in the pig. *Biol Reprod* 39: 473-487.

Milici AJ, L’Hernault N and Palade GE (1985) Surface densities of diaphragmed fenestrae and transendothelial channels in different murine capillary beds. *Circ Res* 56: 709-717.

Spadoni I, Pietrelli A, Pesole G and Rescigno M (2016) Gene expression profile of endothelial cells during perturbation of the gut vascular barrier. *Gut Microbes* 7: 540-548.

Spadoni I, Zagato E, Bertocchi A, Paolinelli R, Hot E, Di Sabatino A, Caprioli F, Bottiglieri L, Oldani A, Viale G, Penna G, Dejana E and Rescigno M (2015) A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* 350: 830-834.

Stan RV, Tse D, Deharvengt SJ, Smits NC, Xu Y, Luciano MR, McGarry CL, Buitendijk M, Nemani KV, Elgueta R, Kobayashi T, Shipman SL, Moodie KL, Daghlian CP, Ernst PA, Lee H-K, Suriawinata AA, Schned AR, Longnecker DS, Fiering SN, Noelle RJ, Gimi B, Shworak NW and Carrière C (2012) The diaphragms of fenestrated endothelia: gatekeepers of vascular permeability and blood composition. *Dev Cell* 23: 1203-1218.

P25

Salmonella in breeding pig herds - differences between sows and weaned piglets

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Introduction

Salmonella spp. continue to be prevalent in the pig production chain in Germany. It was the purpose of this study to compare *Salmonella* isolates from sows and weaned piglets in breeding pig herds that were collected during a national monitoring program.

Methods

In the framework of a national monitoring program composite fecal samples were collected from sows and weaned piglets and tested for *Salmonella* according to ISO 6579. Isolates were serotyped and tested for antimicrobial resistance to 14 substances using broth microdilution in concordance with the prescriptions of Commission Implementing Decision 2013/652/EU. Only farms that provided samples from sows and weaners were included in the analysis.

Results

Overall, prevalence of *Salmonella* spp. in the herds was 14.4% (51/353 herds). It was higher in weaners (10.5%) than in sows (5.4%). While among sows *S.* Derby was the most frequently encountered serovar, *S.* Typhimurium was most prevalent in weaners. In only 5 of 353 farms included in the analysis *Salmonella* spp were found in both, sows and weaners. Moreover, in 4 of these farms serovars differed between the groups of pigs and in only 1 farm monophasic *S.* Typhimurium was detected in both populations. In concordance with the serovars, AMR was higher in isolates from weaners than in those from sows.

Conclusions

Using only two composite fecal samples probably provides only limited sensitivity for the detection of *Salmonella* in pig herds. Results indicate that the prevalence of *Salmonella* in breeding pig farms is a complex issue and that transmission of *Salmonella* from sows to weaners is not straightforward. Controlling *Salmonella* in breeding pig herds therefore requires a complex approach that addresses both, *Salmonella* in the sows and potentially independent circulation of *Salmonella* among weaners.

P49

Characterization of Campylobacter coli isolated from pig, sheep, poultry, wild bird, river and shellfish using MALDI-TOF and comparison of their protein spectra to identify relationships between sources

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Introduction

The sanitary quality of shellfish-harvesting areas is a key issue in France, the leading shellfish producer in Europe. *Campylobacter* spp was detected in coastal catchments and shellfish-harvesting areas in Brittany and Normandy, France (Rincé et al., 2018). This pathogen is excreted by many animals whether wild birds or livestock (Mughini-Gras et al., 2016). These participate in the contamination of the environment directly or via manure spreading.
Comparison of PFGE profiles or MLST types have proved their effectiveness in determining the origin of human cases of campylobacteriosis or surface water contamination (Denis et al., 2009; Denis et al., 2011a; Clark et al., 2011; Jonas et al., 2015; Mughini-Gras et al., 2016). Identify the sources of shellfish contamination with these techniques is possible but these latter are expensive and long to implement. An alternative may be the typing of strains by MALDI-TOF MS. Links of MALDI-types of *Campylobacter* to their MLST types were described (Zautner et al., 2013). Moreover, the MALDI-TOF MS technique is easy to perform and inexpensive.
In this study, we characterize *Campylobacter coli* isolated from pig, sheep, poultry and shorebird fecal samples, river water samples and shellfish batches using MALDI-TOF and compare their protein spectra to identify relationships between sources and the source of shellfish contamination. We focused on *C. coli*, the only species detected in pig in France (Denis et al., 2011b).

Material and Methods

We considered 144 *C. coli* isolated from feces of pigs (60), sheep (15), poultry (30), and shorebirds (10) and from river water samples (24) and shellfish batches (5). They were isolated in Brittany and Normandy, which are the two main areas of shellfish production in France with an important livestock production. After culture on blood agar (24h, 37°C) in microaerophilic conditions, proteins of each strain were extracted as recommended by Bruker. Then, 1 µl of each extract was deposited 8 times on spots of MSP 48 target polished steel plate and included in 1 µl of IVD matrix HCCA. The steel plates were sent to MALDI-TOF Platform of Anses where four reads per spot were realized (32 protein spectra per strain). Under BioNumerics, an average spectrum of protein was obtained for each strain, and all the average spectra were clustered in a dendrogram using UPGMA method and Pearson's coefficient.

Results

The strains were distributed in 14 clusters (Tab1). *Campylobacter coli* of pigs were mostly distinguishable from *C. coli* of other animal reservoirs. They never clustered with *C. coli* from poultry. Seventy percent of pig strains clustered together; the others (28.3%) clustered with sheep (3) strains and, one pig strain (1.6%) with sheep (12), shorebird (4) and river water (3) strains. Sheep strains (80%) clustered with wild bird strains (4) and river water strains (3). Poultry strains (86.6%) clustered with shorebird (3), river (11) and shellfish (2) strains. Shorebird strains (90%) clustered with river water strains (19) and shellfish strains (2). Finally, two strains of shellfish were grouped with strains of river water, shorebird and poultry; the three other strains were only grouped with *C. coli* of river water.

Table 1: Clustering with 93% of similarity of average protein spectrum under BioNumerics

Cluster	Pig	Sheep	Poultry	Wild bird	River	Shellfish	Total
C1	2						2
C2	17	3					20
C3			1				1
C4	39						39
C5	1						1
C6	1	12		4	3		20
C7			26	3	11	2	42
C8				1	3		4
C9					3	2	5
C10					2	1	3
C11			2				2
C12			1				1
C13				1	2		3
C14				1			1
Total	60	15	30	10	24	5	144

Discussion and Conclusion

Although in France, *C. coli* is rarely isolated from shellfish, it is important to identify the animal reservoir that causes this *C. coli* contamination. Especially since the water, from rivers of the upstream catchments, arriving in these shellfish-harvesting areas mainly contains *C. coli* (Rincé et al., 2018).

With the use of MALDI-TOF, we observed that *C. coli* of pigs were mostly distinguishable from *C. coli* of other animal reservoirs, and particularly from *C. coli* isolated from poultry. PFGE already highlighted that *C. coli* of pigs differed genetically from *C. coli* of poultry in France (Denis et al., 2009) while MLST showed common STs in these two reservoirs in other countries (Mughini-Gras et al., 2016).
Our study suggests also that pig is very weakly involved in river contamination by *C. coli* as already described by PFGE or MLST in other studies (Denis et al., 2011a, Mughini-Gras et al., 2016). This may explain why *C. coli* of pigs was not linked to shellfish.
C. coli of sheep clustered with pig strains. Another study showed that very few STs of *C. coli* of ruminants shared common STs with *C. coli* of pigs (Mughini-Gras et al., 2016).
Our study suggests that poultry, shorebirds and sheep could contribute to the contamination of rivers by *C. coli*. This is consistent with MLST results (Mughini-Gras et al., 2016) showing that *Campylobacter* in surface water were mostly attributed to wild birds and poultry followed by ruminants. The contamination of the rivers by *C. coli* can thus contribute to that of shellfish.

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References

Clark CG, Taboada E, Grant CC, et al. (2012) Comparison of molecular typing methods useful for detecting clusters of *Campylobacter jejuni* and *C. coli* isolates through routine surveillance. J Clin Microbiol. 50:798-809.
Denis M., Chidaine B., Laisney M-J, Kempf I., et al. (2009) Comparison of genetic profiles of *Campylobacter* strains isolated from poultry, pig and human *Campylobacter* infections in Brittany, France. Pathologie Biologie, 57:23-29.
Denis M., Tanguy M, Chidaine B, Laisney MJ, et al. (2011a) Contamination by *Campylobacter* spp. of river water destined for human consumption in Brittany, France. Pathologie Biologie, 59:256-263.
Denis M., Henrique E., Chidaine B ., et al. (2011b) *Campylobacter* from sows in farrow-to-finish pig farms: Risk indicators and genetic diversity. Vet Microbiol. 154:163-170.
Jonas R, Kittl S, Overesch G, Kuhnert P. (2015) Genotypes and antibiotic resistance of bovine *Campylobacter* and their contribution to human campylobacteriosis. Epidemiol Infect. 2015 Aug;143(11):2373-80.
Mughini-Gras L., Penny, C., Ragimbeau C., Schets F., et al. (2016) Quantifying potential sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli. Water research, 101:36-45.*
Rincé A., Balière C., Hervio-Heath D., Cozien J., et al., (2018) Occurrence of Bacterial Pathogens and Human Noroviruses in Shellfish-Harvesting Areas and Their Catchments in France. Frontiers in Microbiology, Volume 9, Article 2443.
Zautner AE, Masanta WO, Tareen AM, Weig M, et al., (2013) Discrimination of multilocus sequence typing-based *Campylobacter jejuni* subgroups by MALDI-TOF mass spectrometry. BMC Microbiol. 2013 Nov 7;13:247.

P50

The potential of phytogenic feed additives in disease prevention and reduction of medications

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Introduction

Weaning is a stressful period in the life of pigs with increased susceptibility to environmental and pathogenic challenges (Pluske & Hampson, 1997). These challenges can cause a severe decrease in growth performance and an increase in the need for medications, causing overall considerable economic losses. In the past, antimicrobial growth promoters (AGP) like antibiotics and zinc oxide have been used to counteract these problems (Vondruskova et al., 2010). Increasing occurrence of antimicrobial resistances and a negative environmental impact of these feed supplements, however, raised the need to investigate alternatives to prevent disease development in livestock animals. Phytogenic feed additives (PFA), based on plants and plant derived products like essential oils, have the potential to support health and well-being of animals, in particular during critical periods of their lives (Yang et al., 2015). Consequently, the hereby presented current studies aimed to characterize the modes of action and benefits of PFA with regard to post-weaning diarrhea (PWD) in piglets.

Material and Methods

Three studies have been conducted to evaluate the potential of PFA on piglets' health. In study 1, the effects of fimbriae expression on the surface of pathogenic *Escherichia coli* was studied using an *in vitro* mucus adhesion assay. In brief, microtiter plates coated with ileal mucus of piglets were incubated with a radioactive labelled F4+ fimbriated *E. coli* strain and four different phytogenic substances at non-growth inhibiting concentrations (sub-MIC). Unbound bacteria were removed by washing and the measured remaining radioactivity corresponded to proportion of adhered bacteria.
In study 2 a trial with 132 piglets (6 per pen, 11 pens per treatment) was conducted to determine gut barrier integrity (Aumiller et al., 2018). Piglets received diets with or without supplementation of a phytogenic additive. At day 14 and day 42 one piglet per pen was slaughtered and samples from distal small intestine were used for an *ex vivo* FITC-4kDa permeability assay.
Study 3 was carried out in a commercial farm with history of high incidence of PWD. Piglets were assigned to two groups (200 male and 200 female animals per group): Whereas one group was fed an unsupplemented diet, a PFA was added to feed of the second group. Mortality, appearance of diseases and use of medication was recorded.

Results

Study 1: In untreated microtiter wells nearly 20% of radioactive labelled pathogens adhered to the mucus coating, indicating the occupation of all available receptor sites in the wells. Test substance 1 increased attachment to the mucus whereas substance 2 had no effect, and substances 3 and 4 reduced mucosal attachment compared to the control.
Study 2: Permeability for the FITC-4kDa marker was reduced in the PFA group at day 14 by 69.3% (P=0.049) compared to the control group. At day 42, a non-significant reduction was observed (difference of PFA to control -28.2%; P=0.465).
Study 3: Overall mortality was at a low level with 1.75% in the control and 1.0% in the PFA group. Occurrence of PWD (118/82 in control/PFA group) was reduced by 30.5% (P<0.001). Respiratory disorders (46/44 in control/PFA group, P=0.833) and other diseases (7/8 in control/PFA group, P=0.761) were not affected by treatment. Antibiotic treatments against PWD (82/45 in control/PFA group) were reduced by 45.1% with supplementation of the PFA.

Discussion and Conclusion

The occurrence of PWD in piglets is frequently associated with the presence of F4-positive *E. coli* strains (Fairbrother et al., 2005). These fimbriae are important for intestinal adhesion and colonization of *E. coli* and previous studies demonstrated, that quorum sensing is involved in their expression (Sturbelle et al., 2015). The results of study 1 strongly suggest, that several phytogenic substances are capable to influence adhesion behavior of F4 fimbriated *E. coli in vitro* at sub-MIC concentrations. It can be speculated, that this is a result of interference with bacterial quorum sensing. Reduced adhesion of pathogenic *E. coli* protects the intestinal tract from loss of gut barrier integrity. For this purpose a PFA was formulated, using efficient substances from study 1 and evaluated regarding its effect on gut barrier integrity in piglets in study 2. The reduction of FITC-4kDa in the *ex vivo* permeability assay suggests, that the prototypes were able to support the integrity of the piglets' small intestinal barrier at day 14 after weaning. During further maturation of the animals, this effect was nearly lost, although numerically lower permeability for FITC-4kDa was also seen at day 42. Using the same PFA in study 3 revealed its potential to protect piglets from the outbreak of PWD, indicated by both,

a significantly reduced number of sick piglets and reduction of medications needed to treat these animals. Due to the specific formulation of the PFA for PWD prevention no additional benefits could be detected with regard to respiratory and other disorders between control group and PFA treated animals.
In summary, it can be concluded, that specific PFAs are suitable to support post-weaning piglets against *E. coli* associated PWD. Application of these products can therefore reduce the negative economic impact of post-weaning health issues.

References

Aumiller T, Michiels J, Vinyeta E, van der Klis JD, Müller AS (2018): Influencing growth performance and intestinal barrier integrity with phytogenics. Proceedings of the 14th International Symposium on Digestive Physiology of Pigs (DPP2018), Poster 214.
Fairbrother JM, Nadeau E, Gyles CL (2005): Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Animal Health Research Reviews, 6(1), 17–39.
Pluske J and Hampson DJ (1997): Factors influencing the structure and function of the small intestine in the weaned pig: A review. Livestock Production Science, 51, 215-236. doi: 10.1016/S0301-6226(97)00057-2
Sturbelle RT, de Avila LF, Roos TB, Borchardt JL, da Conceição Rde C, Dellagostin OA, Leite FP (2015): The role of quorum sensing in Escherichia coli (ETEC) virulence factors. Veterinary Microbiology 180, 245-252. doi: 10.1016/j.vetmic.2015.08.015. Epub 2015 Aug 21
Vondruskova H, Slamova R, Trckova M, Zraly Z, Pavlik I (2010): Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. Veterinarni Medicina, 55(5), 199–224.
Yang C, Chowdhury MAK, Huo Y and Gong J (2015): Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens, 4(1), 137–156. doi: 10.3390/pathogens4010137

AMR IN THE PORK CHAIN

P26

Monitoring of antimicrobial susceptibility of E. coli and Salmonella from pigs in the Netherlands, 2016-2018

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GD Animal Health (AH) monitors antimicrobial susceptibility (AMS) of pathogens from different animal species. Previously, AMS testing was performed by agar diffusion using tablets; in 2012 GD AH switched to broth-microdilution and minimal inhibitory concentrations (MICs) are being determined since.
The objective of the present study was to analyse the *in vitro* AMS of *E. coli* (ECO) and *Salmonella* isolates originating from clinical submissions and post-mortem examinations from pigs, between January 2016 and December 2018.

MICs of in total 18 antimicrobials were assessed , MIC_{50} and MIC_{90} values were determined (results shown for ECO) and MICs were interpreted as susceptible, intermediate and resistant using CLSI veterinary breakpoints (when available).
ECO isolates (n=905) showed relatively high levels of resistance to the (according to the Dutch Pig Formulary) 1st choice antimicrobials tetracycline and trimethoprim/sulfamethoxazole (≥54%) and the 2nd choice antimicrobials spectinomycin and ampicillin (indicator of amoxicillin) (≥42%). ECO were well susceptible to the 2nd choice antimicrobials apramycin, gentamicin, amoxicillin-clavulanic acid, flumequine, colistin (≤2% resistance) and neomycin (≤9% resistance). Also for the 3rd choice antimicrobial enrofloxacin resistance was very low (≤1%) (see Table 1 and Table 2 for more details).
Dilution series applied for each individual antibiotic are marked green and red; green refers to the 'susceptible' and red to the 'resistant' range (where applicable, 'resistant' includes both

Table 1: MIC distribution (%) for enteropathogenic ECO isolates (n=270) originating from pigs submitted for post-mortem examination at GD AH and faecal samples submitted to the laboratory of GD AH, 2018

Antibiotic	Enteropathogenic E. coli (n=270)											
	MIC-values (µg/mL)											
	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Amoxicillin/Clavulanic acida	**0.0**	**0.4**	**9.6**	**26.7**	**25.6**	**36.3**	**1.5**	**0.0**	**0.0**			
Ampicillin	**0.0**	**0.0**	**11.5**	**23.3**	**8.5**	**0.7**	**0.0**	**0.0**	**55.9**			
Apramycin						**95.9**	**3.0**	**1.1**	**0.0**			
Cefepime			98.9	0.4	0.0	0.4	0.0	0.4	0.0			
Colistin		**86.3**	**10.0**	**1.1**	**0.7**	**1.1**	**0.4**	**0.4**				
Cefotaxime			99.3	0.0	0.0	0.7						
Enrofloxacin	**96.3**	**3.3**	**0.4**	**0.0**	**0.0**							
Florfenicol				4.1	48.1	35.2	12.6					
Flumequine				**93.7**	**4.8**	**1.5**	**0.0**	**0.0**				
Gentamicin				**98.5**	**0.7**	**0.4**	**0.4**					
Neomycin					**93.3**	**0.0**	**0.4**	**6.3**				
Sulfamethoxazole									**14.1**	**1.9**	**0.7**	**83.3**
Spectinomycin						**0.4**	**1.9**	**35.6**	**20.0**	**8.5**	**33.7**	
Streptomycin				**27.4**	**9.3**	**4.1**	**6.3**	**8.1**	**13.3**	**31.5**		
Tetracycline	**0.0**	**1.5**	**36.3**	**7.8**	**0.0**	**0.4**	**0.7**	**53.3**				
Tiamulin	0.0	0.0	0.0	0.0	0.0	0.4	0.4	3.0	96.3			
Tilmicosin	0.0	0.0	0.0	0.0	0.0	0.0	0.4	12.6	87.0			
Trimethoprim	0.0	38.5	1.1	0.4	0.0	0.0	0.0	60.0				
Trimethoprim-Sulfamethoxazoleb	**39.3**	**0.0**	**0.7**	**0.4**	**0.0**	**59.6**						
Tylosin	0.0	0.0	0.0	0.0	0.0	100.0						

'intermediate susceptible' and 'resistant'). To the right of the dilution ranges shown in green and red, percentages of isolates with a MIC value higher than the highest concentration of the dilution range are mentioned in red. The percentage of isolates mentioned at the lowest concentration of a dilution range, refers to isolates with a MIC value equal to or lower than the lowest concentration evaluated in the specific dilution range. In bold the antibiotics mentioned in the Dutch treatment Formulary for Pigs for enteropathogenic ECO infections are shown.

