

Friedrich-Loeffler-Institut Federal Research Institute for Animal Health

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Cover picture: Fluorescence-microscope image of cells infected with Variegated Squirrel Bornavirus

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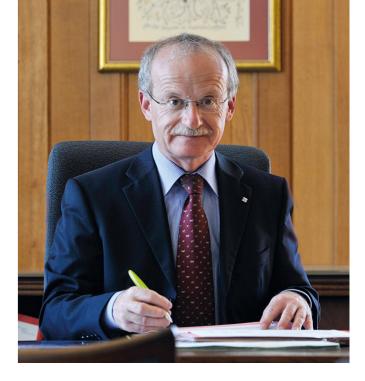
Dear Reader

For more than 100 years the Friedrich-Loeffler-Institut (FLI) has conducted research in the field of animal health. Over this time period scientific communication has undergone considerable changes. Not only has it left the ivory tower and moved to the heart of society. The speed at which scientific results are communicated and publicized also has steadily increased.

At the same time, the interest of society in scientific issues has grown and with it our responsibility as a federal research institute to communicate comprehensibly what we are doing and what we are able to do, and to explain complex scientific contexts and their background. This can be done by publications, media contributions, and in a public dialogue.

At the FLI, 850 staff members conduct research for the health and well-being of food-producing animals. However, research on animal health also is research for the protection of humans from zoonotic infections which affect both animals and humans. As a consequence of climate change and worldwide trade and travel formerly "exotic" infections rapidly become commonplace and globalization also holds for epidemics. Furthermore, questions relating to species-appropriate animal husbandry and responsible handling of farm animals become more and more important.

To meet these challenges successfully, innovative research approaches, transdisciplinary cooperation, and worldwide networks are needed. For this purpose, the eleven specialized FLI institutes cooperate closely with each other and are involved in numerous projects with national and international research institutions.



To generate scientific data, assess risks, and communicate results objectively and transparently, is only one of our tasks. As a scientific research institute we also act as advisers for decision makers at the national and international level and support political actions and legislation with our scientific findings.

The aim of this brochure is to describe our diverse activities comprehensibly, and above all to pique your curiosity for our scientific work.

I hope you enjoy reading this brochure

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Prof. Dr. h.c. Thomas C. Mettenleiter President of the FLI

At headquarters on Insel Riems, six of the eleven specialized FLI institutes conduct research in the fields of epidemiology, immunology, infectology, molecular virology and cell biology, novel and emerging infectious diseases, and diagnostic virology. The Institutes of Bacterial Infections and Zoonoses and of Molecular Pathogenesis are located in Jena. With the Institutes of Animal Nutrition, Farm Animal Genetics, and Animal Welfare and Animal Husbandry in Braunschweig, Mariensee, and Celle, respectively, the FLI has the full range of expertise needed to deal with the multifaceted aspects of animal health.

Research for animal and human health

The Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), conducts research for the health and well-being of food-producing animals and for the protection of humans from infections which can be transmitted between animals and humans.

This is the focus of 850 staff members distributed over five sites in Germany. The headquarters of the FLI are located on the island of Riems close to Greifswald. At this site, research concentrates on the prevention, diagnosis, control, and epidemiology of viral infections. Laboratories and experimental animal facilities up to the highest biosafety level 4 are available. The infectiologist Friedrich Loeffler founded the FLI at this site in 1910 – thus it is the oldest virus research institute worldwide. In Jena, basic and applied research on bacterial diseases is conducted. Furthermore, the Institutes of Animal Welfare and Animal Husbandry in Celle, of Animal Nutrition in Braunschweig, and of Farm Animal Genetics in Mariensee address classical farm animal sciences.

