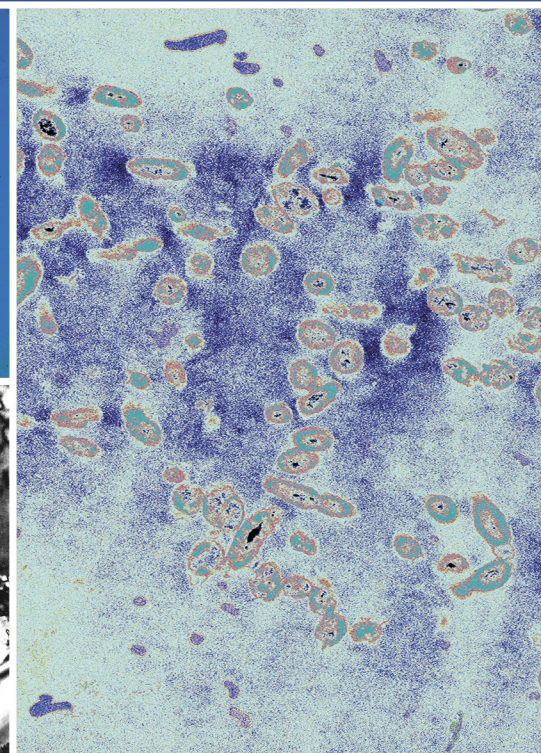


Workshop

on Arthropod-Borne Diseases
transmitted by ticks, mites, fleas, and lice



15th – 16th November 2018
Greifswald - Isle of Riems, Germany

Mosquitos
Fleas
Virus
Ticks
Mites
Bacteria
Lice
Zoonoses
Arthropod-Borne Diseases



Welcome to the

Workshop on Arthropod-Borne Diseases transmitted by ticks, mites, fleas, and lice

15th - 16th November 2018

Friedrich-Loeffler-Institut, Federal Institute for Animal Health
Conference Room
Südufer 10, D-17493 Greifswald - Isle of Riems, Germany

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General Information

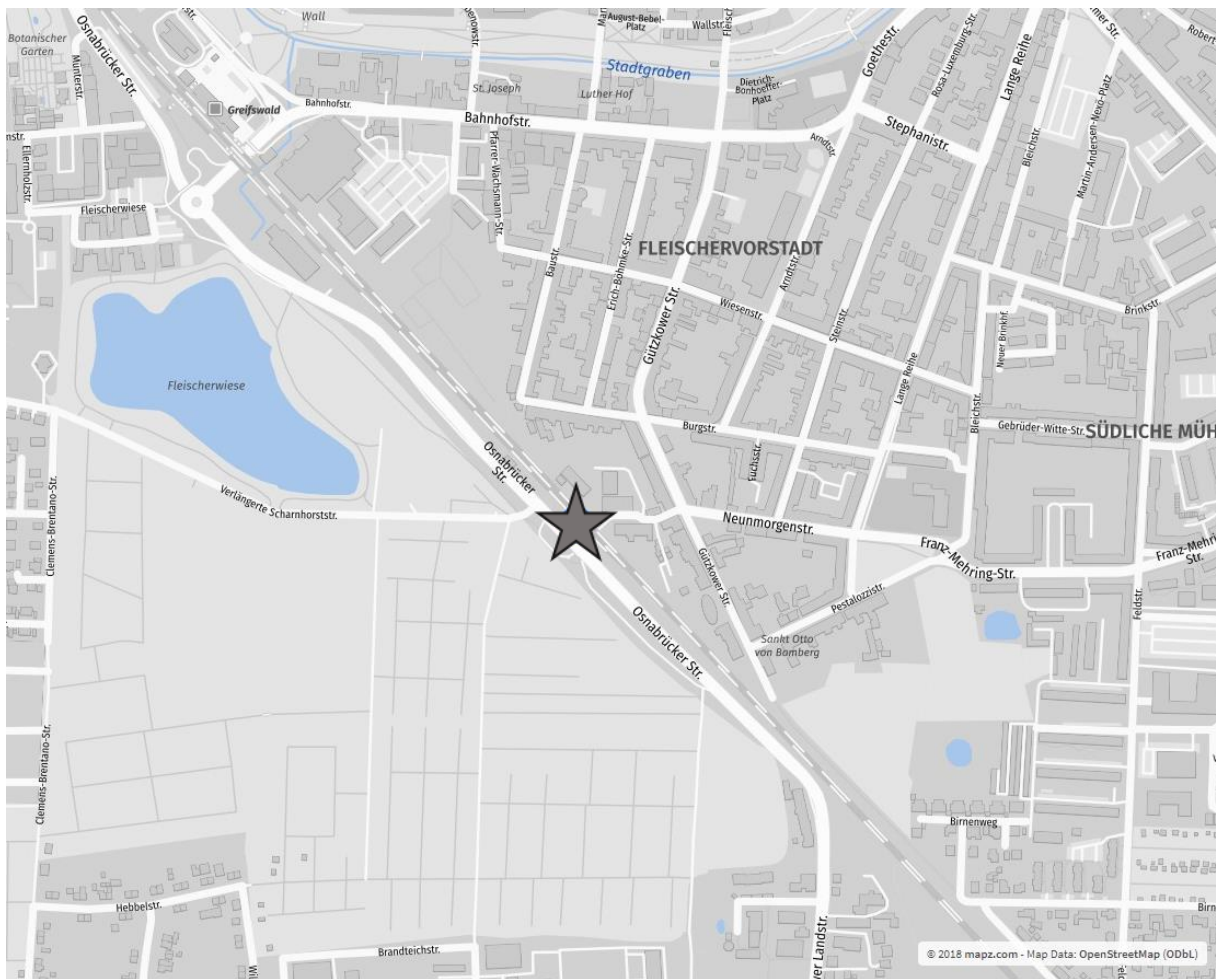
Notes for Speakers

Hand over your presentation to our workshop assistants, please, at the latest before a new topic starts.