[a] *Only the concentration of amoxicillin, tested in a 2:1 ratio (amoxicillin : clavulanic acid), is mentioned;*

[b] *Only the concentration of trimethoprim, tested in a 1 :19 ratio (trimethoprim : sulfamethoxazole) is mentioned.*

Similar results were found for *Salmonella* Typhimurium (STY; n=47) and other group B *Salmonella* isolates (SGB; n=101): increased levels of resistance to trimethoprim/sulfamethoxazole (≥28% of STY, ≥13% of SGB isolates), high levels of resistance to tetracycline (≥46% of STY, ≥63% of SGB isolates) and high levels of resistance to the 2nd choice antimicrobial amoxicillin (ampicillin is tested) (≥54% of STY, ≥73% of SGB isolates). For the 2nd choice antimicrobials apramycin, flumequine, neomycin, amoxicillin-clavulanic acid the percentage of resistant isolates was low (0-3%). No STY or SGB isolates tested resistant to enrofloxacin.

Among ECO, STY and SGB from pigs, high levels of resistance to the 1st choice antimicrobials are found, whereas emergence of resistance to 2nd and 3rd choice antimicrobials appears to be (very) limited. Hence, also resistance against antimicrobials of high interest for human health (colistin) is (very) low. Interpretation of MICs for ECO and *Salmonella* is strongly hampered by the lack of CLSI-defined clinical veterinary breakpoints. More veterinary breakpoints are needed to overcome this problem and to conduct a clinically reliable monitoring of AMS.

Table 2: MIC50 and MIC90, and percentage susceptible, intermediate and resistant for enteropathogenic ECO isolates from post-mortem examination at GD AH and faecal samples submitted to the laboratory of GD AH, 2018, 2017 en 2016

	E. coli (n=270), 2018			E. coli (n=339), 2017			E. coli (n=296), 2016		
Antibiotic	MIC50	MIC90	R	MIC50	MIC90	R	MIC50	MIC90	R
	(µg/mL)	(µg/mL)	(%)	(µg/mL)	(µg/mL)	(%)	(µg/mL)	(µg/mL)	(%)
Amoxicillin/Clavulanic acida	4	8	0	4	8	0.3	4	8	0.0
Ampicillin	>32	>32	55.9	>32	>32	60.2	>32	>32	58.8
Apramycin	≤8	≤8	1.1	≤8	≤8	0.0	≤8	≤8	0.0
Cefepime	≤1	≤1	0.4	≤1	≤1	0.9	≤1	≤1	0.3
Colistin	≤0.5	1	1.9	≤0.5	≤0.5	1.5	≤0.5	≤0.5	2.4
Cefotaxime	≤1	≤1	0.7	≤1	≤1	0.9	≤1	≤1	0.7
Enrofloxacin	≤0.25	≤0.25	0	≤0.25	≤0.25	0.3	≤0.25	≤0.25	0.0
Florfenicol	4	>8	47.8	4	8	48.4	4	8	38.5
Flumequine	≤2	≤2	0	≤2	≤2	1.5	≤2	≤2	0.3
Gentamicin	≤2	≤2	0.4	≤2	≤2	0.0	≤2	≤2	0.0
Neomycin	≤4	≤4	6.3	≤4	≤4	8.6	≤4	≤4	6.8
Sulfamethoxazole	>256	>256	83.3	>256	>256	76.1	>256	>256	74.0
Spectinomycin	64	>128	42.2	64	>128	49.9	64	>128	42.9
Streptomycin	32	>64	53	32	>64	56.6	64	>64	57.6
Tetracycline	>16	>16	54.1	>16	>16	66.1	>16	>16	69.9
Tiamulin	>32	>32	99.3	>32	>32	99.7	>32	>32	98.3
Tilmicosin	>32	>32	99.6	>32	>32	99.1	>32	>32	98.6
Trimethoprim	>16	>16	60	>16	>16	65.2	>16	>16	64.2
Trimethoprim-Sulfamethoxazoleb	>4	>4	59.6	>4	>4	64.6	>4	>4	63.9
Tylosin	>4	>4	Rint	>4	>4	Rint	>4	>4	Rint

P28

Antimicrobial resistance of Yersinia enterocolitica O:3 isolated from tonsils and lymph nodes of slaughtered pigs in Croatia

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Introduction

Human yersiniosis presents one of the main foodborne zoonoses in European Union (1). The main carriers of pathogenic Yersinia enterocolitica are pigs, and meat can be contaminated during slaughter processing. Very limited research of Y. enterocolitica in the context of food chain are available in Croatia. First published study (2) showed low prevalence (0.08%) of pathogen in pork meat, processed meat and surface swabs of meat processing units. However, authors isolated pathogenic Y. enterocolitica strain resistant to main clinical antibiotics relevant at the time of study (1990ties). Recent studies in Croatia (3, 4) evaluated the prevalence of Y. enterocolitica in food chain, including pig tonsils and mandibular lymph nodes, pork meat and meat preparations, thermally processed and fermented meat products, raw milk and unpasteurized milk cheeses. Y. enterocolitica O:3 strains were only recovered from 26 tonsils (33.33%), 8 mandibular lymph nodes (10.25%) and retailed pork meat (6.25%).

Since antimicrobial resistance (AMR) in food chain is one of a leading One Health issues, the aim of presented study was to evaluate it in Yersinia enterocolitica O:3 strains collected from tonsils and mandibular lymph nodes of slaughtered pigs in Croatian abattoirs.

Materials and Methods

Pig tonsils (n=78) and mandibular lymph nodes (n=78) were sampled on slaughter-line and subjected to microbiological testing for presence of Y. enterocolitica, as reported elsewhere (2). Three different types of abattoirs were selected, and pigs were originated from individual households, medium-size family farms and large farms.

Presumptive colonies (n=49) were selected from CIN and CHROMagarTM Y.enterocolitica and subjected to MALDI-TOF MS identification (Bruker Daltonik, Bremen, Germany) and serotyping (Statens Serum Institute, Denmark). Antimicrobial susceptibility was tested by disk diffusion method toward levofloxacin, ciprofloxacin, ampicillin, cephalothin, cefotaxime, tetracycline, nalidixic acid, ceftazidime, trimethoprim/sulfamethoxazole, chloramphenicol and streptomycin. Antimicrobial susceptibility/resistance of strains was assessed following EUCAST/CLSI guidelines.

Results

All selected colonies were identified by MALDI-TOF MS as Yersinia enterocolitica and belonged to O:3 serotype. The majority of strains was resistant toward ampicillin (91.6%) and cephalothin (85.4%), followed by chloramphenicol (31.2%), nalidixic acid (31.2%), streptomycin (27.0%), tetracycline (8.3%) and trimethoprim/sulfamethoxazole (2.0%). Only one strain was susceptible to all antimicrobial agents tested. Y. enterocolitica strains from medium-scale farms were mostly resistant to ampicillin and cephalothin, while strains collected from large farms were additionally resistant to chloramphenicol, nalidixic acid and streptomycin. Multiresistance (resistance to three or more agents) was found in 17 strains (35.4%). Higher prevalence of multiresistant Y. enterocolitica was evident in pigs originated from large farms (Table 1).

Discussion and Conclusion

Y. enterocolitica strains are usually resistant to penicillin, ampicillin, and first-generation cephalosporins. First-line drugs used against the bacterium include aminoglycosides and trimethoprim-sulfamethoxazole and other effective drugs include third-generation cephalosporins, tetracyclines and fluoroquinolones (5). The presence of resistant Y. enterocolitica in pigs at slaughter has been studied in recent years in many European countries (6, 7), but not in Croatia. Similar to our results, Fois et al. (6) reported the most common resistance to ampicillin and cephalothin in slaughtered pigs in Sardinia, Italy. In Latvia (8), additional resistance of all tested Y. enterocolitica was found toward erythromicin and sulphamethoxazole. Bonardi et al. (7) in North Italy also reported high level of resistance against sulphonamides in slaughtered pigs. In contrast, the resistance level toward sulphonamides in our study was low, as reported by other authors in Switzerland or Germany (9, 10). Opposite to other studies (11, 12), our isolates showed relative high resistance (about 30%) toward chloramphenicol, nalidixic acid or streptomycin. In conclusion, AMR in *Y. enterocolitica* of slaughtered pigs in Croatia is comparable to data from other European countries. The majority of strains were susceptible to clinically relevant antimicrobial agents.

References

1. EFSA and ECDC (2018): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J 16(12):5500, 262 pp.

Table 1: Number of (multi)resistant Y. enterocolitica strains and resistance profiles

	Slaughterhouse 1 (medium-size farms)	Slaughterhouse 2 (large farms)	Slaughterhouse 3 (medium-size farms)
Tested strains	15	26	8
Resistant strains	14	26	8
Multiresistant strains	2	15	0
Dominant resistance patterns	AMP-KF (n=10)	AMP-KF-NA-C-STR (n=11)	AMP-KF
Multiresistance patterns	AMP-KF-TET, AMP-KF-TET-C, AMP-KF-NA	AMP-KF-TET-NA-C-STR, AMP-KF-NA-C-STR, TET-NA-CAZ-TST, KF-NA-C-STR, NA-C-STR	-

2. Hadžiosmanović M, Noso M, Živković J (1992): Prevalence of Yersinia enterocolitica in meat and meat products. Stočarstvo 46, 141-152. (In Croatian)
3. Zdolec N, Dobranić V, Filipović I (2015): Prevalence of Salmonella spp. and Yersinia enterocolitica in/on tonsils and mandibular lymph nodes of slaughtered pigs. Folia Microbiol 60, 131-135.
4. Zdolec N, Dobranić V, Kazazić S, Šimunić I, Dumbović Z (2017): Isolation and identification of Yersinia enterocolitica strains from food production chain. Book of Abstracts 7th International Congress "Veterinary Science and Profesion". Faculty of Veterinary Medicine, University of Zagreb, pp 99.
5. Web site. https://emedicine.medscape.com/article/232343-treatment
6. Fois F, Piras F, Torpdahl M, Mazza R, Ladu D, Consolati SD, Spanu C, Scarano C, De Santis EPL (2018): Prevalence, bioserotyping and antibiotic resistance of pathogenic Yersinia enterocoliticadetected in pigs at slaughter in Sardinia. Int J Food Microbiol 283, 1-6.
7. Bonardi S, Bruini I, D'Incau M, Van Damme I, Carniel E, Bremont S, Cavallini P, Tagliabue S, Brindani F (2016): Detection, seroprevalence and antimicrobial resistance of Yersinia enterocolitica and Yersinia pseudotuberculosis in pig tonsils in Northern Italy. Int J Food Microbiol 235, 125-132.
8. Terentjeva M, Bērziņš A (2010): Prevalence and Antimicrobial Resistance of Yersinia enterocolitica and Yersinia pseudotuberculosis in Slaughter Pigs in Latvia. J Food Protect 73, 1335-1338.
9. Fredriksson-Ahomaa M, Stolle A, Stephan R (2007): Prevalence of pathogenic Yersinia enterocolitica in pigs slaughtered at a Swiss abattoir. Int J Food Microbiol 119, 207-212.
10. Bucher M, Meyer C, Gro¨tzbach S, Wachneck S, Stolle A, Fredriksson-Ahomaa M. (2008): Epidemiological data on pathogenic Yersinia enterocolitica in southern Germany during 2000-2006. Foodborne Pathog Dis 5, 273-280.
11. Simonova J, Borilova G, Steinhauserova I (2008): Occurrence of pathogenic strains of Yersinia enterocolitica in pigs and their antimicrobial resistance. Bull Vet Inst Pulawy 52, 39-43.
12. Bonardi S, Paris A, Bassi L, Salmi F, Bacci C, Riboldi E, Boni E, D´incau M, Tagliabue S, Brindani F (2010): Detection, Semiquantitative Enumeration, and Antimicrobial Susceptibility of Yersinia enterocolitica in Pork and Chicken Meats in Italy. J Food Protect 73, 1785-1792.

P29

Phenotypic and genotypic characteristics of Escherichia coli with non-susceptibility to quinolones isolated from environmental samples on pig farms

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Introduction

In the last decade, the growth of the pig-farming industry has led to an increase in antibiotic use, including several used in human medicine, e.g. (fluoro)quinolones. Data from several studies suggest that there is a link between the agricultural use of antibiotics and the prevalence of antibiotic-resistant bacteria in the pig farm environment, including (fluoro)quinolone resistance. This poses a threat to human and animal health. Our goal was to phenotypically and genotypically characterise 174 *E. coli* showing non-susceptibility to quinolones isolated from environmental samples from pig farms.

Material and Methods

Antimicrobial susceptibility testing (AST) was performed using the disk diffusion method. PCR and sequence analysis were performed to identify chromosomal mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and the isolates were screened for the presence of the plasmid-mediated quinolone resistance (PMQR) genes *aac-69-Ib-cr*, *qepA*, *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS*.
Strain relatedness was assessed by phylogenetic classification and multilocus sequence typing (MLST).

Results

Antimicrobial susceptibility testing by the disc diffusion method showed that 81% (n=141) of the strains were resistant and 19% (n=33) were intermediately resistant to nalidixic acid. Furthermore, 36.2% (n=63) of the isolates were also resistant to ciprofloxacin.
Additional antimicrobial resistance was most frequently observed for streptomycin (72.4% / n=126), tetracycline (60.9% /n=106), sulfamethoxazole/trimethoprim (50% /n=87), ampicillin (46.6% /n=81), kanamycin (19.5% /n=34), chloramphenicol (15.5% / n=27), and gentamicin (14.4% /n=25), respectively (Table 1). Resistance to all other tested antibiotics was detected for at least one isolate, except to nitrofurantoin.
Of the 174 isolates analysed in this study, 68.4% (n=119) were resistant to three or more classes of antibiotics and therefore categorised as MDR. The most frequent MDR combinations detected were SXT-TE-STR (n=15), AM-SXT-TE-S (n=10) and AM-SXT-STR-K (n=8) (Table 1). *E. coli* strains resistant to four and five antibiotics were the most prevalent (21.3% and 19.0%, respectively).
Of 141 isolates with a nalidixic acid resistant phenotype, 98.6% (n=139) possessed at least one nucleotide mutation in the QRDR of *gyrA*. Thereof, 49.6% (n=70) showed single amino acid substitution at codon Ser83, namely Ser83 to Leu (n=67), or Asp87 to Tyr (n=2), or Asp87 to Gly (n=1). Further, 48.9% (n=69) possessed double substitutions at Ser83 to Leu and Asp87 to Asn (n=68) or Tyr (n=1). Two isolates (isolates no. 65 and 106, respectively) tested negative for mutations in the QRDR of *gyrA* (Table 1).
A total of 38 strains possessed one or more PMQR genes, representing 21.8% of the 174 analysed strains (Table 1). Among the 19.5% (n=34) of the isolates with one PMQR gene, twenty (11.5%) possessed *qnrB*, thirteen (7.5%) *qnrS* and one isolate (0.6%) possessed *aac(6')-Ib*, respectively (Table 1). Four isolates (2.3%) possessed a combination of *qnrB* and *qnrS* genes. No isolates tested positive for *qnrA*, *qnrC*, *qnrD* or *qepA*. The occurrence of PMQR positive isolates was remarkably higher in strains exhibiting intermediate resistance to nalidixic acid (90.9% /n=30), than in nalidixic acid resistant strains (5.7% /n=8). Moreover, all *qnrB/qnrS* combinations were detected in intermediately resistant isolates (Table 1). Isolates possessing PMQR were found in 11 (22.9%) of the dust samples 16 (28.6%) of the wipe samples. and 11 (15.7%) of the slurry samples.
Of the 23 farms with reported use of fluoroquinolones, 12 (52.2%) yielded environmental *E. coli* containing PMQR genes. Thereof, the majority (7 farms/58.3%) were farrowing and rearing farms, three (25%) were fattening farms and two (16.7%) were mating and gestation farms (Table 1).
By contrast, of the 32 farms without a history of fluoroquinolone use during the study period, nine (28.1%) tested positive for *E. coli* harbouring PMQR genes. Thereof, five (55.6%) were fattening farms, four (44.4%) were mating and gestation farms, and none (0%) were farrowing and rearing farms.
The majority of the isolates were assigned to phylogenetic groups A (48.3%/n=84) and group B1 (33.3% /n=58). The remaining strains were classified into group C (9.8% /n=17), E (6.9%/n=12), F (1.1%/n=2) and D (0.6%/n=1), respectively. None of the isolates belonged to phylogenetic group B2.
Overall, a total of 50 STs were found. The most common sequence types were ST10 (n=20), ST297

(n=20), ST453 (n=10), ST88 (n=9), ST898 (n=8), ST93 (n=6), ST2197 (n=6), ST737 (n=5), and ST2509 (n=5).