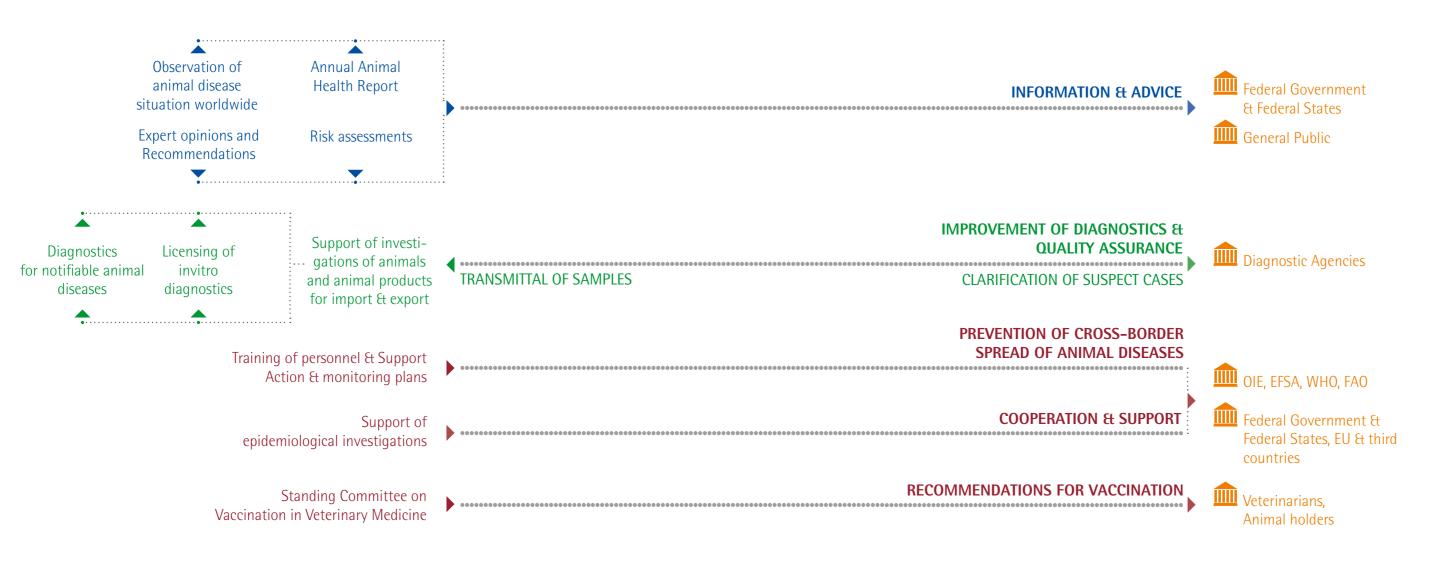
In addition to collaborations with numerous universities and research institutions, the FLI participates in projects of international organizations such as the World Organisation for Animal Health (OIE), the European Food Safety Agency (EFSA), the World Health Organization (WHO), and the Food and Agriculture Organization of the United Nations (FAO) and is a member of various committees. In accordance with the "One Health concept", i.e. the unity of human and animal health and the environment, there is intensive exchange between natural scientists and specialists in human and veterinary medicine. Thus, the FLI also is involved in the German Centre for Infection Research (DZIF) to strengthen this centre's expertise in the field of animal health.

In the frame of policy advice on the national and EU level, the FLI prepares expert reports and advisory opinions. As an independent higher federal authority of the Federal Ministry for Food and Agriculture (BMEL) essential tasks of the institute are defined in Article 27 of the Animal Health Act. This includes the function as National Reference Laboratory for all notifiable animal diseases.



Large photo: FLI site island of Riems; row of photos: FLI sites island of Riems, Braunschweig, Mariensee, Celle, Jena





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On the trail of infectious agents

The filter developed by Shibasaburõ Kitasato made "ultrafiltration" possible. By means of fine-pored porcelain and kieselgur material, solutions could be filtered under vacuum conditions. Bacteria generally do not fit through the pores.

Friedrich Loeffler and Paul Frosch started searching for the causative agent of footand-mouth disease (FMD), they worked with ultrafiltration through porcelain and kieselguhr filters, at that time a "hightech" method. These filters withheld even the smallest bacteria, but not the causative agents of FMD. Based on this observation, the two scientists postulated the existence of novel, even smaller pathogens: viruses. Therefore, Loeffler and Frosch today are rightly regarded as the founders of virus research.