Evening event



Restaurant „KulturBahnhof“
Osnabrücker Straße 3, 17489 Greifswald



Scientific Programme

Thursday, 15th November 2018

10:30 REGISTRATION

11:00 WELCOME OPENING

Cornelia Silaghi, Klaus Henning, Gustavo R. Makert dos Santos
"Historical overview of the Friedrich-Loeffler-Institut"

11:45 KEYNOTE 1

Distribution, ecology, taxonomy and data on the biological control of mosquito vectors in Albania based on a country-wide field survey

Elton Rogozi

Control of Infectious Diseases Department, Institute of Public Health, Tirana, Albania

LUNCH

13:15 KEYNOTE 2

Artificial tick feeding systems: possibilities and challenges

Ard Nijhof

Freie Universität Berlin, Institut für Parasitologie und Tropenveterinärmedizin, Berlin, Germany

TICK-BORNE PATHOGENS I
(Chair: Gustavo R. Makert)

13:45 **Development of an *in vitro* feeding system for the analysis of the vector competence of ticks in the transmission of *Coxiella burnetii***

Gustavo R. Makert et al.

Department of Immunology, Vaccine Technologies Unit, Fraunhofer Institute for Cell Therapy and Immunology IZI, Leipzig, Germany

13:45 **Modelling uptake and organ distribution of *Coxiella burnetii* in ticks - development of an *in vitro* feeding system**

Sophia Körner et al.

Friedrich-Loeffler-Institut, Jena, Germany

14:00 **Emergence of ticks and tick-borne diseases in Northwest Italy**

Aitor Garcia-Vozmediano et al.

Dept. Veterinary Sciences, University of Turin, Italy

14:15 ***Rickettsia* spp. transmitted by arthropods in Baltic region**

Algimantas Paulauskas et al.

Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania

COFFEE BREAK AND ISLAND TOUR

TICK-BORNE PATHOGENS II
(Chair: Klaus Henning)

16:00 **Predicting and mapping human risk of exposure to *Ixodes ricinus* nymphs in northern Europe using climatic and environmental data**

Lene Jung Kjær et al.

Department for Diagnostics and Scientific Advice, National Veterinary Institute, Technical University of Denmark, Lyngby, Denmark

16:15 Tick-borne pathogens detection in African cattle by PCR and a newly developed Reverse Line Blot microarray

Babette Abanda et al.

University of Ngaoundéré, Faculty of Science, Ngaoundéré, Cameroon

16:30 *Borrelia*-infections of ticks removed from humans, 2013-2017

Andrea Springer et al.

Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Germany

COFFEE BREAK WITH POSTER SESSION

BABESIOSIS
(Chair: Cornelia Silaghi)

17:15 Canine babesiosis in Lithuania

Jana Radzijeuskaja et al.

Department of Biology, Vytautas Magnus University, Lithuania

17:30 Canine babesiosis in Belgrade area in period 2015-2017

Ivan Pavlović et al.

Scientific Veterinary Institute of Serbia, Belgrade, Serbia

17:45 Epidemiological investigations into a babesiosis outbreak in a Northern German cattle herd

Andrea Springer et al.

Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Germany

19:30

DINNER AT THE RESTAURANT “KULTURBAHNHOF”
(meals and drinks are not included in the workshop fee)

Friday, 16th November 2018

OTHER VECTORS AND VECTOR-BORNE PATHOGENS
(Chair: Klaus Henning)

09:15 Vector competence of field-collected and laboratory reared mosquitoes for West Nile virus Lineage 1

Elisabeth Wöhnke et al.

Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

09:30 The influence of virus variant selection on *Culicoides* vector competence for bluetongue virus

Silvija Vuckovic et al.

Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

09:45 Effect of a combination of H₂O₂ fluid and permethrin by use of a fogger-technique on permethrin resistant and non-resistant bed bugs (*Cimex lectularius*)

Georg Gerhard Duscher et al.

Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria

- 10:00 **Clinico-pathological presentation of babesiosis and demodicosis in different domestic animals species**
Olatunde B. Akanbi et al.
Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria
- 10:15 **Integrated Pest Management (Schädlingsbiologie) - Update 2018**
Eva Scholl
SchädlingsBiologie Scholl, Bonn, Germany
- 10:30 **Tick, mite and flea infestation of small mammals at three locations in Germany**
Leonie Arnold et al.
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

COFFEE BREAK AND POSTER SESSION

TICK-BORNE PATHOGENS III
(Chair: Cornelia Silaghi)

- 11:15 **What is the significance of *Candidatus Borrelia Kalaharica* in the cause of febrile illness in Nigeria?**
Adefolake Bankole et al.
School of Health, Sports and Bioscience, University of East London
- 11:30 **Molecular survey of two major tick-borne pathogens in *Ixodes ricinus* ticks collected from natural habitats in North East Germany**
Cristian Raileanu et al.
Friedrich Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany
- 11:45 **Tick infestation and occurrence of *Anaplasma phagocytophilum* and piroplasms in cattle in the Republic of Serbia**
Ana Vasic et al.
Friedrich Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany
- 12:00 **Tick-borne pathogens detected in ticks from passerine migratory birds in Lithuania**
Vesta Matulaitytė et al.
Vytautas Magnus University,
- 12:15 FINAL DISCUSSION

LUNCH AND DEPARTURE

ABSTRACTS

KEYNOTE 1

Distribution, ecology, taxonomy and data on the biological control of mosquito vectors in Albania based on a country-wide field survey

Elton Rogozi, Enkelejda Dikolli, Perparim Kadriaj, Vjola Jani, Gjergji Sino, Juliana Nanaj, Klajdi Topulli, Silva Bino

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Introduction

Previous studies performed during 1918-1919 by foreign authors and 1975-1997 by local ones have reported the presence of a significant number of mosquito species in Albania. Mosquito borne diseases are a serious problem in human and animal health worldwide. The study of the ecology and distribution of mosquitoes in the country is an important tool for their surveillance and control. Biological control of larval instars is strongly recommended as a safe technique to control the mosquito population, and what is most important is not affecting the other aquatic invertebrate species.