Discussion and Conclusion

Quinolone non-susceptible *E. coli* are widespread in the environment of Swiss pig farms. In particular, isolates showing intermediate resistance to nalidixic acid frequently possess transmissible PMQR genes. This is worrisome, since the presence of *qnr* genes may increase the ability of bacteria to acquire point mutations in the gyrase and topoisomerase IV genes, resulting in high level resistance to (fluoro)quinolones. Furthermore, plasmids harbouring *qnr* genes may contribute to the horizontal spread of antibiotic resistance in livestock and in the environment. In pig farms which are part of sow pool systems, inter-farm measures that aim to reduce the risk of spreading resistant bacteria and resistance genes from one stage of production to the next need to be assessed and promoted. Our data further show that farm environments contain commensal MDR *E. coli* as well as *E. coli* with zoonotic potential. In particular, we demonstrate for the first time the presence of EPEC O80:H2 in an environmental sample from a pig farm.

P30

Temporal dynamics of enterobacteriaceae antimicrobial resistance at the human-pig interface in Peri-Urban Kampala

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Antimicrobial resistance (AMR) leads to increased mortality, morbidity and health expenditure. Globally, there is an increasing concern over AMR which is claiming 700,000 people every year and this is projected to 10,000,000 people by 2050. The recently documented AMR rates paint an increasingly alarming scenario for Uganda, and if strategic measures are not taken to halt and reverse the trends, treatment options for infectious diseases will always become more limited to many financially constrained Ugandans

A longitudinal study of linked human-pig pairs in Kampala and Wakiso Peri Urban setting was carried out to determine antimicrobial sensitivity profile of Enterobacteriaceae at the human-pig interface within a six months period. Purposive sampling was done to select pig farmers to be included in the study based on type of pigs kept. I selected farmers who had breeding sows or boars that were to be kept for more than one year. Here, we visited 35 pairs (human and pigs) for every two months, and for six months, we collected approximately 220 fecal samples. In addition, metadata i.e. house hold demographics Nutrition, Pig management, disease occurrences and antibiotic use by using a mobile deployed questionnaire was collected.

I found a 72% mono resistance prevalence for all isolates recovered, predominated by resistance to trimethoprim/sulfur, tetracycline and amoxicillin. 45.1% of the isolates were resistant to more than one antibiotic (multidrug resistant), dominated by *E.coli* (60%) and Klebsiella (36%). We observe evidence of AMR phenotype exchange/sharing in one among six pig farmers in Kampala, which reaffirms the occupational risk they represent to the general population. These findings taken together indicate that a highly dynamics flux in resistance prevalence generally increased over the six months period at the human-pig interface. In the short term, further investigation using granular molecular methods are needed to understand the observed dynamics. In the medium and long term, we need to understand behavioral drivers of antibiotic usage in order to limit the irrational use that is driving the observed resistance profiles. From a public health point of view, farmers are likely to be the source of animal generated resistance for the general population, therefore, occupational health experts need to focus on identifying critical control for transmissions arising from this group.

P31

Quantitative investigation of ESC producing E. coli in the Danish pork meat chain with estimation of the full burden of bacteria carrying blaCTX genes

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Introduction

Third and fourth generation cephalosporins are considered critical important antibiotics for treating serious infections in humans and the presence of extended-spectrum cephalosporinase (ESC)-producing bacteria in the food animal production is therefore a serious concern internationally (EFSA, 2011).

In 2010, the Danish pig industry introduced a voluntary stop for use of critically important antibiotics belonging to the group of cephalosporins. A decline in ESC resistance in pigs after the stop of using cephalosporins has been shown. By a selective enrichment procedure, Agersø and Aarestrup (2013) showed a significant reduction of the presence of ESC producing E. coli in caecal samples from pigs at slaughter, with a prevalence reduction from on average 10.9% in 2009/2010 (N=1193) to 3.5% in 2011 (N=777). The DANMAP procedure for testing random E. coli isolates from pigs and pig meat has not been designed to describe actual occurrence or differences between the years 2009-2017 (DANMAP 2009-2017).

This project aimed to provide a quantitative estimate on the prevalence and concentrations of ESC resistance carried in E. coli and in the total microbiome in the pig meat chain from slaughter to retail (Figure 1). A retail exposure assessment was defined as the quantitative occurrence of ESC resistance in 100 gram meat cuts.

Materials and Methods

During 2015 to 2018 the Danish pork chain has been investigated qualitatively and quantitatively for ESC producing E. coli and Enterobacteriaceae. The level of resistance carried by animals into slaughter was measured on caecal content (N=266). The contamination of the carcass at slaughter was measured from carcass swabs of 1400 cm^2. The contaminations at cutting (N= 288) and retail (N=529) were measured from meat cut samples of 100 cm^2. Extended-spectrum cephalosporinase (ESC)-producing E. coli and Enterobacteriaceae were culture quantified by direct plating on cefotaxime (FOT) and tetracycline (TET) containing media. A more sensitive qualitative culture analysis for ESC producing E. coli using pre-enrichment according to the standard procedure for the harmonised EU surveillance on antimicrobial resistance was also carried out.

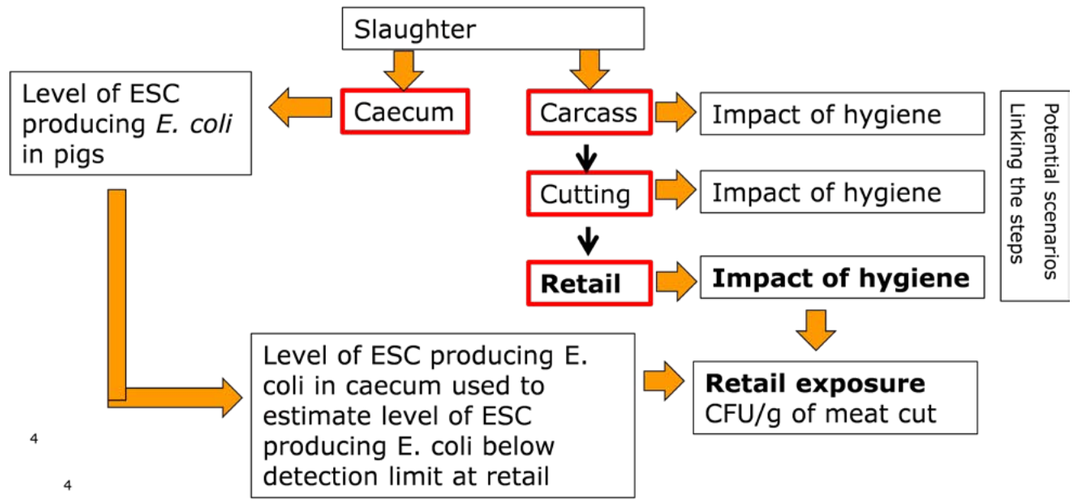

Figure 1: Diagram of elements in the ESC retail exposure assessment. Red boxes: points of data generation

To quantify the total bacterial population carrying specific resistances, qPCR was performed using primers specific for tetA , tetB, bla$_{CTX}$ genes, and for uidA (E. coli). The regression of qPCR C$_T$ values against E. coli cell counts was used to design standard curves, which enable linking of a qPCR C$_T$ value to a corresponding cell count. By this method, concentrations of bacteria carrying bla$_{CTX}$, tetA and tetB genes were estimated.

As the resistance genes analysed by qPCR target all bacteria carrying the gene, the joined data set can be used to analyse to what extent resistance occurs within E. coli compared to the total bacterial population, and how the bacterial population structure changes over the pig meat chain. The principle for quantification of the total pool of bla$_{ctx}$ genes in a sample is illustrated in Figure 2.

Maximum likelihood methods and Tobit regressions (Lorimer and Kiermeyer, 2007) were used to determine quantitative levels of ESC producing E. coli and TET resistant E. coli below the detection limit (Figure 3), which enables us to do a comparative assessment of E. coli and the total number of bacteria carrying specific ESC genes in the meat at retail. To substantiate modelling at retail, data generated at slaughter was included to support the analysis.

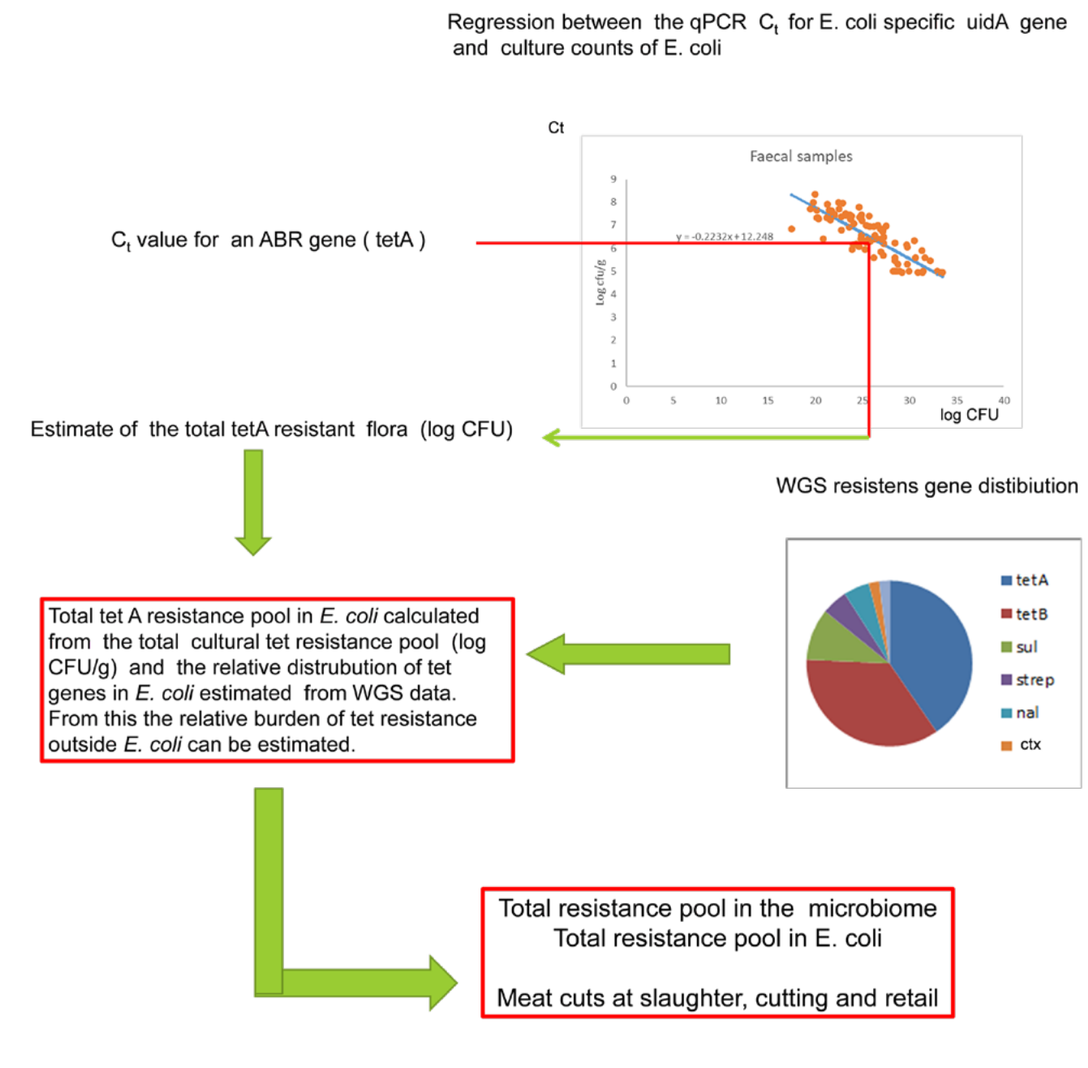

Figure 2: Principle for quantification of blactx resistance gene pool in E. coli and in the total microbiome in pig meat at slaughter, cutting and at retail

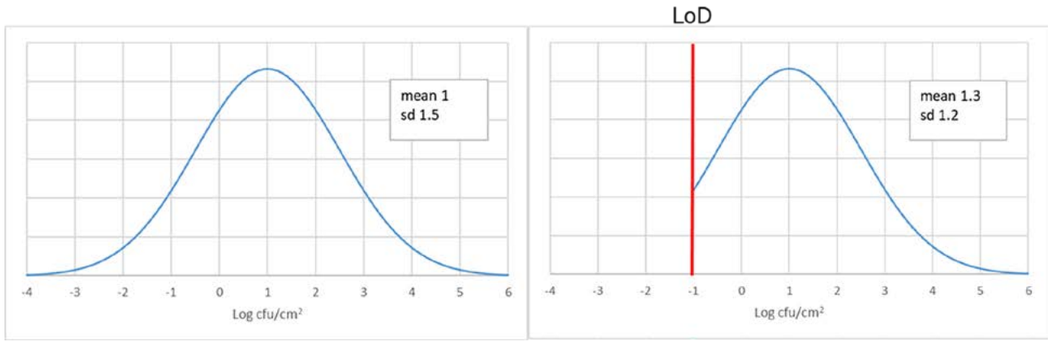

Figure 3: The (hypothetical) true distribution of concentrations (left) and the mean and standard deviation found, if the values below the LoD were not taken into account

Results

In feces, on carcasses, at cutting and at retail the observed prevalence of cefotaxime resistant E. coli was 32% , 2%, 1% and 1%, respectively. The observed mean log concentrations were 2.3 log cfu/g, 2.4 log cfu/1400 cm², -0.4 log cfu/cm², and at retail it was below the detection limit. To estimate the concentrations of ESC producing E. coli at retail we used the concentrations of E. coli and ESC producing E. coli in faecal samples for modelling mean concentration of ESC producing E. coli estimated to be -5.2 log CFU/cm2, with standard deviation 1.47. Calculating back to portions this would imply 0.2% of 100 g portions of raw pig meat at retail to be contaminated with at least 1 CFU ESC producing E. coli. The prevalence of meat being contaminated with ESC producing E.coli at 10 or 100 CFU/ 100 g was estimated to be 0.01% and 0.001%.
To compare the ESC carriage in all bacteria to that of E. coli, using Tobit regression, we estimated that the qPCR based prevalence of 100 g portions contaminated with bla_{CTX} carrying bacteria at slaughter, cutting and retail to be 4.0%, 3.6% and 0.9% respectively compared to the culture based prevalence of ESC producing E. coli of 3.6% , 3.5% and 0.2%. This indicates that a significant part of ESC resistance in pig meat is carried by E. coli.

Discussion

Despite the ban of cephalosporins for almost 10 years in the pig production, ESC resistance prevails in the Danish pork industry. The reason for this persistence is not clear, but co- and cross-resistance may play a role (Jensen et al., 2018). The use of any beta-lactam antibiotics in the primary production such as ampicillin or penicillin will select for existing ESC producing bacteria. Also, if the ESC genes do not hamper the ecological fitness of the bacteria, ESC producing bacteria may be able to sustain intestinal colonisation in pigs without any selective pressure as indicated for ESC producing E. coli in broilers (Mo et al. 2016).
Using the direct culture methods, the quantitative occurrence of ESC producing E. coli at retail was below detection limits and made it impossible to assess the retail exposure based on available culture data. This led to a novel approach, using Tobit regression, to extrapolate quantitative distributions for ESC producing bacteria below culture detection limits at retail. This extrapolation incorporated the use of data from slaughter and cutting plants to construct retail distributions. By defining a retail exposure as the level of contamination of 100 gram portions, we estimate that only 1 in 500 portions will be contaminated with at least 1 CFU ESC producing E. coli and 1 in 100.000 portions will be contaminated with more than 100 CFU. Based on qPCR amplification, we also suggest that E. coli is a major carrier of ESC genes in pig meat.

References

Agersø, Y, and Aarestrup, FM (2013). Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended spectrum cephalosporinase producing Escherichis coli in slaughter pigs. Journal of Antimicrobial Chemotherapy, 68, 569-572. Jensen, LB, Birk, T, Høg, BB, Larsen, LS, Aabo, S, and Korsgaard, HB (2018). Cross and co resistance among Danish porcine E. coli isolates, Research in Veterinary Science, 119, 247-249. Lorimer,M.F., Kiermeier, A., 2007. Analysing microbiological data: tobit or not tobit? Int. J.Food Microbiol. 116 (3), 313–318. Mo, S.S., Norström, M., Slettemeaås, J.S., Løvland, A., Urdahl, A.M., and Sunde, M. (2014). Emergence of AmpC-producing Escherichia coli in the broiler production in a country with a low antimicrobial usage profile. Veterinary Microbiology, 171, 315-320.

MONITORING AND SURVEILLANCE SYSTEMS IN THE PORK CHAIN

P32

Detection of Salmonella antibodies in asymptomatic fattening pigs varies depending on the test and the matrix used

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Introduction

Human salmonellosis is a common meat-borne infection in Europe including Finland. Around 10% of the domestic cases are due to contaminated pork in Finland. *Salmonella* infection in fattening pigs is mostly asymptomatic and therefore identification of *Salmonella*-positive pigs is usually not possible at the slaughterhouse. Serological testing has been established in some European countries to identify fattening farms producing *Salmonella*-infected animals. The presence of antibodies to *Salmonella* can easily be detected by commercial ELISA kit using blood or meat juice samples collected at the slaughterhouse (Felin et al. 2015, 2019). However, reliable and comparable commercial ELISA tests are of major importance for serological monitoring (Felin et al. 2017). In this work, presence of *Salmonella* antibodies were studied in blood and meat juice samples of Finnish fattening pigs with two commercial ELISA tests.

Material and Methods

In total, 146 blood samples of fattening pigs originating from 29 farms (1-10 samples per farm) and 94 meat samples from 66 farms (1- 5 samples per farm) were selected for this study. The blood samples were collected from pigs at farm at the end of the fattening period before arrival to the slaughterhouse (Felin et al. 2019) and meat samples of diaphragm muscle were collected from fattening pigs at slaughter during *Trichinella* sampling (Felin et al. 2015). The samples were stored at -70°C until testing. Presence of *Salmonella* antibodies was studied with two commercial ELISA tests: Pigtype® Salmonella Ab (Qiagen, Leipzig, Germany) and PrioCheck® Porcine Salmonella kit (Thermo Fischer Scientific, Waltham, MA USA). Statistical analyses were performed using SPSS Statistics 24. Correlation between the ELISA tests was estimated by calculation of Spearman's rho. Additionally, Cohen's kappa value was calculated to test the level of agreement between the ELISA tests.