At the end of the 19th century, when

Portrait Friedrich Loeffler

Friedrich Loeffler and Paul Frosch. 1898:

"Therefore the assumption cannot be denied that the effects of the filtrates are not the effects of a solute, but the effect of pathogens capable of reproduction. These, of course, would then have to be so small that they would be able to pass through the pores of a filter that retains even the smallest bacteria ..."

The foot-and-mouth disease virus belongs to the family of picornaviruses, which are among the smallest known viruses with a size of 25-30 nanometers (1 nm corresponds to 1 millionth of a millimeter). It consists of an icosahedral protein shell containing the genetic material. As an infectious disease with both economic and animal health significance, FMD continues to represent a great challenge for animal disease control.

Certainly, Loeffler and Frosch would have been glad to observe these 'tiniest of living beings' themselves. However, this only became possible by the development of electron microscopy in the 1930s which allowed the first view into the nanoworld of viruses. The FLI has been working with this technology since 1944 and still uses it to characterize viruses. Cell culture techniques have since then allowed virus replication outside the infected animal.

From ultrafiltration to modern diagnostics

Another milestone was set by methods which do not detect the complete infectious agents themselves but their genetic material (DNA/RNA) instead. As a molecular diagnostic method, especially polymerase chain reaction (PCR) has accelerated diagnostics from the 1990s on. Genome segments from saliva, nasal secretions, blood or tissue samples are amplified millions of times and detected. PCR delivers reliable results within hours - a particularly important aspect in animal disease diagnostics.

In addition, PCR paved the way for rapid and easy complete sequencing of genetic material. Meanwhile, "high throughput sequencing" methods are so sophisticated that even unknown pathogens can be detected based on smallest genome segments which are compared to known signatures. Using this method scientists of the FLI were able to first detect the previously unknown Schmallenberg virus in cattle in 2011 and a novel bornavirus in

variegated squirrels in 2014, which also infected and killed several humans.

In addition to the increasingly efficient pathogen analysis, the FLI also develops detection systems for rapid diagnostics, so-called "point-of-care" or "pen-side tests".

Modern diagnostic procedures rely

on targeted and sensitive methods

that are often based on the detec-

tion of the genetic material of animal

pathogens. Decisive here is correct

preparation of the sample according

to the requirements of the detection method. Also important is the deve-

lopment of the appropriate software

for analysis of the huge quantities of

data that are generated.

Without elaborate laboratory technology, infectious agents can be detected quickly and reliably on site. Thus, the FLI also supports countries where no appropriate laboratory facilities are available.

An end of further enhancements and new developments is not in sight. The FLI will continue its research work to develop rapid and efficient diagnostics for the protection of animals and humans.



The island of Riems in the 1930s and today - headquarters of the oldest and at the same time one of the most modern research institutes for virus diseases of animals

Prof. Dr. h.c. Thomas C. Mettenleiter, born in 1957 in Göppingen, studied biology in Tübingen and earned a PhD in genetics in 1985. After a research fellowship at the Vanderbilt University Medical School in Nashville, USA, he worked at the Federal Research Centre for Virus Diseases of Animals in Tübingen until 1993 and qualified as university lecturer in 1990. In 1994, he became head of the Institute of Molecular Virology and Cell Biology, as it is named today, on the island of Riems. In 1996, he was appointed as President of the FLI.

3 Questions to the President



What is the biggest challenge as president of a federal research institute?

To keep the team together. Not only are there five sites with their own history and development. There also are 850 staff members in eleven different specialized institutes. To be visible "at home" and at the same time fulfill our task to support and guide research in the field of animal health in Europe and worldwide is the biggest challenge.

Global trade and travel routes, warm temperatures and mild winters support the rapid spread of animal diseases. Can we win this battle?

It is indeed a continuous struggle, but we have certainly achieved great successes. Nevertheless, it remains a challenge at the regional, national, international and global level. To prepare ourselves as well as possible for the unknown and to do our best for the health of humans and animals is our core task.

What do you find so fascinating about viruses?

At the age of fifteen I saw a picture of a bacteriophage in Hoimar von Ditfurth's book "Im Anfang war der Wasserstoff" that I couldn't get out of my mind. I wanted to explore and understand these "smallest creatures" myself. The universe of viruses is infinite and only a fraction of it has been explored so far. For me, virus research remains the most exciting activity that I can imagine.