Aim of the study

The aim of this study was to collect broad field data (all coastal area, some eastern and northern regions) on the species presence and distribution, as well as to minimize the mosquito concern through their biological control in larval stage in Albania.

Material and methods

Mosquito collection was performed during 2010-2012 by sampling of larvae in stagnant water and adults with CDC light traps, resting catch and human landing catch. During the period May-October 2015 a program on the integrated biological mosquito larval control was performed in the coastal and urban areas of the country. *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* in granular and water dispersible granules formulations were used as biological agents. The monomolecular film as well was used as physical control barrier. Eight different techniques were used following a different protocol formulary varying once in two or three weeks. Finally, a randomized questionnaire was performed to get a first impact of the inhabitants on the program of mosquito control

Results and discussion

The collected material was identified to species level and a total of 21238 mosquito specimens were identified. From the total 15119 (71.19 %) were males and 6164 (28.81 %) were females. The entomological material was classified in 8 genera: *Culex* genus 12672 specimens represented with 14 species (59.65 %); *Anopheles* genus 609 specimens in 12 species (2.85 %); *Aedes* genus 1139 specimens in 3 species (5.35 %); *Ochlerotatus* genus 6149 specimens in 7 species (28.81 %); *Culiseta* genus 671 specimens in 3 species (3.15 %); *Orthopodomyia* genus 7 specimens one species (0.03 %); *Coquillettidia* genus 9 specimens one species (0.04 %) and *Uranotaenia* genus 27 specimens one species (0.12 %). In total 42 species were reported throughout the country, where 11 species were reported for the first time. The most abundant species were as followed *Cx. pipiens* (46.2 %); *Ae. vexans* (1.38 %), *Ae. albopictus* (4 %), *Oc. caspius* (28.6 %), *Cx. impudicus* (1.98 %), *Cx. theileri* (2.09 %). A total of 3040 hectares were totally treated, with a two weeks period in twelve treatments with biological agents only. As a result from the questionnaire: 61 % of the population

answered that they did not have any concern by mosquitoes; meanwhile 39 % had had a concern. 82 % answered that there had been a low density of mosquitoes; meanwhile 18 % answered there had been a high or similar density. 85 % of the population is satisfied with the program; meanwhile 15% of them are not satisfied. 98 % of the population would like the program to be carried out the followed years; meanwhile 2 % did not like. 91 % of the population thought that even the community themselves affect the mosquito control process, and 9 % of them did not think so.

Conclusions

Our study showed a high species diversity on mosquitoes in Albania with a higher density along the coast, wetlands and lagoons areas. The invasive species like *Ae. albopictus* were present in most of the country starting from the coast up to 1260m altitude and were absent in the East part of the country in a region with altitudes above 700m. *Cx. pipiens* was found in all the studied areas and stations from the coastal areas to altitudes up to 1500m a.s.l. The program of mosquito control with biological agents is a very quick, effective and a safe method to control the larval mosquito population. It has a positive impact in increasing the life quality of people and tourists mostly in the coastal areas. It is strongly recommended that this integrated biological technique for the larval mosquito control is suggested to follow up as a continuous program in the country and worldwide.

Key Words: Mosquito, taxonomy, ecology, distribution, biological control, invasive species, Albania.

KEYNOTE 2

Artificial tick feeding systems: possibilities and challenges

Ard Nijhof

Freie Universität Berlin, Institut für Parasitologie und Tropenveterinärmedizin, Berlin, Germany

In vitro methods for feeding hematophagous arthropods have been developed for numerous arthropods and effective artificial feeding systems (AFS) are being used for (i) the colonization and maintenance of some species, (ii) the mass rearing of insects for use in control programs (e.g., the sterile insect release programs), and (iii) the facilitation of entomological studies. The latter includes the evaluation of the *in vitro* efficacy of test compounds against hematophagous arthropods, studies on arthropod physiology and pathogen transmission. Public demand for the use of alternatives to animal experimentation, as enunciated in the 3R concept (Replacement, Reduction, and Refinement of animal experiments) has further stimulated the development and usage of AFS.

The first report on AFS for ticks dates back over a century ago and describes the use of rat diaphragm or skin as membranes, stretched over a tube filled with a heated solution. Ticks pierced through the membrane and were able to feed (Hindle & Merriman, 1912. *Parasitology*, 5 (3), 203-216). Since these type of membranes are prone to decay, various alternatives were developed and significant advances in the *in vitro* feeding of both soft and hard ticks have been made since. Despite all improvements, the feeding success of ticks in AFS is in general still lower than that of ticks feeding on laboratory animals. Further optimization of AFS for ticks will be required to fully benefit from their potential in tick research.