Results

The OD% values varied in blood samples (146) between 18 and 116 (median=29, mean=33) using Pigtype and between 1 and 70 (median=20, mean=21) using PrioCheck (Table 1). The OD% values varied in meat juice samples (N=94) between 9 and 55 (median=19, mean=21) using Pigtype and between 0 and 71 (median=9, mean=15) using PrioCheck (Table 1).
There was no correlation (P>0.05, Spearman's rho) between the tests. Using the cut-off OD% values of 20, 30 and 40, the detection rate of *Salmonella* antibodies in blood samples was clearly lower with PrioCheck compared to Pigtype (Table 2). There was a fair agreement (Cohen's kappa=0.247, P< 0.0001) between the tests when blood samples were studied using the cut-off OD% value of 30. In meat juice samples, higher detection rates were obtained with PrioCheck compared to Pigtype when the cut-off OD% values of 30 and 40 were used (Table 2).

Discussion and Conclusions

The key element of *Salmonella* control programs in Europe is the classification of fattening pig herds according to seroprevalence at slaughter measured by ELISA. ELISA test are typically reliable, accurate and cost effective. However, there is a lack of correlation between serological and microbiological results for detection of individual *Salmonella*-positive pigs and there is variability associated with the use of different ELISA kits and matrices (Mainar-Jaime et al. 2018).
In this study, we could show that blood samples had a clearly higher mean and median OD% values than meat juice samples, which also influenced the seroprevalence using different cut-off levels. This demonstrates that the matrix influences the OD% values and the seroprevalence, and therefore, the cut-off value should be adjusted depending on the matrix used. We could also show that the seroprevalence was clearly higher in blood samples with Pigtype compared to PrioCheck using cut-off OD% values of 20, 30 and 40. Blood samples were studied with a newer Pigtype test than meat juice samples. The newer Pigtype test detected antibodies to more antigens (serotypes), which may explain the higher detection rates compared to PrioCheck test, especially when the cut-off value of 40 was used. Interestingly, higher detection rates were obtained in meat juice samples with PrioCheck compared to Pigtype when cut-off OD% values of 30 and 40 were used. One reason can be the equation for the calculations of OD% values, which differs between the two tests and may influence the results. There was no clear correlation between the ELISA tests in

our study, which further complicates the comparison of serological results if they have not been studied with same methods.
This study show that the ELISA test used can strongly affect the results. This study also demonstrate that the cut-off value should be adjusted depending on the test and matrix used.

References

Felin E, Hälli O, Jukola E and Fredriksson-Ahomaa M (2019): Assessment of the feasibility of serological monitoring and on-farm information about health status for the meat inspection of fattening pigs. Prev. Vet. Med., 162, 76-82.
Felin E, Jukola E, Raulo S and Fredriksson-Ahomaa M (2015): Meat juice serology and improved food chain information as control tools for pork-related public health hazards. Zoonoses Public Health, 62, 456-464.
Felin E, Näreaho A and Fredriksson-Ahomaa M (2017): Comparison of commercial ELISA tests for the detection of *Toxoplasma* antibodies in meat juice of naturally infected pigs. Vet. Parasitol. 238, 30-34.
Mainar-Jaime RC, Casanova-Higes A, Andrés-Barranco S and Vico JP (2018): Looking for new approaches for the use of serology in the context of control programmes against pig salmonellosis. Zoonoses Public Health, 65, 222-228.

Table 1: OD% values in blood and meat juice samples using Pigtype and PrioCheck commercial ELISA tests

Sample	ELISA test	Mean	Median	SD	Min	Max
Blood	Pigtype	33	29	15	18	116
(N=146)	PrioCheck	21	20	11	1	70
Meat juice	Pigtype	21	19	7	9	55
(N=94)	PrioCheck	15	9	16	0	71

Table 2: Detection rates of Salmonella antibodies in blood and meat juice of fattening pigs using different tests and OD% values

Matrix	Test	Detection rate		
		OD%≥20	OD%≥30	OD%≥40
Blood	Pigtype	95%	49%	23%
(N=146)	PrioCheck	51%	19%	3%
Meat juice	Pigtype	50%	5%	2%
(N=94)	PrioCheck	26%	14%	9%

P33

Cross-linking existing official and private (business-owned) data for creating a trusted third party administered data information system as public-private-partnership tool for improving the welfare and health of pig herds (PPP-InfoS)

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Introduction
Along the pork food chain many data are generated which potentially give information about animal health and animal welfare. In the context of the project “PPP-InfoS” existing official data and the existing production management data from farmers and slaughterhouse operators were used for the creation of a data information system. This information system serves as a tool for early warning and prevention of health and welfare deficits in pig herds. A continuous improvement process of animal health and animal welfare is supposed to be realized by an aggregation of data into several animal health scores for a benchmark tool.

Material and Methods
After an intensive literature research, several animal-related health indicators for fattening pigs have been identified. Subsequently, by means of a questionnaire, various stakeholders of the pork food chain have been asked regarding the availability of animal health related data and the data flow between the stakeholders. The result is a list of animal health indicators that are standardized and electronically retrievable.
The identified meaningful and usable indicators were weighted on the basis of an expert survey and summarized into animal health scores. These scores depict various areas of animal health and provide a benchmarking system for the health status of pig farm units participating. The scores were validated on the basis of a questionnaire on the actual health status of fattening pigs and an anonymized data set provided by QS Quality and Safety GmbH.
Based on a research of legal requirements and previous interviews with the stakeholders of the pork food chain, different use cases for a data information system have been created. In addition, a concept has been developed that allows a secure exchange of information while maintaining data protection.

Results and Outlook
A detailed concept for a public-private-partnership data information system for improving the welfare and health of pig herd farm units has been developed. The most important use cases were already implemented by the Balvi GmbH in a demonstrator to illustrate the project result.
Efficient use of existing electronically-integratable animal health data through cross-linking is an important step in achieving a steady improvement in animal health. Our elaborated concept is a valuable instrument for the further development of a marketable information system.

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rentenbank

P34

Use of serology as a tool for control programs of Salmonella sp. in pork production chain

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Introduction

Brazil had regulamented in 2018 a self-control program with official verification of *Salmonella* sp. in pig carcasses for slaughterhouses submitted to Federal Inspection Service. This proposal was recently presented to the sector and will stimulate the agroindustry's to develop systemic strategies for the control and monitoring of *Salmonella* sp., which at some point should also be directed to pre-harvesting stage. In this context, the serology may help the production system to predict the risk of entry and dissemination of *Salmonella* sp. in slaughter environment, as well to evaluate control measures in the field and the herd sanitary evolution.

The objective of this research was to determine the correlation between serology, by seroprevalence and optical density values, with pigs shedding *Salmonella* sp. in faeces at the slaughter.

Material and Methods

The experiment was carried out on 20 growth and fattening pig farms, belonging to the same agroindustry integration system, with approximately 500 animals each. The pig herds were selected based on the following standard:

1- based on historical data of persistent infection by *Salmonella*sp.;

2- according to the housing program for piglets in the agroindustry.

Blood was collected from 30 animals by farm on the housing day. At the slaughterhouse, blood and portions of the ascending colon of were taken from 30 and 20 animals respectively. Serological analysis was performed using a commercial-ELISA (Herd Check Swine *Salmonella*®IDEXX Laboratories, ME, USA). The faeces were submitted to the isolation protocol of *Salmonella* sp. (ISO 6579: 2002). All statistical analyzes were performed using commercial software SAS®9.3: 2012. The association between the isolation of *Salmonella* sp. in the faeces with seroprevalence and with the intensity of the serological reaction measured by the optical density variability (% OD), through logistic regression.

Results

Considering 1200 blood serum collected, and a cut-off point of 20% OD, the seroprevalence at the time of housing ranged from 15 to 22%, and at slaughter rose to 75-80% in all batches, while optical density, in%, ranged from 10 to 22% to 75 to 95%. A total of 113 pigs (28,32%; IC 95%) were shedders *Salmonella* sp. in the faeces.

The correlation between the prevalence of pigs shedding *Salmonella* sp. in feces with the seroprevalence and optical density values was positive and significant ($p \leq 0.05$). For every 10 units of increase in seroprevalence (using a cut-off point of 40% OD), there was a 30.3% increment in the percentage of shedders(Figure 1), and for every increase of 10% in the% of OD value (Figure 2), was estimated a 15.6% increment of pigs shedding *Salmonella* sp.

Discussion and Conclusions

The antibody research, performed using a commercial available indirect enzyme-linked immunosorbent assay -ELISA (Herd Check Swine *Salmonella*® IDEXX Laboratories, ME, USA), considering 20% of the cut-off, has a low seroprevalence in the housing, varying from 15 to 22%, and at the time of slaughter it increased to 75 and 80%,which confirms that at this stage of production occurs the spread of bacteria among the animals and, consequently, an amplification of the infection. Previous studies have also found that pigs are infected at some point during the fattening period (Berends et al., 1996; Beloeil et al., 2003; Kranker et al., 2003). As the growth and fattening period lasts on average 110 days, the animals have the opportunity to become infected and seroconvert increasing the seroprevalence in the final phase of production.

From this perspective, biosafety is an essential component in the control of *Salmonella.* External biosecurity reduces the probability of introduction in the herd, while internal biosecurity reduces the spread of infection between stages and batches of pigs. Different measures of biosafety be they internal or external, as well as control of other enteric pathogens, were associated with seroprevalence of the herd and their deficiency increased the prevalence of *Salmonella* in pig farms (Arguello et al., 2018).

For being an indicative of the spread of bacteria in the herd, serology has been used as an indicator of risk in the batch of animals for the introduction of bacteria in the slaughter environment. It enables discrimination between herds in a concise, quick and inexpensive way. Serologically positive individuals, per se do not represent risk, however, in this study a rise of 10% in the herd seroprevalence was followed by an increase of 30% in the possibility of *Salmonella* sp. faeces excretion. In this way, the seroprevalence of the lot has a positive correlation with the excretion level in the faeces.

These data support the serology as a useful tool to discriminated pigs' batches in a *Salmonella* control program to be adopted by the agroindustry. Measures such slaughter logistics orientation according to% of seroprevalence, monitoring of sanitary herds evolution, standardisation of sanitary pyramids; checking of control programs already implemented and evaluations of specific strategies against *Salmonella* sp. at the pre-harvest level can be based in serology database.

References

Arguello H, Manzanilla EG, Lynch E et al (2018): Surveillance Data Highlights Feed Form, Biosecurity, and Disease Control as Significant Factors Associated with *Salmonella*Infection on Farrow-to-Finish Pig Farms. Frontiers in Microbiology. 9, 1-9.

Beloeil PA, Chauvin C, Proux K et al (2003): Longitudinal serological responses to *Salmonella* enterica of growing pigs in a subclinically infected herd. Preventive Veterinary Medicine. 60, 207-226.

Berends BR, Urlings HAP, Snijders JMA et al (1996): Identification and quantification of risk factors animal management and transport regarding in *Salmonella*spp. in pigs. International Journal of Food Microbiology.30, 37-53.

Kranker S, Alban L, Boes J et al (2003): Longitudinal Study of *Salmonella*enterica Serotype Typhimurium Infection in Three Danish Farrow-to-Finish Swine Herds. Journal of Clinical Microbiology. 41, 2282-2288.

SAS INSTITUTE INC. System for Microsoft Windows, Release 9.4, Cary NC, USA, 2002-2012. (cd-rom).

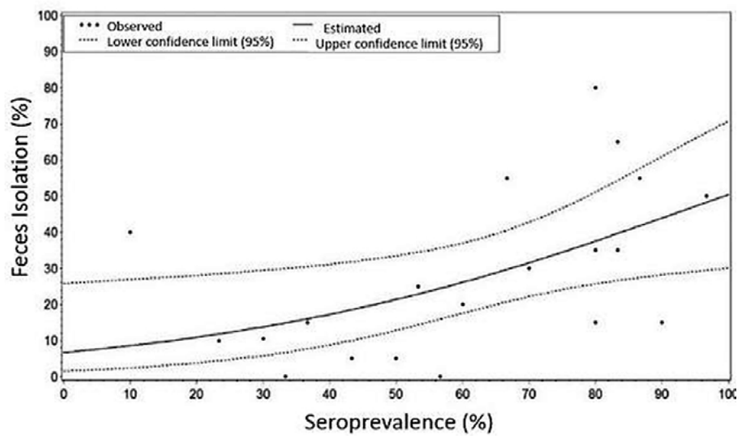

Figure 1: Percentage of isolation of Salmonella sp. in feces due to seroprevalence

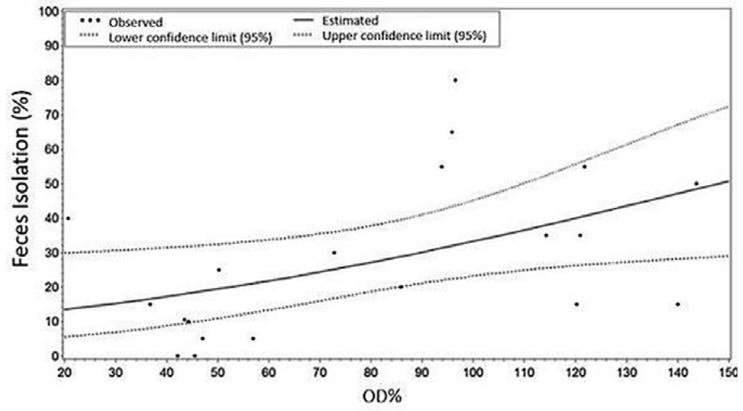

Figure 2: Percentage of isolation of Salmonella sp. in feces as a function of mean optical density

P35

Swine transit analysis in main regions of Minas Gerais state, Brazil, 2014

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Brazil stands out as the fourth largest world pig herd and third pork exporter until October 2018. The relevance of national pig farming in the economic and social scenario at global levels is noteworthy. The activity is characterized by concentrating its ventures in country main regions, especially South and Southeast, to reduce production costs and facilitate the supply chain logistics. The animal`s movements are intense, and the transit dynamics can be a risk factor for diseases entrance and spreading. Minas Gerais (MG) is responsible for the largest number of pig slaughtering among southeast states. The study objective is to characterize and analyze the pig traffic in Minas Gerais state, with emphasis on the animals movement among the state`s main regions, also identifying the traffic purposes: slaughter, fattening and reproduction. Data collection was performed through the Animal Transit Guides (GTAs), a mandatory document that accompanies all loads of live animals, whose information includes the animals number and traffic purpose in 2014. The GTAs were stored in state database, Instituto Mineiro de Agropecuária (IMA), the official organization responsible for the animal health and inspection. The software Pajek 1.24 was used for network design.A total of 84,595 GTAs were issued in MG in 2014, corresponding to 7,263,066 pigs on movement. Which 95,13%, 6,909,309 animals, were destined to state counties. The remaining had other states as destination. Animals for fattening from other main regions or states, with no inferior health status, were most representative category among all activities. The destination of a greater number of animals was to Triângulo and Alto Paranaíba, main state regions. Thus, those are the most vulnerable to pathogens introduction. Belo Horizonte area, main state city, received the largest pig volume, mainly for slaughter. The study allowed better visualization and characterization of animals transit purpose within the state. It is concluded that the tools used by the epidemiological surveillance system state body, can help in the health risks descriptions and allow the development of mitigation actions. In addition the methodology used can be expanded to other regions of the country.

P36

A Salmonella database to monitor and centralize regulatory own-checks results (CE) n°2073/2005 obtained by slaughterhouses

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Salmonellosis is a major cause of foodborne outbreaks caused by bacteria in Europe. In 2014, the European Commission reinforced the survey of this contaminant in the pig industry by the competent authority. In this context, French General Directorate for Food required a new system to centralize regulatory own-checks results for *Salmonella* in pig carcasses.
In 2014, the national pork trade association (INAPORC) has funded the development of a web application for collecting and analyzing the microbiological own checks performed by the French pig slaughterhouses. That year, among the three proposed procedures for the supervision of European regulation (EC) n°854/2004 modified by regulation (UE) n°218/2014, the French General Directorate for Food chose to collect results of regulatory own-checks (EC) n°2073/2005 performed by French pig slaughterhouses. The annual output results are transmitted to EFSA according to Directive 2003/99/EC for the monitoring of animal diseases and infections.
This innovative approach has been validated by the representatives of French pig slaughterhouses, the national pork trade association and the French Institute for pig and pork industry (IFIP).
In 2015, the IT team in cooperation with the fresh and processed meat department of IFIP has developed the web application: https://pdc.ifip.asso.fr .
Since January 2016, the French pig slaughterhouses have been able to input their microbiological own checks data into the web application. In December 2016, all the 166 approved French pig slaughterhouses sent their data to the database: https://pdc.ifip.asso.fr . Since 2017, a computer data retrieval system has transmitted to the French General Directorate for Food the results of regulatory own-checks for *Salmonella*. In addition, this web application allows pig slaughterhouses to monitor their process hygiene criterion in particular by cumulative attributes control charts.
In 2018, the application was improved by allowing data implementation of own checks data of ruminant animals (cattle, sheep, goats and horses) and with an additional module allowing data implementation of chemical and physical hazards for every species.

P37

Biosecurity in Italian pig farms - monitoring as a basis for targeted improvements

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Introduction

Monitoring biosecurity of pig farms is pivotal for farmers and veterinary authorities; particularly, when considering emerging and re-emerging diseases such as porcine epidemic diarrhoea (PED) and African swine fever (ASF). Both PED and ASF outbreaks may have severe consequences on pig production (Niederwerder and Hesse 2018; Sánchez-Cordón et al., 2018) and, in case of ASF, also result in bans on export. Improving biosecurity may also be essential to reduce antimicrobial use (AMU) without compromising production (Postma et al., 2017). In line, reducing AMU in livestock is part of the Italian national plan against antimicrobial resistance (Anon., 2017).
The aims of this study were to investigate biosecurity levels in Italian pig farms and identify potential areas for improvement as this has not been done before.