Mission fowl plague – on the track of the culprit

Fowl plague can occur unpredictably, spread rapidly and cause millions of dollars in economic damage as well as immense animal suffering. The pathogen type and its pathogenic properties often decide on the economic existence of farmers. In order to minimize the spread of the pathogen and the harm done to humans and animals, the most rapid diagnosis possible is required to either rule out the animal disease or to take countermeasures as quickly as possible.

The outbreak investigations, for which the affected federal states can request assistance of the FLI's epidemiological advisory group, require almost criminological precision work: In order to identify possible causes and the time period of infection, animal holders, employees and veterinarians are surveyed in detail. In the end, in many cases, the most likely causes of introduction are identified, but determination of a unique cause is seldom. Very often the "lone perpetrator" as in a crime thriller does not exist.

To enable animal disease diagnostics directly on site, the FLI has converted a minibus into a mobile laboratory, which is equipped with all neces-

sary utensils for the diagnosis of various animal pathogens and is ready to roll within 30 minutes. The epidemiological advisory group will be able to use the mobile laboratory flexibly in the frame of future epidemiological missions.

Most of the laboratory work is still done in the reference laboratories of the FLI. Every year, thousands of samples from all over Germany and the world are examined in the National and OIE Reference Laboratory for Avian Influenza. To find out how the viruses came to Europe, their genetic material is analyzed and compared with existing virus sequences.

Infection studies provide information on whether and under which conditions transmission to mammals is possible and which factors are responsible for the pathogenic effects of fowl plague viruses. These results have many uses including the development of vaccines.

Another tool to prevent and control animal diseases are risk assessments that serve as a source of information for the general public and decision makers. They can help prevent and control animal diseases before they spread.

In case of an outbreak, the responsible veterinary agency takes samples from livestock or poultry.

Before handing them over to the mobile laboratory, the sample containers are disinfected. Infectious agents possibly present in the samples will be inactivated on site.

Since PCR diagnostics is carried out in a closed system, contamination of the workplace with pathogens is excluded. "The result of our investigations can decide on the economic existence of farmers."

Dr. Timo Homeier-Bachmann,
Epidemiologist at the FLI on the island of Riems



What does a pig need?Behavior-based requirements in theory and daily practice

Pigs by nature are very curious. They explore their surroundings with their mouths and muzzles. The surroundings also include the tails of their fellow species – especially if there are too few other stimuli or the animals are overwhelmed by too many behavioral requirements. The consequences can be injuries, infections and pain. Tail docking, i.e. removal of the tail in young piglets, does not prevent tail biting satisfyingly. It is associated with immediate pain and is allowed in exceptional cases only.

Individual triggers for tail biting are hard to identify. Rather, it is the interplay of numerous factors affecting the pig – from a comfortable lying area to the health status and quality of care. The "tail-biting intervention program" provides support in identifying problem fields: it is a management assistance program that provides an overview of the holding. It was developed by the Institute of Animal Welfare and Animal Husbandry of the FLI in Celle and has been passed on to agricultural advisors and veterinarians in training courses. The latter in turn help farmers to analyze factors such as microclimatic conditions in the animal housing, feeding conditions, or alternative behavioral enrichments, and to provide more animal-friendly holding conditions. Approximately 300 holdings have participated in the project to date.

"There is no universal solution. The farmer has to decide individually which measures to take."

Dr. Sabine Dippel,
Biologist and expert in livestock sciences at the Institute
of Animal Welfare and Animal Husbandry in Celle

In addition to tail biting in pigs, the institute investigates the behavioral requirements of livestock and explores the question of how animal-friendly holding conditions and humane slaughtering can be implemented in practice. The Institute, together with the Institute for Farm Animal Research of Wageningen University (Netherlands) and the Department of Animal Science of the University of Aarhus (Denmark), has been designated as the first EU Reference Center for Animal Welfare. The research results gathered in the individual investigations and projects provide the basis for assessments and expert opinions related to animal welfare. However, higher requirements, e.g. in space and care, are not necessarily reflected in the price policy for meat, eggs and milk. It is precisely this conflict between the needs of animals and daily practice in agriculture that makes the topics animal welfare and animal husbandry so important.