DEVELOPMENT OF AN *IN VITRO* FEEDING SYSTEM FOR THE ANALYSIS OF THE VECTOR COMPETENCE OF TICKS IN THE TRANSMISSION OF *COXIELLA BURNETII*

Gustavo R. Makert^{1,2}, Sophia Körner^{1,3}, Katja Mertens³, Klaus Henning³, Martin Pfeffer⁴, Sebastian Ulbert¹

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The transmission of *Coxiella burnetii* and the role of ticks as vector and reservoir for Q fever are still unclear. The understanding of different possible ways by which *C. burnetii* can be transmitted is decisive for its efficient prevention. As alternative to the aerosolic route, the role of ticks as transmission vectors should be investigated in detail. To evaluate the transmission of *Coxiella*, a silicone-membrane *in vitro* feeding system based on previously published protocols was developed for *Ixodes ricinus* and *Dermacentor* spp. ticks. First, for the proof of principle, ticks will be fed artificially with *Coxiella burnetii* Nine Mile phase II. After the development of this artificial feeding system, this methodology will be extended to assess the transmission of *C. burnetii* by ticks: The distribution, excretion and reinfection of the agent will be investigated by quantitative realtime PCR (qPCR) and fluorescence microscopy. The results will allow us to judge the role of *Ixodes* and *Dermacentor* ticks in the transmission of *Coxiella* and to develop better tools for monitoring its occurrence in nature.

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Dieses Projekt wird finanziert durch das Ministerium für Bildung und Forschung (BMBF) unter der Projektnummer 01KI1726G als Teil des Nationalen Forschungsnetzes zoonotische Infektionskrankheiten.

Predicting and mapping human risk of exposure to *Ixodes ricinus* nymphs in northern Europe using climatic and environmental data

Lene Jung Kjær¹, Arnulf Soleng², Kristin Skarsfjord Edgar², Heidi Elisabeth H. Lindstedt², Katrine Mørk Paulsen^{3,4}, Åshild Kristine Andreassen³, Lars Korslund⁵, Vivian Kjelland^{5,6}, Audun Slettan⁵, Snorre Stuen⁷, Petter Kjellander⁸, Madeleine Christensson⁸, Malin Teräväinen⁸, Andreas Baum⁹, Kirstine Klitgaard¹, and René Bødker¹

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⁵ Department of Natural Sciences, University of Agder, Kristiansand, Norway

⁶ Sørlandet Hospital Health Enterprise, Research Unit, Kristiansand, Norway

⁷ Department of Production Animal Clinical Sciences, Section of Small Ruminant Research, Norwegian University of Life Sciences, Sandnes, Norway

⁸ Department of Ecology, Wildlife Ecology Unit, Swedish University of Agricultural Sciences, Grimsö, Sweden

⁹ Department of Applied Mathematics and Computer Science, Technical University of Denmark, Lyngby, Denmark

In recent years, focus on tick-borne diseases has increased as diseases such as Lyme disease and tick-borne encephalitis have become more common and represent a health problem in many parts of Scandinavia. More effective prevention of infections requires a better understanding of the factors affecting the vector abundance as well as human exposure to the vectors. Hence, there is a great need for analyses and models that can predict how vectors and their associated diseases are distributed now and possibly in the future.

As a part of the ScandTick Innovation project, we surveyed tick nymphs at 159 sites (forests and meadows) in Denmark, southern Norway and south-eastern Sweden. At each site we measured presence/absence, and used the data obtained along with environmental data from satellite images to run Boosted Regression Tree machine learning algorithms to predict overall distribution in southern Scandinavia. Together with the predicted distribution maps, we used human density maps to identify and plot areas with high risk of exposure to ticks.

The predicted distribution and the spatial variation found corresponded well with known distributions of ticks in Scandinavia (sensitivity: 91 %, specificity: 60 %), and we found that the model was predominantly temperature-driven. Because presence was strongly correlated with forested habitats the risk areas were much larger in Sweden and Norway compared to Denmark. When combining these distribution maps with human population density maps, we were able to quantify the proportion of people living in areas with tick presence in Scandinavia. We found that although tick nymphs were restricted to a small proportion of the modelled area, high proportions of the human populations (67-79 %) lived within these same areas. The model suggests that a potential future range expansion of *I. ricinus* in Scandinavia is likely but may only affect a relatively small additional proportion of the human population.

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Tick-borne pathogens detection in African cattle by PCR and a newly developed Reverse Line Blot microarray

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Alfons Renz³, Albert Eisenbarth^{3,4}

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Piroplasmoses and rickettsioses are diseases reducing the value of ruminants worldwide, thus having a high economic impact on livestock and farmers.

Important tick-borne diseases in Africa include piroplasmoses (*Babesia*, *Theileria*) and rickettsioses (*Anaplasma*, *Ehrlichia*, *Rickettsia*). The epidemiological study focuses on the detection of those genera in a cattle-rearing area in North Cameroon.

Each positive status can be from a primary or chronic infection or of a carrier stage only detectable by highly sensitive molecular tools, whereas microscopy is generally not sensitive enough to detect asymptomatic carrier animals. Due to environmental factors and vector pressure, cattle can be infested simultaneously by a variety of tick-borne pathogens. The commonly used molecular-diagnostic techniques such as conventional or real-time PCR have shown limitations both in the detection scope and sample capacity.

The PCR-based reverse line blot (RLB) technique and next-generation sequencing are therefore appropriate methods addressing such limitations with similar advantages. The latter is a powerful tool in terms of detection rate, number of screened species and samples, including quantifiable data. However, this high-throughput approach requires advanced laboratory infrastructure and bioinformatics capacity mostly not achievable on a routine basis in endemic developing countries. Our newly designed and developed RLB “low density” microarray has the potential to be the application of choice for veterinary laboratories with limited infrastructure to allow rapid, sensitive and specific pathogen detection of the above mentioned genera.

The microarray based DNA hybridization technique has been already used for tick-borne pathogen detection, but requires a high level of expertise and skills by the user. Here we present the prototype chip for piroplasmoses and rickettsial bacteria with standardized, simple workflow, and the results on prevalence of African cattle breeds in North Cameroon. After successful validation, the microarray chip shall be commercially available for the establishment in laboratories of Cameroon and other African countries.