Material and Methods

Between Jan. 2017 and Jan. 2018, two researchers visited 124 pig farms during trial studies for the development of a monitoring system, called ClassyFarm, by the Italian Ministry of Health. All farms included were involved in the ClassyFarm trial on a voluntary base. Biosecurity was measured using Biocheck.UGent 2.1 (available at https://www.biocheck.ugent.be/), a risk-based survey which quantifies in percentages the on-farm biosecurity and provides a score for external biosecurity (all measures to prevent introduction of infection) and internal biosecurity (all measures to prevent spread of infection in the herd). Total biosecurity is calculated as the average of external and internal biosecurity. The survey encompasses six subcategories for external biosecurity and six for internal biosecurity (Fig.1). The relationships between farm size and biosecurity (total, internal and external) were investigated using Spearman´s rank-order correlation.

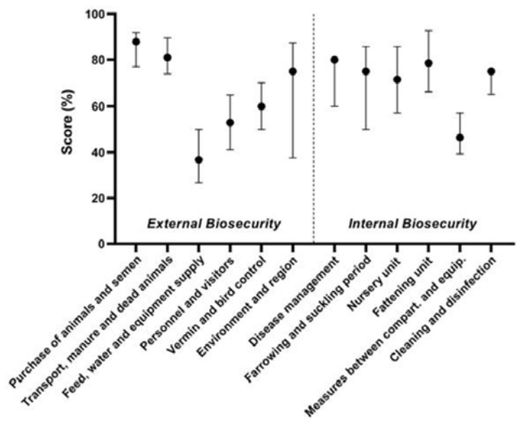

Figure 1: Median and interquartile ranges of 124 Italian pig farms of Biocheck.UGent 2.1 subcategories

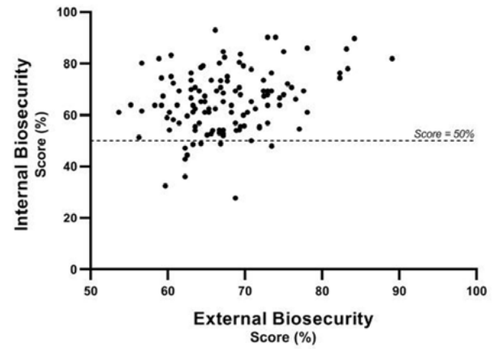

Figure 2: External and internal biosecurity of 124 Italian pig farms (Biocheck.UGent 2.1)

Results

The median yearly number of reared pigs in fattening farms was 7562 (range 1091-77349) while the median number of sows in the other farms was 490 (range 180-2600). The median total biosecurity was 66.3% (range 47.0%-86.1%), external biosecurity 67.2% (range 53.6-89.1%), and internal 65.3% (range 27.8-93.0%). Median scores below 50% were found in two subcategories, one for external biosecurity: "*Feed, water and equipment supply*" (36.7%; range 10.0-100%); and one for internal biosecurity: "*Measures between compartments and use of equipment*" (46.4%; range 17.8-92.9%). Figure 1 illustrates the median score and interquartile ranges of each subcategory.

The correlation between external and internal biosecurity (Fig. 2) was weak (ρ = 0.25) but significant (P = 0.006). A weak negative correlation (ρ = -0.25, P= 0.02) was found between size of fattening farms and internal biosecurity.

Discussion and Conclusion

Since the sample size was limited, results of this study should be interpreted with caution. Furthermore, the farms involved in this study were part of a convenience sample which may not be entirely representative of the Italian pig production.
External biosecurity was, on average, lower than what has been reported in countries such as Belgium, Denmark, Germany, Sweden, and the Netherlands (Filippitzi et al., 2017). This warrants attention due to the re-emerging of ASF in Europe (Sánchez-Cordón et al., 2018). Biosecurity levels of feed, water and equipment supplies were particularly poor, and this may lead to introduction of different pathogens which may increase AMU. These results highlight the importance of promoting good practices such as keeping trucks and transporters outside the clean areas, buying feed with proper hygienic standards, and monitoring the quality of drinking water.
Internal biosecurity was generally higher than in other countries (Filippitzi et al., 2018); nevertheless, biosecurity between compartments and equipment management were generally poor which may facilitate spread of highly contagious agents once introduced into a herd (e.g. PED virus). Hence, target measures should be promoted such as keeping proper disinfection baths between compartments and using compartment-specific equipment.
A detailed knowledge of biosecurity areas of improvement may guide policies of veterinary authorities and allow for targeted education of famers and vets. Finally, an important step towards better identification of areas of improvement could be applying a risk-based scoring system, such as Biocheck.UGent, to a sample of farms which is believed to be representative of the national pig production.

References

Anonymous (2017): Piano Nazionale di Contrasto dell'Antimicrobico-Resistenza (PNCAR) 2017-2020. Available at http://www.salute.gov.it/imgs/C_17_pubblicazioni_2660_allegato.pdf. Last accessed 23 April 2019.

Filippitzi ME, Brinch Kruse A, Postma M, Sarrazin S, Maes D, Alban L, Nielsen LR and Dewulf J (2018): Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. Transboundary and emerging diseases, 65(2), 381-398.

Niederwerder MC and Hesse RA (2018): Swine enteric coronavirus disease: a review of 4 years with porcine epidemic diarrhoea virus and porcine deltacoronavirus in the United States and Canada. Transboundary and emerging diseases, 65(3), 660-675.

Postma M, Vanderhaeghen W, Sarrazin S, Maes D and Dewulf J (2017): Reducing antimicrobial usage in pig production without jeopardizing production parameters. Zoonoses and public health, 64(1), 63-74.

Sánchez-Cordón PJ, Montoya M, Reis AL and Dixon LK (2018): African swine fever: A re-emerging viral disease threatening the global pig industry. The Veterinary Journal, 233, 41-48.

P38

Salmonella prevalence across different pork value chains in Hanoi, Vietnam

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Introduction

In Vietnam, pork and pork products still play an important role in food security which contributes more than 56% of total meat intake [1]. Each Vietnamese consumed approximately 29,1 kg pork/year, among the highest in the world [2]. However, along with the development, the pork value chain has been criticised for its quality degradation and lessened the trust of community, in which microbiology contaminated pork has been a critical issue. Previous studies reveal that salmonella contaminated in pork was so prevalence with 44.4% to 70.7% of pork in markets positive with Salmonella [3, 4], leading to human salmonellosis with 17.7% cases out of whole Vietnamese population [5].

Hanoi is the second biggest city in the country. To response to the high demand of consumer, pork is allocated through many distribution channels, from high-end to common level. However, evidences on the current state of pork across different value chains is still limited. Therefore, this research attempts to determine the Salmonella prevalence in various pork retail type and generate the clear evidence, which will contribute to the ambitious goal to combat with food safety issue in Vietnam.

Methods

Row pork was collected in retails from different actors in Cau Giay District, Hanoi from July to November 2018. Each sample was coded with identification and linked to checklist codes. Every sample was put a aseptic plastic bag with information sticked in, then all samples were preserved in cool boxes and transported to the Laboratory before 24 hours following [6]. Totally, there were 211 samples collected from both tradition (traditional market, wet market) and modern retail (supermarket, convenient store, boutique shop). Salmonella was detected by qualitative method (following ISO 6579:2017) and quantitative method (MPN method). Checklist was also used to observe the hygiene of pork shop and practice of retailers.

Results

Out of 211 pork samples, the percentage of sample positive with Salmonella was high with 63%, the average *Salmonella* concentration was 13.2 MPN/g. The modern retail showed a better result in Salmonella with the counterpart; however, supermarket was seen as the worst value chain with 82.9% positive sample while this figure in boutique shop was just 31.8%. Results from observation revealed that pork in modern retail was often wrapped, kept at cool cabinet and sometimes visible stamp by meat inspection authority. By contrast, pork from traditional retail mostly stemmed from suburban areas in the vicinity of Hanoi, be transported with the average distance of 31 km and sold without cover. In addition, hygiene practices of pork sellers were also poor, e.g., only 16% using gloves and 3% wearing hat and no separation between pork and intestines.

Discussion and Conclusion

The rate of Salmonella infection in our study (66.9%) in traditional retail was in line with a study of Nhung et al (2017) conducted in Ho Chi Minh city, Vietnam with 72.7% of pork in wet market and 68.4% in supermarket positive with Salmonella [7]. However, the finding of Toan et al (2013) showed that there was only 25% pork in traditional market in Hanoi contaminated with Salmonella. This situation can be attributed to the unhygienic along the value chain,

Table 1: Salmonella prevalence across different value chains

Value chain	Sal Y/N	Sal MPN (Mean ± SD)
Modern retail (105)	56.19	15.8 ± 35.2
Supermarket (35)	82.86	16.8 ± 35.4
Convenience store (48)	45.83	19.5 ± 40.0
Boutique shop (22)	31.82	0.5 ± 1.1
Informal retail (106)	66.87	11.1 ± 25.7
Traditional market (54)	81.48	9.1 ± 19.7
Wet market (52)	59.62	13.9 ± 32.5
Total (211)	63.03	13.2 ± 30.2

from slaughtering, transporting to selling. Although the number of convenient stores and boutique shop with high food safety standard has increased significantly recently in Hanoi, traditional markets still be a key actor in pork value chain when 80% of pork was delivered to consumer via them [1]. This emphasize the need of improve the hygiene practice for pork seller in traditional retail.

However, our result showed that although the hygienic practices at modern retail was quite good (e.g. pork was covered, storage in cool temperature), pork still high contaminated with Salmonella, which might be caused by potential high contamination during slaughter, processing and an extended storage time in the shop. The observed lower Salmonella contamination in boutique shops and management practice may be further explored in terms of its feasibility for other pork retail.

References

1. Unger, F., Hung Nguyen-Viet, Pham Duc Phuc, Sinh Dang Xuan, Pham Van Hung, Huyen Le Thi Thanh, Tuyet-Hanh Tran Thi, Makita, K. and Grace, D, Food safety in the pork value chain and pathways towards safer pork in Vietnam, in 15th International Symposium of Veterinary Epidemiology and Economics. 2018: Chiang Mai, Thailand.
2. OECD, Meat consumption (indicator) 2019.
3. Dang-Xuan, S., et al., Risk factors associated with Salmonella spp. prevalence along smallholder pig value chains in Vietnam. Int J Food Microbiol, 2018. 290: p. 105-115.
4. Nhung, N.T., et al., Antimicrobial residues and resistance against critically important antimicrobials in non-typhoidal Salmonella from meat sold at wet markets and supermarkets in Vietnam. Int J Food Microbiol, 2018. 266: p. 301-309.
5. Dang-Xuan, S., et al., Quantitative risk assessment of human salmonellosis in the smallholder pig value chains in urban of Vietnam. Int J Public Health, 2017. 62(Suppl 1): p. 93-102.
6. TCVN:4833-1, Meat and meat products sampling techniques - Kỹ thuật lấy mẫu thịt và sản phẩm thịt. 2002.
7. Nhung, N.T., et al., Antimicrobial residues and resistance against critically important antimicrobials in non-typhoidal Salmonella from meat sold at wet markets and supermarkets in Vietnam. International journal of food microbiology, 2018. 266: p. 301-309.

P51

CUTTING EDGE TECHNOLOGY: DIRECT FIELD TESTING USING A PORTABLE INSTRUMENT WITHOUT NUCLEIC ACID EXTRACTION

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Introduction

Worldwide, Central Reference Laboratory systems are being overwhelmed by the volume of African Swine Fever (ASF) samples being submitted. The surge volume is causing substantial delays in the reporting which confounds real-time decision making for control efforts. Here we demonstrate results of cutting edge technological developments that facilitate on site testing for Food and Mouth Disease (FMD) and African Swine Fever (ASF) viruses.

The NAHLN laboratories in the US utilize an ASFV real-time PCR based on a publication from Zsak, et al., 2005 which was designed and developed by Tetracore in 2000. The 16 year old design was evaluated in-silico in 2016 and modernized with an additional primer probe set to increase the potential for detection of contemporary strains of ASFV. The modernized test was evaluated in the field in collaboration with the National Veterinary Institute (SVA) in Uppsala, Sweden, an OIE Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine and the National Animal Disease Diagnosis and Epidemiology Center (NADDEC) in Entebbe, Uganda.

The World Organization for Animal Heath (OIE) Terrestrial Manual Chapter on FMD (3.1.8) recognizes two independent real-time RT-PCR assays, one targeting the 5'UTR and the other targeting the 3D region of the viral genome. The FMDV 3D assay was designed and developed in 2000 and published by Tetracore (Callahan et al, 2002). As viruses evolve over time it is prudent to periodically review the assay design against contemporary FMD sequences. In 2016 the 3D assay was updated with an additional primer / probe set that was added to the original assay design. The modernized test was then validated in collaboration with the Pirbright Institute, an OIE reference laboratory for FMD.

Materials and Methods

For ASF detection, this study was conducted in three stages over three years (2015-2017) as part of a project under the OIE in partnership with the SVA and NADDEC. Stage one focused on the adaptation of the magnetic beads-based protocol for nucleic acids extraction from 64 blood samples. In stage two, two sample testing strategies were tested in parallel: (1) direct testing of samples diluted in PBS were tested by the dried-down ASFV PCR kit with internal control (IC) (Tetracore Inc., Rockville, Maryland) on the portable real-time PCR thermocycler T-COR 8™ (Tetracore Inc.), and (2) samples underwent nucleic acid extraction and were tested by the OIE recommended Universal Probe Library (UPL) assay (Fernández-Pinero et al. 2013) on a Stratagene Mx3000P at NADDEC.

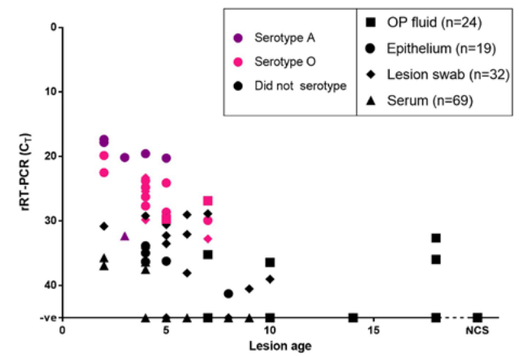

Figure 1: T-CORTM 8 field evaluation results for the 144 samples tested in East Africa. Shapes represents sample type. Colours represent T-CORTM 8 serotyping results. Samples were tested directly (no extraction) in duplicate

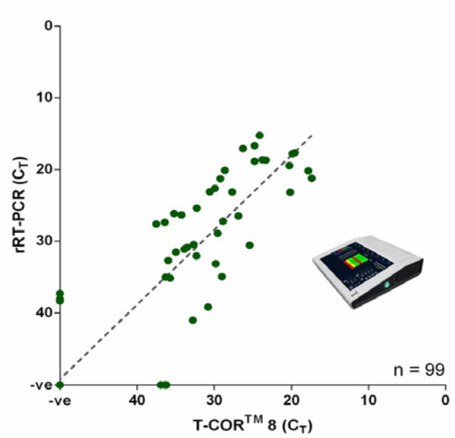

Figure 2: Comparison between laboratory based and field-based rRT-PCR

This parallel testing approach was also evaluated with selected samples in two villages in Northern Uganda during a 3-day outbreak investigation in 2016. In the third and last stage, further comparison of two diluents was performed by testing 46 blood samples in an austere lab setting in affected villages.

In another field study in Africa (Kenya, Tanzania, and Ethiopia), Tetracore's field deployable FMD detection assay was validated by utilizing epithelial tissue suspensions, serum, esophageal–pharyngeal (OP) fluid and oral swabs. The positive FMD samples from the study were then tested with a serotype specific field deployable Real-Time PCR assay, which covers the following serotypes O, A, Southern African Territories [SAT] 1 and 2. The positive results were confirmed by sequencing at the Pirbright Institute. Additionally, a robust sample preparation method for serum, esophageal-pharyngeal fluid and epithelial suspensions was developed to negate the need for RNA extraction prior to rRT-PCR.

Results

ASF - Pigs from two of the five suspected outbreak sites investigated were positive for ASFV using the ASF kit on the T-COR 8™. For blood diluted in PBS, inhibition was prevalent in 20-fold diluted and present in some 40-fold diluted samples. Archived samples were also tested and in total samples for twenty-two pigs were positive for ASFV out of sixty-nine tested.

These results matched those of the reference method in the lab at NADDEC with 100% correlation. Overall, the portable platform performed on par with the reference method.

FMD - The final rRT-PCR protocol and associated lyophilized reagents were field evaluated in three endemic settings (Kenya, Tanzania and Ethiopia), consistently detecting both clinical and subclinical FMD infections. Results of 145 samples tested in three test sites combined showed a 100% correlation between lab-based and field-based results.

The field studies in Africa showed that the reagents can be successfully lyophilized and stored under extreme conditions.

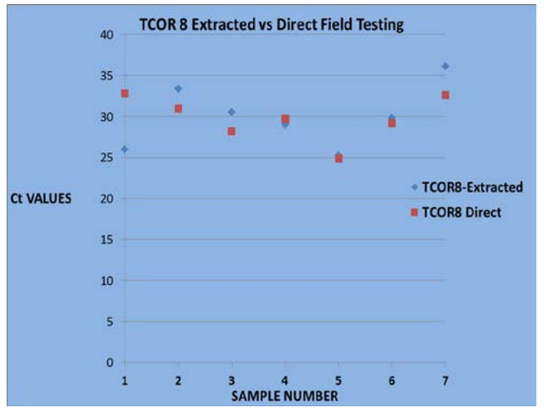

Figure 3: Comparison of direct and extracted whole blood samples

Discussion and Conclusion

Current delays in reporting from Centralized Reference Laboratories confound real-time decision making for animal control and disease containment efforts. These studies showed that confirmation of an outbreak can be performed on-site within 1.5-2 hrs, which would allow for real-time decisions to be made on animal control measures and containment efforts. The experience of performing the PCR assays in remote areas highlighted several factors that need to be carefully considered before deployment of portable technology: including biosafety issues, simplicity and effectiveness of sample preparation and turn-around time. Technical advances demonstrated are: dried assays stored at ambient temperatures; new chemistries that allow direct testing; and mobile, fieldable PCR instruments. Decentralized, on-site testing methods are undergoing validation efforts with OIE Reference Laboratories to enable the adoption of the technology by National Animal Disease Control authorities. The study demonstrated that the results of testing of samples at the point of care in remote field situations correlate very well with data generated from the same samples tested in OIE laboratories using reference methods.