Tuberculosis of cattle – long known disease with new relevance

Tuberculosis of cattle is a bacterial infectious disease occurring worldwide that is transmissible to many animal species and to humans. Thanks to the highly effective control measures initiated in the 1950s, many European countries today are considered to be tuberculosis-free. Germany was recognized officially by the EU as free of tuberculosis in 1996. Nevertheless, isolated outbreaks still occur repeatedly.

In addition, due to the wide spread of the pathogen in natural reservoirs, there are some European countries that have great problems in controlling the disease. These include the UK with

the reservoir host badger, the Alpine region with infected red deer populations, or Spain where the disease occurs in wild boar. France also has been reporting an increasing number of outbreaks. Cattle holdings in Africa, Asia, Australia, New Zealand, North and South America are also affected. Therefore, monitoring of imported cattle and other susceptible animals is extremely important. The Refrence Laboratory for bovine tuberculosis at the FLI in Jena processes up to 750 samples a year to clarify suspect cases.



In the past, tuberculosis of cattle was primarily transmitted to humans through untreated raw milk. Pasteurization of milk now protects consumers from a possible transmission. Today, many people are only aware of tuberculosis as a human problem. However, the long known tuberculosis of cattle has now gained new relevance and should not be underestimated. Infection is not always obvious. Even clinically inapparent animals can transmit the pathogen to humans and from animal to animal. In Germany, the last cases of infection were detected during slaughter inspection of cattle. It is important to preserve the knowledge and awareness of this animal disease and also to improve prevention and control outside Germany.

"Thanks to the highly efficient control measures taken since the 1950s many farmers today are no longer aware of tuberculosis of cattle." Dr. Stefanie A. Barth, Veterinarian and head of the Reference Laboratory for bovine tuberculosis at the FLI Jena

Mosquitoes – blood-sucking transmitters of pathogens as a global challenge

Today diagnostic methods for novel infectious diseases such as Bluetongue disease, African swine fever and West Nile fever are more efficient and rapid than ever before. However, the animal diseases themselves are able to spread just as quickly and efficiently. So-called vectors, transmitters such as mosquitoes and ticks, which spread via international trade and travel routes and by increasing global warming, make the fight against pathogens a global race against time. To make things worse, knowledge concerning the potential of invasive and indigenous bloodsuckers to transmit unknown pathogens is very limited.

Germany-wide mosquito monitoring investigates the spread of invasive and indigenous mosquitoes. Mosquitoes caught in net traps and other sampling vessels, larvae collections and trapped insects provide information about the mosquito species present in different regions of Germany. The Citizen Science Project "Mückenatlas" also allows citizens to participate in re-

search and send in their own mosquito finds.

Over 360,000 mosquitoes from submissions and collections have already been analyzed. The question of how indigenous mosquito species are able to efficiently ingest, replicate and transmit pathogens is also investigated at the FLI on the island of Riems. From here, the National Expert Commission "Mosquitoes as Transmitters of Pathogens" is coordinated. This commission discusses questions relating to monitoring and control of biting midges as vectors of infectious agents, bundles various fields of expertise and develops recommendations for action and action plans. This mainly concerns handling of the Asian tiger mosquito, which is already present in southern Europe and has recently been detected in Germany as well. The mosquito, which is only a few millimeters in size, can transmit at least 26 different viruses. These include e.g. Dengue, Chikungunya and Rift Valley fever viruses, which can be fatal to humans. An immediate and effective control of the tiger mosquito before it becomes endemic in Germany is all the more important.

> "We should try to prevent the establishment of the Asian tiger mosquito in Germany for as long as possible."