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This study is based on a young researcher's grant of Dr. Albert Eisenbarth as PI

***Borrelia*-infections of ticks removed from humans, 2013-2017**

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Borreliosis is the most prevalent tick-borne disease of humans in Europe. In Germany, several genospecies of the *Borrelia burgdorferi* sensu lato complex as well as *B. miyamotoi* are present. This study investigated *Borrelia* prevalence and genospecies distribution in ticks removed from humans and sent as diagnostic material to the Institute for Parasitology, University of Veterinary Medicine Hannover, from 2013-2017. A quantitative real-time PCR was carried out and *Borrelia*-positive samples were subjected to a reverse line blot (RLB) for (geno-)species differentiation. In total, 20 % (510/2547) of ticks were *Borrelia*-positive. Prevalence was highest in adult ticks (27.1 % [191/704]), followed by nymphs (17.6 % [298/1706]) and larvae (4.6 % [3/65]). Between sampling years, prevalence ranged from 17.2 % (2014) to 24.1 % (2015). Overall, 271/475 positive samples (57.1 %) could be differentiated by RLB. *B. afzelii* was detected in 30.5 % of cases (145/475), followed by *B. garinii*/*B. bavariensis* (13.3 % [63/475]). *Borrelia miyamotoi* was detected in 7.4 % (35/475), *B. valaisiana* in 5.9 % (28/475), *B. spielmanii* in 4.6 % (22/475), *B. burgdorferi* sensu stricto (ss)/*B. carolinensis* in 2.3 % (11/475), *B. lusitaniae* in 0.6 % (3/475) and *B. bissettae* in 0.4 % (2/475) of positive ticks. Thirty-nine ticks (8.2 %) were coinfecting with two different (geno-)species. To further differentiate between *B. bavariensis*/*B. garinii* and *B. burgdorferi* ss/*B. carolinensis*, Sanger sequencing was carried out. Sequencing was successful for 52/63 *B. bavariensis*/*B. garinii*-positive samples, revealing 50/52 *B. garinii*-infections, while only 2/52 samples contained *B. bavariensis* DNA. Furthermore, 6/12 *B. burgdorferi* ss/*B. carolinensis*-positive samples could be differentiated; all of them were identified as *B. burgdorferi* ss. Comparison of the (geno-)species distribution between ticks removed from humans in 2015 and questing ticks collected in the same year revealed a significantly higher *B. afzelii*-prevalence in diagnostic samples than in questing ticks.

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Canine babesiosis in Lithuania

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Canine babesiosis caused by *Babesia canis* is an emerging infectious disease in Europe. Although previously uncommon, canine babesiosis has become quite frequent in Lithuania during the past decade. In the last few years an increasing number of cases with a wide variety of clinical signs have been recorded throughout the country. Expansion of *B. canis* in Baltic countries is associated mainly with the expanding range of the main vector - *D. reticulatus* and international travel. Genetic variability and antigenic variation are important mechanisms for the survival of *Babesia* parasites in their vertebrate hosts. Difference in the virulence of *B. canis* are associated with observed genetic heterogeneity among *B. canis* strains. We have investigated the genetic diversity and distribution of *B. canis* strains isolated from naturally infected dogs in Lithuania. Blood samples from dogs suspected of babesiosis were collected across Lithuania during 2014-2017. PCR-RFLP assay and sequence analysis based on 18S rRNR gene and Bc28.1 gene (which encodes a GPIanchored merozoite surface antigen) were used for molecular characterization of *B. canis* strains. The molecular analysis indicates the presence of genetically heterogenic strains of *B. canis* in Lithuania: five genotypes of *B. canis* were identified based on 18S rRNR gene, while ten different Bc28.1 gene sequence variants which divided into three genetically distinct groups were identified. The distribution of *B. canis* genotypes in Lithuania was not homogenic and showed similarity to North - East and South - West Europe findings.

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CANINE BABESIOSIS IN BELGRADE AREA IN PERIOD 2015-2017

Ivan Pavlović¹, Vlada Antić², Vladimir Terzin³, Dragana Petković⁴, Dragana Terzin³, Ljubomir Ćurčin⁵, Branislav Pešut⁶, Klara Ćurčin⁵, Ana Vasić⁷, Nemanja Zdravković¹

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Babesiosis is a tick-borne disease of dogs caused by protozoan parasite belonging to genus *Babesia*. In Serbia *Babesia canis* and *B. gibsoni* are present in past decades. Spread of babesiosis in dogs in Belgrade area has been continuously examined since 1997. In this paper we present the results of babesiosis in dogs in period 2015-2017.

Total of 1085 dogs' blood samples with clinical signs of babesiosis (anemia, haemoglobinuria, fever, pale of mucous membranes etc.) or infested with ticks were examined. We used capillary blood for examination, this blood films were air-dried, fixed in absolute methanol (>99.8%) for 1 minute and stained with 10 % Giemsa stain for 20-30 minutes. *Babesia* species were identified with microscopy using the schemes of Riek and Soulsby.

The positive results were found in 34.93 % (379/1085) of suspected animals. Throughout years the following results were obtained: in 2015 babesiosis was established in 31.34 % (121/386) of examined dogs; in 2016 in 32.06 % (126/393) and during 2017 in 33.84 % (132/390) of suspected animals. Dominant *Babesia* species occurred during our examination was *B. canis* established in more than 95 % of positive cases.

The number of positive findings of *Babesia* spp. in dogs increased each year within the investigated period. The dynamics of occurrence of dog babesiosis was monitored from January to December. It was noted that the increase in incidence of dog babesiosis started in the interval March-April. May was the month of infection maximum, decreasing gradually until July. The autumn infection peak occurred in September, disappearing completely in December. The appearance of Babesiosis in dogs coincided with the seasonal dynamic of vector ticks especially *Rhipicephalus sanguineus*, *Dermacentor reticulatus* and *D. marginatus*.