MEAT INSPECTION AND TECHNICAL SLAUGHTER SOLUTIONS

P39

Prevalence of gastric ulceration in Italian heavy pigs

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Introduction

Gastric ulcerations in pigs are a pathology linked with nutritional, genetic and management factors. The stomach condition is generally scored using the classification proposed by Robertson et al. in 2002 which implies a 4 classes classification (0=no lesions, 1=parakeratosis, 2=mild ulceration, 3=severe and haemorrhagic lesions).

Materials and Methods

The sample size was estimated on the basis of previous studies (Gottardo et al., 2017), reporting a percentage of serious injuries (score 2-3) in pigs equal to 21%. Assuming a 95% confidence level, a power of 80% and 140 as the maximum number of animals slaughtered per batch, 91 is the number of pigs per batch that were to be taken. Since the aim of the study was also to compare the percentage of pigs with injuries between batches, assuming an average percentage of serious injuries of 21%, a standard deviation of 5%, an error of 1% and a confidence level of 95%, the animals from at least 96 batches had to be selected.
Sampling was performed in two large slaughterhouses placed in Lombardy region in a three months period from November 2018 to January 2019. Stomachs were analized by two trained veterinarians after stomach washing, around 45' after the stunning and bleeding of the animals.

Results and Discussion

103 batches (from 77 different farms) of 91 animals each were assessed for a total of 9371 animals. Class 0 to 3 were reported in 20.3%, 30.7%, 42.2% and 6.8% of the cases respectively. The prevalence of severe gastric injuries (score 2-3) was 47%, significantly higher than the 21% found by Gottardo et al. in 2017. Nevertheless, also the percentage (20.3%) of totally unaffected (score 0) animals was higher than the previous study (16.8%), globally showing a different distribution of the lesions within the animals.

Conclusions

The environmental and management factors leading to these findings have to be studied and further investigated together with the use of non-steroidal anti-inflammatory drugs that can be a further factor worsening the stomach mucosa conditions.

References

Robertson ID, Accioly JM, Moore KM, Driesen SJ, Pethick DW, Hampson DJ. (2002) Risk factors for gastric ulcers in Australian pigs at slaughter. Prev Vet Med. 15;53, 293-303.
Gottardo F, Scollo A, Contiero B, Bottacini M, Mazzoni C, Edwards SA. (2017) Prevalence and risk factors for gastric ulceration in pigs slaughtered at 170 kg. Animal, 11:11, 2010-2018.

P40

Retrospective study of hypodermic needles and other metallic physical hazards detected in the dismantling of pig carcasses by metal detectors

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Introduction

The objective of this study was to compare the occurrence of hypodermic needles with other metallic physical hazards, during the dismantling of pig carcasses.

Methods

- Data was collected over a period of 5 years and 3 months (63 months), from December 2012 to March 2018. The data included: gauge, length, localization and date of occurrence.

Other metallic physical hazards:

- Data was collected over a period of 2 years and 8 months, from January 2015 to August 2018, not including the year of 2017. The data included: localization, date and type or brief description.

Results

In a period of 63 months a total of 26 hypodermic needles were found , but in a much smaller period of 32 months a total of 23 other metallic hazards have been found.
Hypodermic needles:
Localization - 57,69% (n=15) were in the neck muscles and 23,07% (n=6) were found in the shoulder, making these the most affected parts of the carcass;
Length - Most of the needle fragments were between 2,4 cm and 2,6 cm, 84,62% (n=22);
Gauge - 57,69% (n=15) were 16 G and 26,92% (n=7) were 17 G;
Other physical hazards:
Type or brief description - the majority of hazards found were just simple metal fragments with no evident shape 73,91% (n=17), the second most frequent type were both steel filings and washers of steel mesh gloves 8,70% (n=2), the least common were metal bearings and metal filaments 4,35% (n=1);
Localization - the most common was the spare ribs 52,17% (n=13), the neck was the second most common 13,04% (n=3), followed by the ribs and belly both with 8,70 (n=2), the least common place was the shoulder, tenderloin and the ribbon all three with 4,35% (n=1).

Conclusions

The fact that the neck and shoulder were the parts with the highest number of hypodermic needles is possibly because of the preferential zones for IM and SC administrations are precisely in those two parts.
Although the frequency of needles per month (0,413) is much lower then the other metal objects (0,719), it still constitutes a large part of all the physical metal hazards identified.
If we consider all the objects found, we have a monthly average of more than 1 hazard / month, which clearly reflects the importance of equipment such as metal detectors and x-ray machines in the food industry and consumer protection.
It is essential to reflect on the potential impact on food safety arising from the use of hypodermic needles.

P41

Variation of reported meat inspection findings of slaughter pigs in Finland

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Introduction

Meat inspection data can be used for animal health and welfare monitoring (Horst et al., 2019; Stärk et al., 2014). However, the reported meat inspection findings can vary between slaughterhouses due to various reasons. The variation can be related to slaughterhouse arrangements such as slaughter line speed and light, lesion coding and recording methodology, but also personnel inspecting the carcasses (Horst et al., 2019). In Finland, abscesses, arthritis, milk spots, pericarditis, pleuritis and pneumonia of slaughter pigs are monitored in meat inspection (MAF 6/EEO/2012). According to the decree, the slaughterhouse operator is obliged to deliver the data to the pig producer from each batch. In addition, the official veterinarian has to notify the regional state administrative agency responsible for the animal welfare control of the farms where the proportion of arthritis, abscesses or tail bites exceed twice the mean of the slaughterhouse. The aim of this study was to assess the variation of these reported meat inspection findings in pig slaughterhouses in Finland.

Material and Methods

Yearly meat inspection data recorded in 2013–2018 for animal disease and welfare monitoring in three (A–C) pig slaughterhouses, slaughtering majority (98 % in 2018) of pigs in Finland, was collected from Finnish Food Authority meat inspection database. The occurrence of abscesses, arthritis, milk spots, pericarditis, pleuritis and pneumonia each year was recorded in Microsoft Excel 2016. The differences of the reported occurrences of these lesions between the slaughterhouses was tested using Independent-Samples Kruskal-Wallis Test and Dunn's post hoc test was used to test pair-wise differences in IBM SPSS Statistics 24. The mean occurrence of lesions in the slaughterhouses was calculated in Excel.

Results

The occurrence of all lesions varied between slaughterhouses significantly ($p < 0.05$). The occurrence of pericarditis differed significantly in two slaughterhouse pairs (AB and AC) and abscesses (AC), arthritis (AC), pleuritis (AC) and milk spots (AB) in one slaughterhouse pair. No significant slaughterhouse pair-wise difference of tail bites was observed. The mean occurrence of abscess was 2.9%–3.4%, arthritis 2.9%–3.3%, milk spots 5.6%–7%, pericarditis 2.3%–4.0%, pleuritis 16.6–22.6%, pneumonia 2.2%–2.4%, and tail bites 0.9%–1.7% during 2013–2018 and the occurrence was markedly affected by the slaughterhouse variation.

Discussion and Conclusions

The meat inspection findings vary significantly between the biggest pig slaughterhouses in Finland. Of the recorded lesions, tail bite findings were reported most uniformly. The reported variation between slaughterhouses can be related to the variation in animals and their background. E.g. production systems (conventional vs. organic) can affect the prevalence (Alban et al., 2015; Kongsted and Sørensen, 2017) and farm management practices and farm health and welfare status (Heinonen et al., 2001; Teixeira et al., 2016) of farms delivering pigs to different slaughterhouses can vary. However, the variation of meat inspection data between slaughterhouses has been taken into account in the regulation MAF 6/EEO/2012, where the notification limit is twice the mean of the slaughterhouse. To reduce possible reporting variation, Finnish Food Authority formulated detailed instructions for the meat inspection personnel for the judgement and recording of meat inspection findings used for pig health and welfare monitoring. The effectiveness of the instructions will be assessed later.

References

Alban L, Petersen JV, Busch ME (2015): A comparison between lesions found during meat inspection of finishing pigs raised under organic/free-range conditions and conventional, indoor conditions. Porc. Health Manag. 1, 4. https://doi.org/10.1186/2055-5660-1-4

Harley S, More SJ, O'Connell NE, Hanlon A, Teixeira D, Boyle L (2012): Evaluating the prevalence of tail biting and carcase condemnations in slaughter pigs in the Republic and Northern Ireland, and the potential of abattoir meat inspection as a welfare surveillance tool. Vet. Rec. 171, 621.

Heinonen M, Gröhn YT, Saloniemi H, Eskola E, Tuovinen VK (2001): The effects of health classification and housing and management of feeder pigs on performance and meat inspection findings of all-in-all-out swine-finishing herds. Prev. Vet. Med. 49, 41–54.

Horst A, Gertz M, Krieter J (2019): Challenges and opportunities of using meat inspection data to improve pig health traits by breeding: A review. Livest. Sci. 221, 155–162. https://doi.org/10.1016/j.livsci.2019.02.001.

Kongsted H, Sørensen JT (2017). Lesions found at routine meat inspection on finishing pigs are associated with production system. Vet. J. 223, 21–26. https://doi.org/10.1016/j.tvjl.2017.04.016

MAF 6/EEO/2012. Decree of the Ministry of Agriculture and Forestry 6/EEO/2012 on monitoring swine morbidity.

Stärk KDC, Alonso S, Dadios N, Dupuy C, Ellerbroek L, Georgiev M, Hardstaff J, Huneau-Salaün A, Laugier C, Mateus A, Nigsch A, Afonso A, Lindberg A (2014). Strengths and weaknesses of meat inspection as a contribution to animal health and welfare surveillance. Food Control 39, 154–162.

Teixeira DL, Harley S, Hanlon A, O'Connell NE, More SJ, Manzanilla EG, Boyle LA (2016). Study on the Association between Tail Lesion Score, Cold Carcass Weight, and Viscera Condemnations in Slaughter Pigs. Front. Vet. Sci. 3. https://doi.org/10.3389/fvets.2016.00024.

P42

Application of a mathematical model for prediction of Salmonella enterica behavior during manufacturing of Brazilian pork salami

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Introduction

Salami is a ready-to-eat product commonly consumed in Brazil and the presence of *Salmonella enterica* in this product has been reported (PETER *et al.* 2012). So far, no mandatory formulation and maturation protocol has been established (BRASIL, 2000). Besides, heat treatment is not performed, and bacterial inactivation will depend on the reduction of the pH and water activity during processing. Since mathematical models generate predictions that can be used for determining efficient maturation protocols, the aims of this study were to (i) evaluate the behaviour of a cocktail of *S. enterica* serovars during the maturation process of the Brazilian salami; (ii) test the suitability of the "gamma concept" model according to Coroller et al. (2015) for the prediction of growth/inactivation of *S. enterica* in a baseline study and (iii) perform the validation of this model.

Material and Methods

The salami formulation and maturation protocols were obtained from three Brazilian meat industries. Then, 50 and 20 salami pieces in the baseline and validation study, respectively, were prepared and inoculated with a cocktail of five strains of *S. enterica*: (1) S. Typhimurium ATCC 14028; (2) *S.* Typhimurium, (1) *S.* Infantis and (1) *S.* Derby. Salami pieces were ripened at different scenarios: *i.* fermentation at 30ºC and drying at 20ºC in the baseline study; *ii.* fermentation at 25ºC and drying at 18ºC in the validation study.
Periodical samplings for *S. enterica* quantification, water activity (a_w) and pH analysis were performed during maturation and curves were constructed. For this, on each sample time, *S. enterica* populations were enumerated by direct plating on Xylose Lysine Desoxycholate (Oxoid) added with an agar layer of Tryptic Soy Agar (Oxoid) accordingly to Kang; Fung (2000). Three colonies per sample were isolated and confirmed for *S. enterica*. The water activity of the salami samples was measured in Lab Touch - Aw (Novasin) apparatus, while the pH was measured on pH-meter DM-22 (Digimed).
Counts of *Salmonella* observed in the baseline and validation study were analysed by Bayesian inference to infer the distribution of the concentration per gram. Values of pH and room temperature were fitted by linear interpolation and water activity values where fitted in a differential equation accounting for exponential decay. The values in the baseline study were inserted in the mathematical "gamma concept" model where the bacterial growth was modelled using cardinal values of these variables and Weibull model for bacterial inactivation, according to Coroller et al. (2015). The adjusted growth/inactivation parameters (μ_{opt}, δ1, δ2, α) from the baseline study were applied in the validation of the model and tested with values from validation study.

Results

Observed curves in baseline study showed that *S. enteric*a population increased 1.23 log cfu.g^{-1} in the first 21 hours and decreased 4.95 log cfu.g^{-1} after 941 hours of maturation. In the validation study, an increase of 0.54 log cfu.g^{-1} in the first 26 hours was observed followed by a 5.55 log cfu.g^{-1} inactivation after 1.121 hours of maturation.
The pH drop near to 5.3 occurred at 66 and 48 hours in the baseline and validation study, respectively. In both studies a_w decline ranged from 0.9570 to 0.7568: in the baseline study there was a daily decrease of 0.0050; while in the validation study this value was 0.0042. The a_w represented the threshold in the growth/inactivation interface; when the minimum cardinal value for water activity used in this model (0.951) was achieved (26 hours of maturation), bacterial inactivation started. The parameters (95% IC) adjusted in the model were: (h^{-1}) = 2.54 (2.545 - 3.03), δ1(h) = 1588.06 (1587.99 - 1891.68), δ2(h) = 163299.72 (163292.80 - 194520.80) e α = 0.02158 (0.02157 - 0.02571). The baseline model predicted well the growth/inactivation interface and the tail at the end of maturation. Linear regression of predicted compared to the observed values showed that 97.65% of the variation in the observed values could be explained by the predicted ones in the baseline study; while the application of the fitted parameters in the validation study had 95% of the variation explained.

Discussion and Conclusion

The higher maturation temperature used in the baseline study (30 and 20°C) speeded up the reduction of *S. enterica* in comparison to the validation (25 and 18ºC). These results are in concordance with Hwang et al. (2009), in which *S.* Typhimurium had higher inactivation rates when food was stored at temperatures of 21 and 30 °C when compared to 4 °C. Most of bacteria achieve the maximum growth between a_w values 0.990 and 0.995 and the reduction in a_w caused an increase in the lag phase (BEALES, 2004).

When a_w of 0,951 was achieved in salami, according to the model, *S. enterica* inactivation began and this variable was the most important regarding the start of the bacterial reduction. Thus, the drying step was the most important in the inactivation of the bacteria. Furthermore, in the baseline study the reduction of a_w was higher than in the validation of the model and this fact reflected in the *S. enterica* population reduction. Regarding the adjustment of the model, in the validation study 95% of the variation in the observed values could still be explained by the predicted ones, showing a good fit of the model for different protocols of maturation. In conclusion, this model can be applied in salami manufacture planning at industries in order to diminish the risk of *Salmonella* presence.

References

Beales, N. (2004): Adaptation of microorganisms to cold temperatures, weak acid preservatives, low ph, and osmotic stress: a review. Comprehensive Reviews in Food Science and Food Safety, v. 3, p. 1-20.
Brasil, Ministério da Agricultura e do Abastecimento (2000). Instrução Normativa nº22, de 31 de julho de 2000. Aprova o Regulamento Técnico de Identidade e Qualidade de Salame. Diário Oficial [da] União, Brasília, DF.
Coroller, L., Jeuge S., Couvert O., Christieans S., Ellouze M. (2015)Extending the gamma concept to non-thermal inactivation: A dynamic model to predict the fate of *Salmonella* during the dried sausages process. Food Microbiology, v. 45, p. 266-275.
Hwang C., Porto-Fett AC, Juneja VK, Ingham BH, Luchansky JB (2009) Modeling the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium during fermentation, drying, and storage of soudjouk-style fermented sausage. International Journal of Food Microbiology, v. 129, p. 244-252.
Kang, D H., Fung, D Y (2000) Application of thin agar layer method for recovery of injured *Salmonella typhimurium*. International Journal of Food Microbiology, v. 10, p. 127-132.
Peter C., Haubert L., Werlang GO, Cardoso M., Corbellini LG (2012). Análise microbiológica de salaminhos elaborados sob Inspeção Federal ou Estadual e comercializados em Feiras Modelo de Porto Alegre. XXI Congresso Latino Americano de Microbiologia - ALAM Santos, Brasil.

P43

Pork cutting plant condemnation data: Economic value and potential use as a farm-inoculation surveillance tool

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Introduction

It is known the potential of pig abattoir inspection data as a health and animal welfare surveillance tool. However, the information is scarce regarding the potential of meat condemnation data at the cutting plant as additional information for surveillance purpose.

The objective of this study was to evaluate pork meat condemnation in a cutting plant and analyse its economic value and its potential use as a surveillance tool.

Methods

During a period of 30 labour days (February and March 2018), data from one pork cutting plant was collected, including:

- Daily production volume (units and Kg);
- Number, weigh and cause of condemnation of the condemned parts.

Due to logistic restrictions, the type of lesions or cause of condemnation were classified as abscesses or other lesions. The classification of "abscess" included different stages of abscess formation. The classification of "other lesions" included lesions other than abscesses, for example: bruising, fibrosis, abnormal colour and consistency of the muscle tissue. Also, the samples analysed were restrict to only four parts of the carcass: the neck, the superficial skin and subcutaneous muscle of the neck known as "caluga", the shoulder and the loin.

In order to evaluate with more detail the lesions observed, 13 samples of muscle lesions found during the study period were sent for a histopathological analysis.

Results

In the referred study period, a total of 53 361 deboned carcasses were analysed (corresponding to 504 684 parts of carcass). From those, a total of 2 090 meat units were condemned representing a direct economic loss of 3 343.24 Euros. From those, 421 parts were condemned due to abscess lesions and 1669 parts due to other lesions. The distribution of these condemnation causes per part of carcass and respective cost (Euros) is presented in table 1.