Epidemiological investigations into a babesiosis outbreak in a Northern German cattle herd

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Babesia divergens, transmitted by the tick *Ixodes ricinus*, is the most common cause of bovine babesiosis in Northern Europe and plays a role as a zoonotic pathogen in immunocompromised humans. In endemic areas, most cattle are immunologically protected from clinical disease in case they acquired the pathogen as a calf. Here, we report an unusually large outbreak of clinical babesiosis in a Northern German beef production farm. In May 2018, 21 adult cattle from two suckler cow herds died within a short period, showing classical symptoms of babesiosis. *Babesia divergens* merozoites were detected in blood smears and the species was confirmed by PCR and sequencing. Remarkably, the majority of the affected animals had been raised on the farm, which had not previously experienced any babesiosis outbreak. To investigate the local epidemiological situation, ticks were collected on the farm's pastures by the flagging method, and subjected to PCR for *Babesia* sp. detection. Furthermore, blood samples from all remaining animals, belonging to five different herds, were investigated for presence of *Babesia* DNA. At this point, *Babesia* DNA was still detected in eight animals despite prior treatment with imidocarb. Data on *Babesia* prevalence in the collected ticks, geographical distribution of positive ticks and *B. divergens* exposure of the different herds will be presented.

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Vector competence of field-collected and laboratory reared mosquitoes for West Nile virus Lineage 1

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West Nile virus (WNV) is a zoonotic *Flavivirus* transmitted by several mosquito species amongst which some are widely distributed in northern Europe. The first identification of WNV in Germany was recorded in a Great grey owl (*Strix nebulosa*) near Halle (Saale) in August 2018.

The aim of our research was to investigate the vector competence of indigenous mosquito species in northeastern Germany (field collected flood-water mosquito species (*Ae. cinereus/geminus*) and bridge vector (*Cx. pipiens*)) and of laboratory colony (*Ae. vexans* GR) for WNV lineage 1 under average summer temperature of July and August in northern Germany.

Mosquito eggs/larvae were collected at 14 locations during 2018. Emerging mosquitoes belonged to *Ae. annulipes* complex or *Ochlerotatus punctor* (April-June); *Ae. cinereus/Ae. geminus* and *Cx. pipiens* biotype *pipiens* (mid-June to September).

Vector competence experiments involving WNV lineage 1 (Magpie/Italy/203204; stock titer TCID₅₀= 10⁸/ml) were carried out under BSL3 conditions. Females (7-14 day old) were orally infected through an artificial membrane feeding system for 3 hours or overnight. Viral titers in blood meals ranged from 10^{6.5} TCID₅₀/mL to 10^{6.125} TCID₅₀/mL prior and after feeding. Fully engorged females were incubated at constant temperature of 24°C for 14 days before salivation assay and dissection to determine infection, dissemination and transmission rates of WNV lineage 1 by virological and molecular biology methods (qRT-PCR). Preliminary results showed that 21.6 % *Ae. vexans* GR (62/287), 15.4 % of the *Ae. cinereus/Ae. geminus* (4/26) and 1.8 % of *Cx. pipiens* (9/502) consumed blood. Survival rates revealed the crucial role of waking from anesthesia for *Ae. vexans* (32.1 % survival (17/53)) and *Cx. pipiens pipiens* (62.5 % survival (5/8)), while all *Ae. cinereus/geminus* recovered. 14 day survival ranged from 80 % for *Ae. vexans* to 100 % for *Cx. pipiens* and *Ae. cinereus/geminus*. Transmission and dissemination rates are currently under investigation.

Keywords: West Nile virus lin.1, *Cx.pipiens* biotype *pipiens*, *Ae.vexans*, vector competence, northern Germany

The influence of virus variant selection on *Culicoides* vector competence for bluetongue virus

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Bluetongue is an infectious, non-contagious viral disease of domestic and wild ruminants, caused by bluetongue virus (BTV), (Orbivirus: Reoviridae); a double stranded, segmented RNA virus transmitted by biting midges (*Culicoides* spp.).

BTV can infect all ruminants but causes severe disease mostly in sheep, resulting in significant economic losses to the agriculture sector of many countries. Currently, there are 27 known serotypes of BTV and numerous virus strains per serotype, all of which differ in key characteristics such as virulence and replication ability in the insect vector. Most importantly BTV can exchange genome segments when two BTV strains infect the same cell and such gene reassortment complemented by genomic mutations leads to the constant formation of new variants. Studies using conventional molecular methods have already shown that individual blood-feeding *Culicoides* select different BTV variants from the virus pool present in a viraemic blood-meal. However, the impact of virus variant selection on vector competence is currently unknown.

Therefore, the aim of this project is to determine BTV variant selection in a *Culicoides sonorensis* laboratory model (originally supplied by the Pirbright Institute) from a viral variant pool with next generation sequencing techniques.

For this, laboratory reared *C. sonorensis* will be fed via artificial membrane systems on blood-virus mixtures containing virus variant pools of a highly competent BTV-4 strain and virus variants present within the blood meal will be compared to those selected by fully disseminated insects. Initial experiments will be followed by assessing the impact of viral dosage limits and different vector incubation temperatures on virus variant selection.

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Effect of a combination of H₂O₂ fluid and permethrin by use of a fogger-technique on permethrin resistant and non-resistant bed bugs (*Cimex lectularius*)

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Bed bugs (*Cimex lectularius*) are insects indwelling human buildings such as houses, hotels, cabins etc., that affect human health by biting during the night hours. Although the vector role could not be confirmed for any pathogen so far, their occurrence can lead to dermatological signs such as wheals, redness and pruritus and mental impacts like insomnia and anxiety state. Countermeasures against the bugs are hampered due to their hidden life style and selection for insecticide resistant strains. Additionally their distribution is favoured by reasons of globalisation and traveling of people and transportation of goods.