Table 1 - Distribution of condemnation causes, in units and kilograms, per part of carcass and respective cost.

Most of the abscesses lesions were found in caluga and most of the "other lesions" were mainly observed in the neck muscle, representing the main economic loss (2 909.82 Euros).

For both cases, the results obtained through histopathological analysis revealed traits that may fit with inoculation compatible lesions. These traits included:

- Cellular necrosis;
- Myositis;
- Abscesses;
- Oedema;
- Calcification;
- Fibrosis;
- Cellular infiltrates.

In the case of abscess lesions, those may be related with older inoculation incidents, in opposite to "other lesions", which some may be related with more recent innoculations.

Also, the neck region it is one of the most common inoculating zones for intramuscular and subcutaneous injections in swines, reinforcing the suspicious that this could be the major cause of these lesions. In addition, one of the 13 samples sent to the lab for histological analysis, revealed a strange substance (we suspect of pharmacological residue) around which a granulomatous reaction developed. This sample was from a neck muscle that was classified as "other lesions" and macroscopically it was evident the alteration of the normal texture and colour of the muscle tissue.

Table 1: Distribution of condemnation causes, in units and kilograms, per part of carcass and respective cost

	abscess	abscess	abscess	other lesions	other lesions	other lesions	total	total	total
	units	weight (Kg)	cost (€)	units	weight (Kg)	cost (€)	units	weight (Kg)	cost (€)
caluga	389	272,3	373.44	0	0	0	389	272.3	373.44
neck	3	8,7	5.22	1639	4753,1	2851.86	1642	4761.8	2857.08
shoulder	27	116,91	50.76	17	73,61	31.96	44	190.52	82.72
loin	2	6,74	4	13	43,81	26	15	50.55	30
total	421	404.65	433.42	1669	4870.52	2909.82	2090	5275.17	3343.24

Conclusion

The quality of pork meat is dependent on every production stage, ranging from the primary producer to the final consumer.

This study demonstrates the potential use of neck lesions observed at pork cutting plant for a farm-inoculation risk-based surveillance system. This system could be extremely helpful for the pork meat industry because it gives the possibility to access if suppliers respect the correct practices of intramuscular and subcutaneous inoculation. However, more studies should be performed to prove as accurately as possible the association between these variables.

In addition, the monitorization of muscle lesions in the neck area of the swine carcasses, may decreases the risk of undetected abscess or other lesions reaching the consumer.

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P44

Retrospective study on the occurrence of hypodermic needles and other metallic physical hazards detected by metal detectors in one pork cutting plant

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Introduction

A physical hazard is any extraneous object or foreign matter in a food which may cause illness or injury to a person consuming the product. In addition to biological and chemical hazards, the economic food business operator must determine procedures to control physical hazards. The objective of this study was to evaluate the occurrence of hypodermic needles and other metallic physical hazards in pork meat at the level of a cutting plant.

Methods

For this retrospective study we decided to compare the occurrence of hypodermic needles with the occurrence of other metallic physical hazards, to better understand the real dimension of the problem posed by hypodermic needles.

The data used in this study was collected from the records of quality control team present in the respective cutting plant.

For the needles, data was collected from December 2012 to March 2018 (63 months), and included the following information:

- Needle Gauge;
- Needle length;
- Needle location (carcass part or muscular localization).

Regarding the other metallic physical hazards, data was collected from January 2015 to August 2018, not including the year of 2017 (32 months). The data included the localization and a brief description of the hazard.

Results

For the 63 months period, a total of 26 hypodermic needles were found. From those, the most common localization was the neck muscles (n=15, 57.69%) and the shoulder (n=6, 23.07%). Most (n=22, 84.62%) of the needle fragments were between 2.4 cm and 2.6 cm and had, mainly, a gauge of 16 G (n=15, 57,69%) or 17 G (n=7, 26,92%).

Regarding to the other metallic physical hazards, there were found 23 during the period of 32 months. From those, the majority were just simple metal fragments with no evident shape or origin (n=17, 73.91%). The second most frequent type were both steel filings and washers of steel mesh gloves (n=2, 8.70%). The main localization was the spare ribs (n=13, 52.17%) and the neck (n=3, 13.04%).

Conclusion

The fact that the neck and shoulder were the parts with the highest number of hypodermic needles can be related to the fact that these two anatomical areas are the main preferential areas for inoculation.

As for the other physical hazards, one possible explanation for the fact that the most affect part was the spare rib, is that the spare rib has many bone structures that cause a greater wear upon the cutting blades and machinery.

Although the number of needles per month is practically half of the other metal objects, it still constitutes a large part of all the physical metal hazards identified. If we consider all the objects found, we have a monthly average of more than 1 object / month, which clearly reflects the importance of equipment such as metal detectors and x-ray machines in the food industry and consumer protection.

Additionally, these results, should alert for the importance of good production practices use regarding inoculation procedures in order to mitigate the impact on food safety and consumer confidence arising from the use of hypodermic needles in livestock.

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P45

Cadaverine and putrescine contents in traditional Portuguese pork sausages linked to the addition of starter cultures

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Introduction

Starter cultures are used in meat sausages production mainly for technological reasons but, depending on the species, an added effect against other bacteria, including pathogens, can occur. Lactic acid bacteria (LAB), usually considered non-pathogenic and non-toxic, are the main fermenting microorganisms in starter cultures, but some LAB species can produce biogenic amines (BAs). The biogenic amines content in meat, although not regulated by law, is a meat freshness indicator. The formation and increase of the content of BAs is related to food degradation processes, reason why it is important to control the contents of these amines over product lifetime. Currently, European Commission Regulations (2073/2005, 144/2007, 365/2010) set food safety criteria for histamine in fishery products and no criteria have been established for other BAs or other food products, such as meat, dairy, or other products, despite the presence of important levels of BAs in all types of food and the potential public health concern due to their physiological and toxicological effects, when these products are consumed.

This study aims to ascertain the effect of the addition of a LAB strain, known to be active against some pathogens that can be found in cured meat products, on cadaverine and putrescine production in cured-smoked sausage-like pork products during storage.

Material and Methods

The LAB strain *Lactobacillus plantarum* ST153ch was applied to three cured-smoked products. In "Alheira" it was added together with meat and other ingredients before the curing process and in "Salpicão" and "Lombo" it was included in finished products after slicing. Similar products were produced without the addition of starter culture, here called control samples.

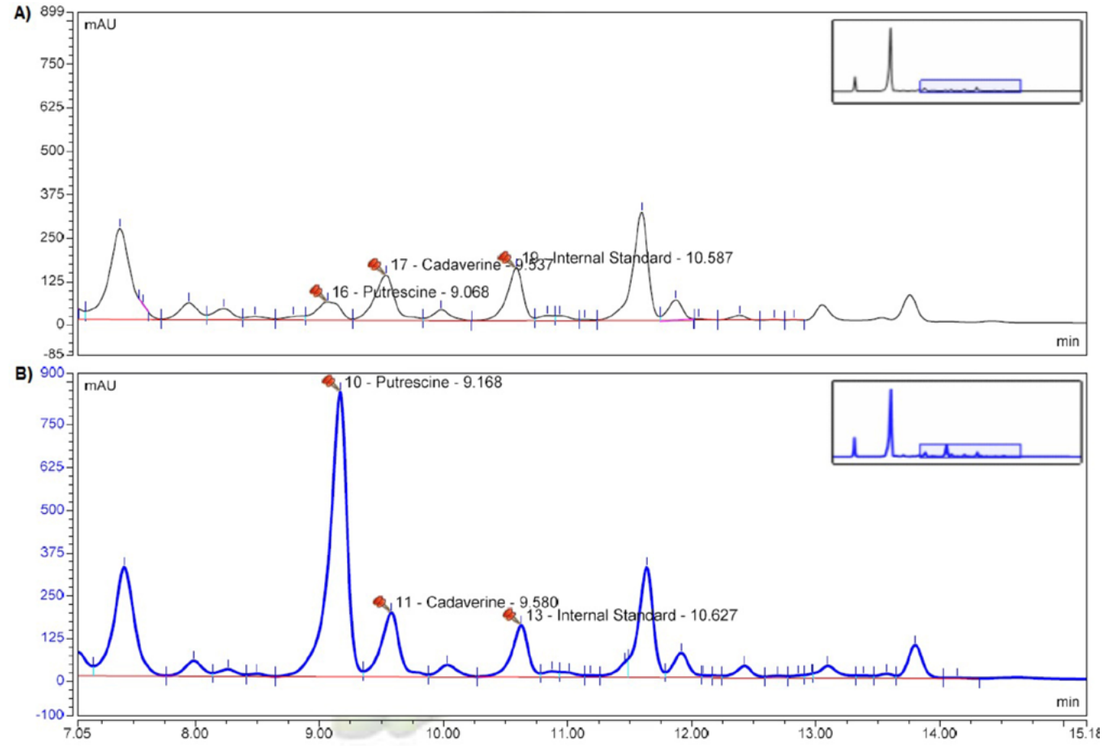

Figure 1: HPLC chromatogram of biogenic amines in "Alheira" control sample (A: 0 days, B: 60 days).

All samples were vacuum packed and stored at 4°C. Putrescine and cadaverine determinations were performed along a storage time of 0, 15, 30, 45, 60 e 90 days for "Alheira" product, and at 0, 15, 30, 45, 75, 90, 120, 165 e 180 days for "Salpicão" and "Lombo" sliced products.
BAs levels were determined by HPLC with an analytical Thermo Ultimate 3000 system and a Dionex Ultimate 3000 diode array detector. Amines were extracted with perchloride acid solution and derivatized with dansyl chloride following J. AOAC International method (AOAC, 1999) with some modifications: Hypersyl ODS C18 column (5mm, 250x4.6 mm I.D., Agilent) conditioned at 40°C, injection volume of 20 mL, flow rate of 1mL/min, UV at 254nm and a mobile phase with a binary mixture of water and acetonitrile. A paired *t*-test was performed for each parameter (putrescine and cadaverine) and for each product ("Alheira", "Lombo" and "Salpicão") over the study time using Microsoft Excel 2016. In this analysis it was used the average of two measurements.

Results

A good separation of putrescine and cadaverine was obtained and two representative chromatograms of biogenic amines in samples with 0 and 60 days of storage for "Alheira" control sample are presented (Figure 1).
The level of putrescine in "Alheira" control product was between 11.4 and 161.3 mg/kg during the storage time, until 90 days. Similar results were observed for this product with added lactic acid bacteria, with a similar rate of increase (Figure 2), the concentration was between 11.9 and 161.2 mg/kg respectively at 0 and 90 days. Concerning the cadaverine content, it remained lower than 41.5 mg/kg (at day 90) and more constant for control and LAB samples (Figure 2).
During storage time there are no significant differences for putrescine concentration between control and LAB products, "Alheira", "Salpicão" and "Lombo" (p=0.068, p=0.976 and p=0,848 respectively using paired *t*-test). Concerning the other biogenic amine, there are no significant differences in cadaverine content for "Lombo" (p=0.759), but there are differences for the other two products, "Alheira" e "Salpicão" (p=0.019 and p=0.047 respectively).
The levels of biogenic amines with storage time are presented in Figure 2. In "Alheira" there is an increase in putrescine values with time, without significant differences between groups. However it is observed a constant level for cadaverine values in LAB samples with significant differences when compared to the control samples which amine concentration increases with time. In "Lombo" sausage there is no tendency for putrescine and cadaverine increase with time, both for LAB and control samples with no significant differences (Figure 2). For "Salpicão", the cadaverine concentration does not change significantly with time for both groups, however for putrescine there is a slightly increase with storage for the LAB group, without much change for the control group.

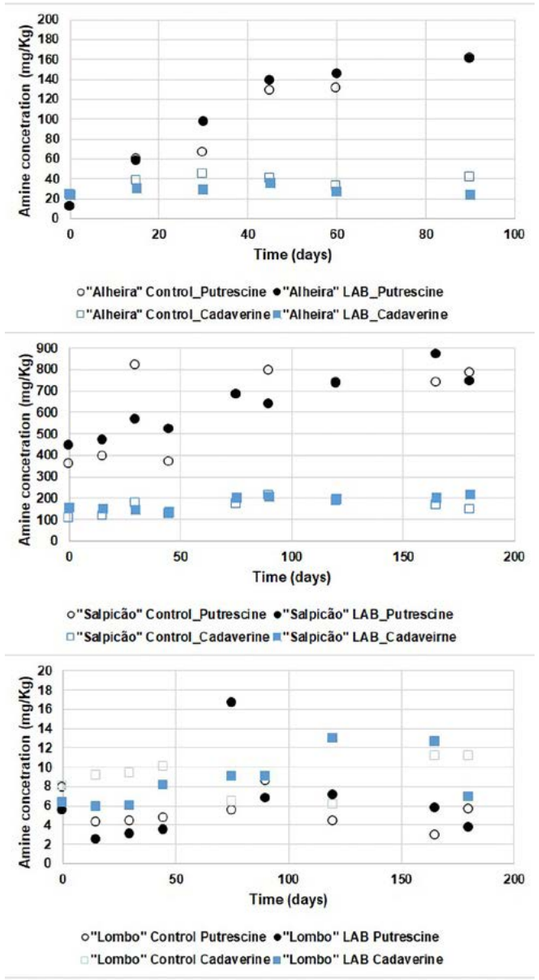

Figure 2: Biogenic amines content for "Alheira", "Salpicão" and "Lombo" in LAB and Control groups

Discussion and conclusion

The tendency observed is that the starter culture addition did not influence the putrescine contents in "Alheira", "Salpicão" and "Lombo" cured products, and did not also influence the level of cadaverine in "Lombo" and in "Salpicão" it is in the limit with p=0.057. However in "Alheira" product the starter culture addition influences the concentration of cadaverine, with a lower concentration in the LAB group.
In this study it can be concluded that the inclusion of LAB starter cultures did not influence the putrescine and cadaverine contents along the storage of all the tested products, except for "Alheira" with a positive effect in cadaverine.
Usual contents of putrescine in fresh pork are < 2, < 5, and 20-40mg/kg, respectively (Kalac, 2006). The amount of cadaverine in fresh meat is at the level of 1 mg/kg but can raise even to 120 mg/kg (Bover-Cid *et al.*, 2006).

References

AOAC Int. (1999): Liquid chromatographic method for determination of biogenic amines in fish and fish products, 82, 1097-1101.
Kalac, P. (2006): Biologically active polyamines in beef, pork and meat products: A review. Meat Science, 73, 1-11.
Bover-Cid, S., Miguelez-Arrizado, J., Latorre Moratalla, L. L., & Vidal Carou, M. C. (2006): Freezing of meat raw materials affects tyramine and diamine accumulation in spontaneously fermented sausages. Meat Science, 72, 62-68.

P46

Food chain information - data based recommendations for a standardised ”relevant period of time” within all EU member states

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Introduction

As part of the food chain information, the farmer has to inform about administered veterinary medicinal products with withdrawal periods greater than zero within a “relevant period of time” before slaughter. This time period, which is not yet defined uniformly within the EU, is fixed at seven days for all farm animal species except for broilers in Germany.

Methods

Within the project “Survey on the treatment of certain farm animal species (in turkeys and pigs [rearing and fattening] and fattening cattle including fattening calves) with veterinary medicinal products with regard to the food chain information, 2nd stage” (grant 2815HS008) data on the usage of veterinary medicinal products with withdrawal periods greater than zero and on the slaughter check findings have been collected and analysed from 43 German fattening pig farms.

Results

The “treatment-free period” and the “withdrawal free period before slaughter” have proved to be particularly meaningful for answering the question of a species specific adaptation of the “relevant period” within the food chain information according to Reg. (EU) No. 853/2004. The median for the shortest withdrawal period before slaughtering is 71 days and the 5% percentile of this shortest waiting time before slaughter is 24 days.
On the basis of these data, for fattening pigs it is recommended to maintain the “relevant period” at seven days.

Conclusions

The presented recommendations for the included farm animals species are an essential part of the political discourse on the definition of an EU-wide uniform “relevant period per animal species” as they were derived on the basis of data representative for Germany and other countries with comparable agricultural structures.

ANIMAL WELFARE

P47

The effect of inulin-feed and improved housing conditions on boar taint reduction

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Introduction

The potential ban on surgical castration in Europe is turning a major advantage of this practice, the elimination of boar taint, into a big challenge for pig industry (Meinert et al., 2017). Raising entire male pigs has some economic advantages as boars possess the advantage of superior growth over castrates, generally leaner carcasses, and compared to castrates less feed is needed in order to achieve the same final weight (Morlein et al., 2015; Wauters et al., 2017). Boar taint is described as an unpleasant odour which becomes especially intense when pork is cooked (Mathur et al., 2012), and is mainly associated with the presence of skatole and androstenone. Skatole (3-methylindole) is a metabolite derived from the amino acid tryptophan produced in the lower gut by intestinal bacterial flora, and androstenone (5α androst-16-en-3-one) is a steroid produced in the testis (Aldal et al., 2005). Introduction of functional ingredients in feed can reduce boar taint. Aluwe et al., (2013), Backus et al., (2016), Byrne et al., (2008) reported that inulin was effective in the reduction of the skatole’s concentration in the hindgut Housing conditions and genetic selection can also have a favourable effect on boar taint reduction (Backus et al., 2016).

Methods

Sixty entire male pigs (progeny of Large White x Landrace gilts sired by Pietrain boars) were raised under controlled housing and feeding conditions in order to determine its effects on boar taint content. Inulin was added to feed 48 days prior to slaughter in three different levels, combined with two housing conditions - normal and improved housing, which consisted in a larger area, easier access to water and environmental enrichment accessories, making a total of 6 sampling groups (Table 1). A quantitative descriptive sensory analysis was performed by 11 trained panellists in two sessions, assessing odour and flavour of skatole and androstenone, on a 1 to 10 scale. A total of eight coded samples, with the six conditions and two replicates to evaluate repeatability. Hardness (Texture analyser), pH, moisture content and intramuscular fat (Soxhlet method) were determined in ham samples. An HPLC method for the simultaneous quantification of skatole and androstenone, adapted from Hansen-Moller, (1994), was performed using the liquid fat extracted from belly’s adipose tissue. ANOVA with a post hoc Tukey’s test was used to investigate the significance of observed differences.