Pyrethroid resistant and non-resistant strains of bed bugs (15 individuals per group) were exposed to a mixture of permethrin and H₂O₂ by the use of a modified fogger (droplet size ~ 1 µm) for 2 and 4 hours and their viability was evaluated in comparison to control groups (without treatment, permethrin alone, H₂O₂ alone). Additionally, the expression levels of mRNA of several detoxification enzymes from the groups cytochrome P450 monooxygenases, glutathione-S-transferases, carboxylesterases were determined by using RT-qPCR of each bug individually.

The killing effect and the expression levels - including the resistant strain - of the mixture (permethrin and H₂O₂) suggest a synergetic effect of the compounds, which might be explained by applying O₂ radicals on organisms and simultaneously increase the oxidative stress due to permethrin.

Although other resistant strains have to be tested, these results are promising and the application on other arthropods can be considered in future.

Arthropod-Borne Diseases: Clinico-pathological presentation of Babesiosis and demodicosis in different domestic animals species

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Introduction: Over the past three decades, tick-borne diseases have increased and now constitute a major health problem in many parts of Europe, Africa and around the world. Babesiosis is responsible for causing fever, anemia, haemoglobinuria, shock, kidney dysfunction and death in affected animals.

Materials and Methods: Some cases of ticks and mites infestation in various species of domestic animals (avian, canine, ovine and bovine) presented to Veterinary clinics and Veterinary Pathology laboratories of the Veterinary Teaching hospital, Faculty of Veterinary Medicine, University of Ilorin, Nigeria were investigated by Clinical examination, Haematology, thin smear microscopy, microbiology and postmortem examination (PM).

Result: All species examined had varying degrees of ectoparasitism by ticks and/or mites. Babesiosis and demodicosis were the confirmed diagnosis. Clinical and pathological manifestation of babesiosis in chicken included weakness, paralysis, tick attachment histiolympocytic dermatitis, intraerythrocytic babesia organisms and erythrophagocytosis. While in the dog, there was anemia, jaundice, PM icterus, splenomegaly, myocardial ecchymosis, and nephropathy. Similarly, there was haemoglobinuric nephrosis, icteric nephropathy and splenomegaly in sheep and calf. In the fattening ram with demodicosis, raised solitary and multi-nodular epidermal skin biopsy showed parakeratotic hyperkeratosis with bacteria colonies. Hair follicles, sebaceous glands and the dermis often times had cross sections of large arthropod consistent with demodex mite and foci of eosinophilic inflammatory exudates.

Discussion: Worthy of note is the tick attachments on these animals on clinical presentation especially on the chicken. Apart from the hemolytic crisis caused by arthropod-borne organisms, local tick attachment leading to contact dermatitis, a delayed hypersensitivity reaction which was evident grossly by epidermal swelling and microscopically by the histiolympocytic dermal infiltration in the chicken is of note. All of the babesiosis cases were fatal in the species examined due to systemic organ lesions, showing that unattended babesiosis or late presentation at the clinic results in fatal outcomes.

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Integrated Pest Management (IPM) - Updated Considerations from the Entomological Practice

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Old realisations

- Pesticide/ antibiotics overuse generates resistance.
- Human population explosion
- Natural resource limitations
- Vector borne diseases kill more humans than wapons in wars.
- *(Past priorities; human holistic entomo-ecological perspective -> SCHOLL 2009)*

New - changing challenges in a changing world, e.g.

- Availability and generation of information easier than ever before.
- The more one knows about a subject, the easier it is. NOW

Since 2017

- Bed bugs do not transmit diseases - they are a luxury problem.
- Impairment of real estate through arthropods
- holistic concepts (parasitic mites, invasive polygynic ants, pigeon ticks,
- individuals with fear of parasites
- *sustainability of chemicals, pharmaceuticals (honorary)*

The old and new question is whose interests are worth it to allow oneself to be exploited. Responsibility of professional smart alecks is to share their treasures.

Conclusion, Consequences, priorities; human holistic entomo-ecological perspective

- Update knowledge permanently
- Translate science into lay person language and share.
- Merge old intuitive low-tech knowledge into new technology
- Revival of alternatives to chemicals - simplicity (e.g. modification of attitude, behavior, structure; barriers, traps; unity of timing, location and action; self help - potentials and limitations; intuition)
- Stand in for limitation of waste of limited resources, and for smart use.

SCHOLL, E. (2009): From Integrated Pest Control to Holistic Risk Reduction - Sustainable Management of Nuisance, Pests, Disease Carriers and Vectors.

http://schaedlingsbiologie.de/files/content/downloads/IPM-Sustainable-Pest-Risk-Reduction_DGMEA-BiKF_2009.pdf

Tick, mite and flea infestation of small mammals at three locations in Germany

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Rodents serve as main hosts for the development and the distribution of several blood sucking ectoparasites such as ticks, fleas and mites. The aim of this study was to investigate the different ectoparasite species prevalence and mean intensity on rodents, to evaluate the rodents' role in the ectoparasites' development and maintenance. Small mammals were trapped during 2012 and 2013 at three different locations in Germany, determined to species level and examined for ectoparasites. In a descriptive analysis the overall number of small mammal species and ectoparasites as well as the number per location were examined. Prevalence and mean intensity were calculated for the overall number of animals caught and stratified for small mammal species. Chi-squared tests were used to test for differences between species.

Overall, 779 animals (689 in 2012, 90 in 2013) were caught during the study period. The rodents (n=775) comprised seven species with *Myodes glareolus*, (n=473) and *Apodemus flavicollis*, (n=247) being the two most common species. Most animals were trapped in the location Cospudener See (n = 498) followed by Tussenhausen (n = 241). Only 36 animals were trapped in Dörnberg Park, all of the species wood mouse *Apodemus sylvaticus*.

Three species of ticks, twelve species of mites and twelve species of fleas were found on the small mammals during the study period. *Ixodes ricinus* was the most prevalent tick species (68 %) but *Dermacentor reticularis* displayed the highest mean intensity of all ticks (8.07), *Laelaps agilis* the most prevalent mite species and had the highest mean intensity (19 %, 6.56) and *Ctenophthalmus agyrtes* the most prevalent flea species and had the highest mean intensity (50 %, 2.26). The distribution and co-occurrence of ectoparasites species will be the focus of further analysis.

Molecular survey of two major tick-borne pathogens in *Ixodes ricinus* ticks collected from natural habitats in North East Germany

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Ixodid ticks are responsible for transmitting the greatest variety of pathogens when compared to other arthropods. *Ixodes ricinus*, the most widespread tick species in Europe and Germany, is known to feed on many different hosts and is capable to transmit numerous pathogens. Most relevant pathogens for which *I. ricinus* is a competent vector are *Borrelia burgdorferi* sensu latu, the agent responsible for Lyme disease and the tick-borne encephalitis virus.

The aim of this study was to assess the prevalence rates with molecular methods of two major tick-borne pathogens in *I. ricinus* ticks from North East Germany. In order to perform the study, we collected ticks by flagging from 17 forest sites in Western Pomerania between April and October 2018. Samples were processed by RNA and DNA extractions, performed from each individual adult tick and from pools of 10 nymphs. RNA samples were tested by RT-qPCR for detection of tick-borne encephalitis virus while DNA was tested by nested PCR followed by sequencing for identification of *Borrelia* species. A total of 2410 ticks were obtained of which 234 were females, 231 males and 1945 nymphs.

So far, after analyzing 249 samples for *Borrelia* spp., 61 (24.5 %) ticks tested positive. The comparison between developmental stages showed a higher prevalence rate in nymphs (34.8 % vs 23.9 % of females vs 14.6 % of males). Sequencing revealed several *Borrelia* species relevant for public health: *B. garinii*, *B. afzelii*, *B. valaisiana* and relapsing fever agent *B. miyamotoi*. RT-qPCR for TBEv is undergoing, no positive samples being detected until now.

To elucidate the potential of pathogen transmission by *I. ricinus* and other relevant tick species in Germany and Europe, a main focus of our perspective research will be the assessment of vector competence for putative and major tick-borne pathogens that constitute a growing threat to human and animal health.

ROAD-KILLED VERTEBRATES AS SENTINEL HOSTS FOR ACTIVE SURVEILLANCE OF TICK-BORNE PATHOGENS

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Tick-borne zoonoses represent a serious threat for human and animal health. Thus, efforts are made to monitor the spatial distribution, intensity and diversity of their causative agents. Nevertheless, most of the studies focus on the tick vectors, whereas the vertebrate hosts remain neglected, mostly due to issues associated with complicated sampling (laborious, skill-demanding, legislative/species protection etc.). The main aim of our project is to verify whether carcasses of accidentally killed (mostly road killed) animals are suitable source of biological material for monitoring of selected tick-borne pathogens. Considering the epidemiological significance, we have focused specifically on urban habitats. Hedgehogs (*Erinaceus europaeus* and *E. roumanicus*), squirrels (*Sciurus vulgaris*) and blackbirds (*Turdus merula*) were chosen as representatives of wild animals that thrive in urban areas, are ordinarily infested by ticks and seem to be suitable hosts for many important zoonotic pathogens. A total of 169 specimens (75 hedgehogs, 22 squirrels and 72 blackbirds) were collected with the help of general public. Altogether 1 267 samples of different tissues were screened by multiplex qPCR assays. The prevalence of the main target pathogens reached for hedgehogs, squirrels and blackbirds respectively was 72 %, 100 %, 58 % for *Borrelia burgdorferi* s.l., 89 %, 82 %, 51 % for *Anaplasma phagocytophilum*, 21 %, 77 %, 3 % for *Bartonella* spp. and 33 %, 4 %, 4 % for *Rickettsia helvetica*. In conclusion, carcasses of accidentally killed vertebrates were confirmed as a useful source of biological material for monitoring of several tick-borne pathogens in urban environments

MONITORING SEASONALITY OF Aedes albopictus EGGS IN NORTH AND SOUTH COAST OF ALBANIA DURING 2015

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Aedes albopictus is already a well stabilized population in Albania and since its first introduction has spread across the country. The Albanian populations of the species originated from the temperate areas are able to survive the cold season through the production of diapauses eggs.

The aim of this study was to monitor *Aedes albopictus* eggs and evaluate the length of the favorable season for their growth in south and north coast areas of the country.

Two study sites were determined and set in the north coast (Velipoje and Shengjin) and another two ones in the south coast (Ksamil and Sarande) with 10 ovitraps in each site. Seed germinating papers were used in ovitraps with 300 ml of water to collect the eggs. Every two weeks the germinating papers with eggs were monitored and collected and further counted under lighted stereomicroscope. The respected data were entered in a database for further analyses.

A total of 9187 eggs were collected in both regions (respectively 31.57 % in north and 68.43 % in south).

Egg density was measured throughout the study period and we concluded that the highest number of eggs was collected in the south part (69.11 %) and less in the northern one (30.89 %). The deposition of eggs in all sites starts from the 22nd week and peaked at week 28 (6-12 July). A second peak was reached in the first week of September (31st Aug-6th Sept). The minimum value (99.1) was reached during the 1st week of October.

Key words: *Aedes albopictus*, oviposition, monitoring, Albania.

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