Results

Results showed that improved housing conditions led to higher hardness and lower pH values ($p < 0.05$). Intramuscular fat was significantly higher for this condition, specifically in group C6%. There were no observed significant differences in moisture content. HPLC results showed that androstenone average levels tended to be lower with higher percentages of added inulin, however without significant statistical differences. Skatole levels were significantly higher ($p < 0.05$) in the groups where no inulin was added (N0% and C0%),

Table 1: Housing conditions, inulin feed composition and number of pigs for each trial

Pen	Housing	Added inulin in feed	Number of pigs	Group code
A	Normal	0%	10	N0%
B	Normal	3%	10	N3%
C	Normal	6%	10	N6%
D	Improved (+Care)	0%	10	C0%
E	Improved (+Care)	3%	10	C3%
F	Improved (+Care)	6%	10	C6%

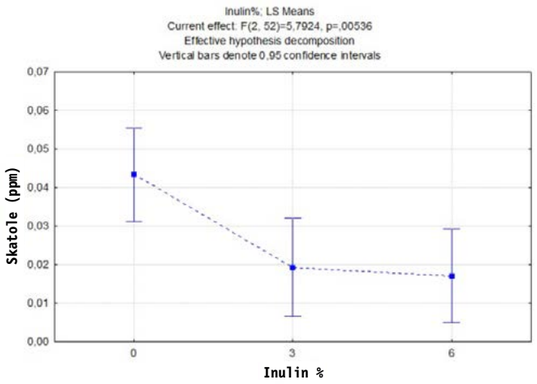

Figure 1: Skatole values (ppm) in belly fat in the feeding groups

and no differences were found between 3% and 6% of added inulin, as shown in Figure 1. Similar results were found by Byrne et al., (2008) and Aluwe et al., (2013), where the addition of inulin lead to the reduction of skatole concentration in fat.

In the sensory analysis, panellists attributed belly samples the highest scores in skatole odour, compared to ham. Samples from NO% group were considered higher in skatole flavour and androstenone odour ($p< 0.05$). Concerning to ham's meat samples, panellists found no differences in odour and flavour between groups. This difference of sensitivity between belly and ham can be explained by the amount of fat in the two meat cuts: due to the lipophilic characteristics of skatole and androstenone, redistribution from blood to fat tissue is easily occurring with prolonged accumulation in fat tissues (Aldal et al., 2005; Wauters et al., 2016).

Conclusion

It can be concluded that housing conditions mostly led to changes in the meat overall quality whereas feed conditions had an extended effect on boar taint reduction. Inclusion of inulin in commercial feeds reduced the skatole concentration, which led to a lower boar taint perception by panellists. Meanwhile, studies on changing feed formulations are being carried out.

Acknowledgements

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References

Aldal, I., O. Andresen, A. K. Egeli, J. E. Haugen, A. Grodum, O. Fjetland, and J. L. H. Eikaas, 2005, Levels of androstenone and skatole and the occurrence of boar taint in fat from young boars: Livestock Production Science, v. 95, p. 121-129.

Aluwe, M., K. C. M. Langendries, K. M. Bekaert, F. A. M. Tuyttens, D. L. De Brabander, S. De Smet, and S. Millet, 2013, Effect of surgical castration, immunocastration and chicory-diet on the meat quality and palatability of boars: Meat Science, v. 94, p. 402-407.

Backus, G. B. C., E. van den Broek, B. van der Fels, L. Heres, V. M. Immink, E. F. Knol, M. Kornelis, P. K. Mathur, C. van der Peet-Schwering, J. W. van Riel, H. M. Snoek, A. de Smet, G. M. L. Tacken, N. I. Valeeva, and C. P. A. van Wagenberg, 2016, Evaluation of producing and marketing entire male pigs: Njas-Wageningen Journal of Life Sciences, v. 76, p. 29-41.

Byrne, D. V., S. M. Thamsborg, and L. L. Hansen, 2008, A sensory description of boar taint and the effects of crude and dried chicory roots (Cichorium intybus L.) and inulin feeding in male and female pork: Meat Science, v. 79, p. 252-269.

Hansen-Moller, J., 1994, Rapid high-performance liquid-chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs: Journal of Chromatography B-Biomedical Applications, v. 661, p. 219-230.

Mathur, P. K., J. ten Napel, S. Bloemhof, L. Heres, E. F. Knol, and H. A. Mulder, 2012, A human nose scoring system for boar taint and its relationship with androstenone and skatole, v. 91, p. 414.

Meinert, L., B. Lund, C. Bejerholm, and M. D. Aaslyng, 2017, Distribution of skatole and androstenone in the pig carcass correlated to sensory characteristics: Meat Science, v. 127, p. 51-56.

Morlein, D., C. Schiermann, L. Meier-Dinkel, J. Trautmann, R. Wigger, G. Buttinger, and M. Wicke, 2015, Effects of context and repeated exposure on food liking: The case of boar taint: Food Research International, v. 67, p. 390-399.

Wauters, J., V. Vercruysse, M. Aluwe, K. Verplanken, and L. Vanhaecke, 2016, Boar taint compound levels in back fat versus meat products: Do they correlate?: Food Chemistry, v. 206, p. 30-36.

Wauters, J., K. Verplanken, V. Vercruysse, B. Ampe, M. Aluwe, and L. Vanhaecke, 2017, Sensory evaluation of boar meat products by trained experts: Food Chemistry, v. 237, p. 516-524.

P48

Wounds on the body and tail biting in finishing pigs: Do these aggression injuries measured at slaughterhouses predict the welfare on farms?

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Introduction

Welfare is one of the most debated themes nowadays. Intensive farming may favour the occurrence of welfare problems such as aggression and tail biting. Aggression is a normal pattern of the pigs' social behaviour; however, if prolonged in time it impairs animal welfare. When aggression occurs, the result may be injuries, pain and in extreme cases, death of the animal. Also, aggressions lead to physiological stress, immunosuppression and reduction of food intake (1).

For pigs, the mixing with unfamiliar animals is a major source of social stress (2). Crowding or limited available space and access to a limited resource (e.g. feed) can trigger the aggressions. The incidence of injuries seems to be more reliable and feasible than behavioural observations (3). The location of the lesions on the animal body can give a more detailed information about the causes of the injuries. Lesions on the head and shoulder area are caused by fights associated with social ranking (4). Lesions on the rear part of the body may be caused by competition for food (5) or by rough handling. These aggressions typically occur on the farm prior to transportation to the abattoir, at loading for transport and unloading of the transport (6).

Tail biting is also considered a major welfare problem in pig production and is an actual highly debated theme. It indicates pain and suffering, not only of the bitten animal, but also of the biting animal. The bitten animal suffers from the pain of the bite itself and of possible secondary infections. The biting animal usually bites due to the experience of some frustration within the group (7). Efforts are being done in order to find solutions for this problem and the quantification of the problem itself is fundamental.

The veterinary community is questioning about the possible use of these aggression injuries, evaluated at slaughterhouse by the Food Business Operator, to assess the compliance of welfare at farm of animals' provenance.

Materials and Methods

This study evaluated comparatively the level of wounds on the body and tail biting in finishing pigs at both farms and slaughterhouse level. They were both recorded in 10 lots from 10 Dutch farms, before their leave to slaughter and after their arrival at the slaughterhouse. A total of 774 and 794 animals were assessed on farms and slaughterhouse, respectively.

The evaluation of the presence of wounds on the body and tail biting was done based on the Welfare Quality® assessment protocol for pigs (8). The measure Wounds on the body was divided into two levels as described in the protocol. The assessor choses a side of the pig's body and counts the number of scratches or wounds in each one of the five regions considered (ears, front, middle, hind-quarters and legs), the tail zone was not considered. Firstly, the lesions are counted, then the number of lesions is turned into a score (a, b, c). Finally, the classification is done first in an individual level and then in herd level, in percentage. More detailed information about the method of classification of the measure Wounds on the body can be consulted on the Welfare Quality® assessment protocol for pigs (8).

The measure *Tail biting* was assessed individually and was recorded when a pig tail had visible fresh blood; had evidence of some swelling and infection; had part of the tail tissue missing and a crust has formed. To compare the results, Wilcoxon tests for paired samples were done.

Photo 1: Pig showing bleeding wounds in the front part of the body

Table 1:

Measures	Farms (average)	After arrival at slaughterhouse (average)	p-value
Wounds on the body scored 1	21%	45%	<0.01
Wounds on the body scored 2	5%	15%	<0.05
Tail biting	7%	5%	0.7

Results

Examples of observed lesions (wounds) in the assessed animals can be seen in picture 1.

The measures *wounds on the body* scored 1 and 2 were statistically different and increased at the slaughterhouse, by 23,9% and 10,9%, respectively (table 1). The bleeding (fresh) wounds were remarkably higher on the slaughterhouse assessments. The measure *tail biting* was not statistically different between farms and slaughterhouse (table 1).

Discussion and Conclusions

The higher results of *Wounds on the body* measured at slaughterhouse is probably a consequence of stress and mixing animals during transportation and permanence in the slaughterhouse barn (2). When mixing the pigs, they tend to fight and mount each other.

The decrease of 1,29% of *Tail biting* at slaughterhouse, suggests that not all tail-bitten pigs observed at farm were sent to the slaughterhouse, fact that could be explained to its possible unfit condition to be transported or to its lower weight.

The results from this study allow to conclude that *Wounds on the body* can't fairly predict the welfare on farms since it may be influenced by the stress caused during transportation and permanence at lairage pens. The measure *Tail biting* can be assessed at slaughterhouse to quantify an eventual welfare problem of the farm.

More studies should be conducted in order to understand, with more detail, the level of tail biting during pig production that can escape to the slaughterhouse sieve.

For most of the farmers, the mitigation of aggression injuries is difficult, requiring additional costs for example regarding space allowance and enrichment material supply. However, a worst welfare is synonym of economic losses and, solving these problems, should be perceived by farmers as a profitable advantage and not an obligation.

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References

1. Fraser D. Aggressive Behaviour. Vet Clin North Am Food Anim Pract. 1987;3(2):285-305.
2. Van Staaveren N, Teixeira DL, Hanlon A, Boyle LA. The effect of mixing entire male pigs prior to transport to slaughter on behaviour, welfare and carcass lesions. PLoS One. 2015;10(4):1-15.
3. Dalmau A, Fabrega E, Manteca X, Velarde A. Health and Welfare Management of Pigs Based on Slaughter Line Records. J Dairy, Vet Anim Res. 2014;1(3):5-10.
4. Luescher UA, Friendship RM, McKeown DB. Evaluation of methods to reduce fighting among regrouped gilts. Can J Anim Sci. 1990;70(2):363-70.
5. Leeb B, Leeb C, Troxler J, Schuh M. Skin lesions and callosities of group housed pregnant Sows-animal related welfare indicators. Acta Agric Scand A Anim Sci. 2001;51(30):82-7.
6. Kelley KW, McGlone JJ, Gaskin CT. Porcine aggression: measurement and effects of crowding and fasting. Anim Sci. 1980;50(2):336-41.
7. Schrøder-Petersen DL, Simonsen HB. Tail biting in pigs. Vet J. 2001;162:196-210.
8. Welfare Quality®. Welfare Quality® Assessment Protocol for Pigs (sows and piglets, growing and finishing pigs). Lelystad, Netherlands; 2009.

Industrial Exhibition

The Industrial Exhibition will take place in the Foyer.

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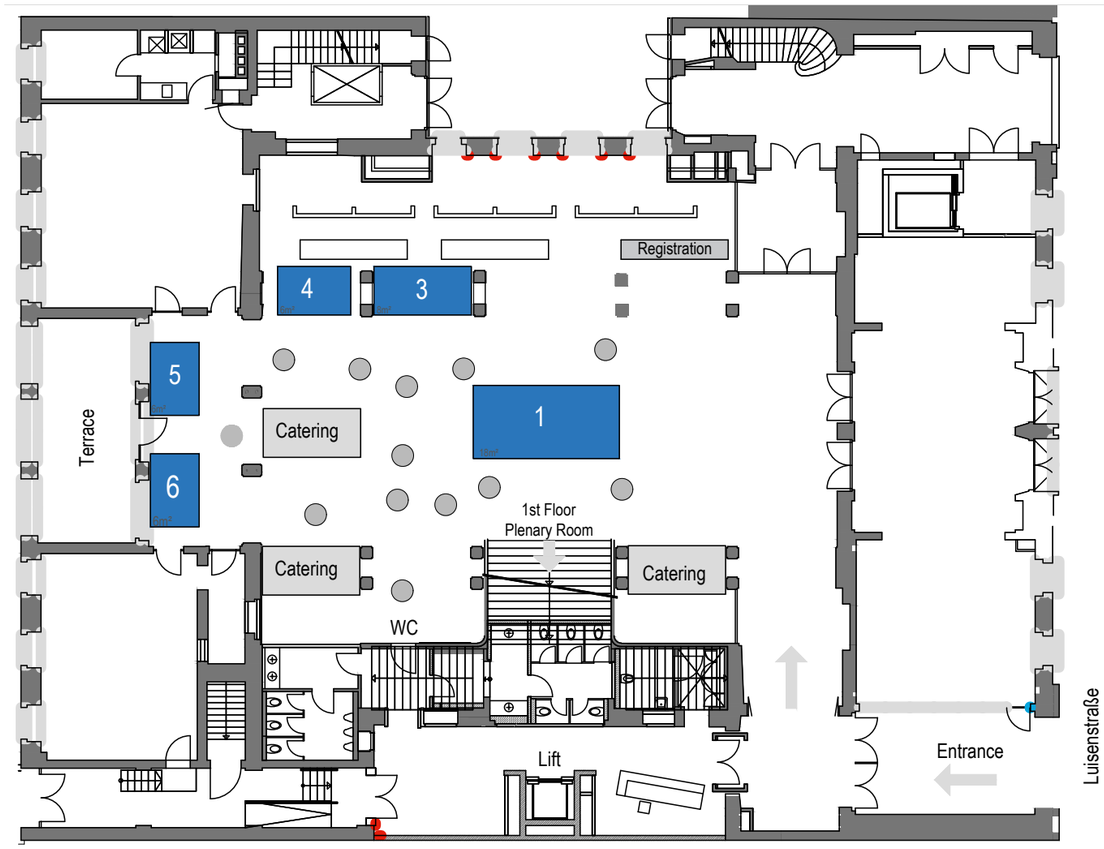

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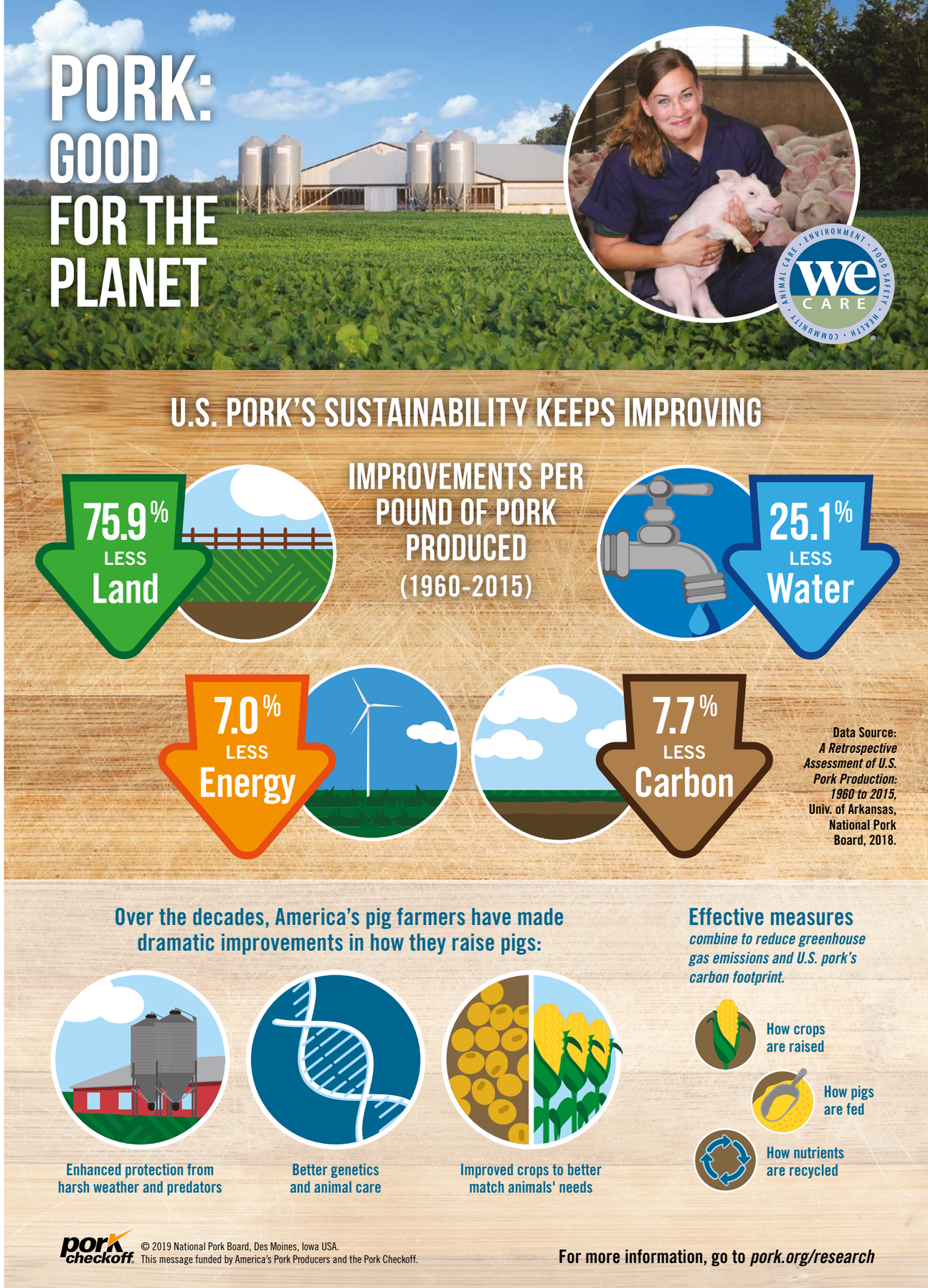

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