Dr. Helge Kampen,
Entomologist at the FLI and head of the National Expert
Commission "Mosquitoes as Transmitters of Pathogens"



High-tech research in the combat against fatal pathogens

Zoonotic pathogens originating in animals can be highly dangerous to humans. There are no vaccines or therapies available for some of these diseases. A number of these highly dangerous pathogens may only be investigated in specialized safety laboratories of the highest biosafety level 4 (S 4). With its new laboratory buildings and animal housing costing approximately € 350 million and completed in 2013, the FLI has a unique infrastructure on the island of Riems. 89 laboratories and 163 animal rooms were built here on an area of approximately 80,000 m². The laboratory and animal housing biosafety level 4 area with a size of almost 350 m² represents the "centerpiece" of the complex.

The new high containment facility provides the opportunity in Europe to investigate highly dangerous animal and zoonotic pathogens directly in the natural host animal and subsequently develop suitable vaccines.

Two animal rooms of 66 m² each make it possible to do research on infections with highly dangerous Ebola, Crimean-Congo Haemorrhagic Fever, and other S4 pathogens in large animals such as pigs and cattle. So far, this type of research work in large animals has only been possible in Winnipeg in Canada and in Geelong in Australia.

Such a high containment facility can only be run under special conditions with regard to building and safety technology. The entire S 4 area represents a "box in box" system. This means that the area with the highest safety level is surrounded by that of the next highest level (3). The scientific staff, the technical staff and the animal caretakers must first pass this area under the provisions of the applicable safety and hygienic measures the clock.

before they can enter the S 4 area. They work in a full protective suit, which is supplied with filtered air via a hose equipped with a safety valve. Therefore, the suit always remains slightly inflated so that no pathogens can enter even if there should be a small leak. The entire building is constantly kept at a pressure below that of the surroundings. It is monitored by the technical services around the clock.



The supply and exhaust air is filtered twice. All wastewater is collected in tanks located below the laboratory, is treated chemically and then thermally and thus sterilized directly in the building before proceeding to the institute's own wastewater treatment plant. The carcass disposal plant is also in the S4 area. For every activity, whether in the animal facility or in the 180 m² laboratory, the employee is in radio contact with a "buddy". This "buddy" is specially trained for these tasks and the special conditions of the S4 area and stays in the adjoining laboratory area to assist the employees in the S4 area from outside and to be able to help in an emergency. When leaving the S4 area, the full protective suit is disinfected thoroughly in a chemical shower; only after this chemical shower the employees are allowed to open the suit and take it off.

"I am not afraid, I have respect for the pathogen and the consequences of an infection. But this is positive, it makes me work attentively and cautiously."

Dr. Sandra Diederich,
Human biologist at the FLI on the island of Riems

200,000 semen samples for the preservation of genetic diversity

The "red list" of endangered livestock breeds in Germany is long. While in the past there were hundreds of different livestock breeds, today the entire livestock production uses just eleven different breeds. The German Genebank of farm animals is a repository of genetic diversity and wants to contribute in the long term to its preservation.

The Institute of Farm Animal Genetics of the FLI in Mariensee coordinates the German Genebank together with the federal states. This results in a Germany-wide network for the preservation and use of old agricultural breeds from poultry to cattle. In close cooperation with breeding associations and insemination centres, semen samples from 25 non-related males per livestock breed are collected from existing herds nationwide. The Genebank can now access more than 200,000 semen samples. Not only semen is frozen permanently, but also embryos and other genetic material are stored in large nitrogen containers. These can be used at a later date, e.g. for conservation breeding programs of breeds which have suffered significant losses due to an animal disease, or for specific research questions, e.g. for molecular genetic characterization of the stored material. An endangered breed can thus be restored and its genetic diversity preserved. In Lower Saxony, among these breeds are the Bentheim Black Pied pig, the German Black Pied cattle, the Leine sheep and the German Sperber chicken. The genetic resources of goat and horse breeds are also stored here at -196 °C.

The Genebank also allows the exchange of material to supplement corresponding collections in Germany and abroad. This creates an archive of livestock history that depicts the diversity of indigenous agricultural animal breeds; a public good, which now remains available for breeding in the long term.



"More than 200,000 samples are archived here. They are intended to preserve what has been lost in the field."

Dr. Martina Henning,
Coordinator and head of the German Genebank at the Institute of Farm Animal Genetics of the FLI Mariensee



Eating for the climate

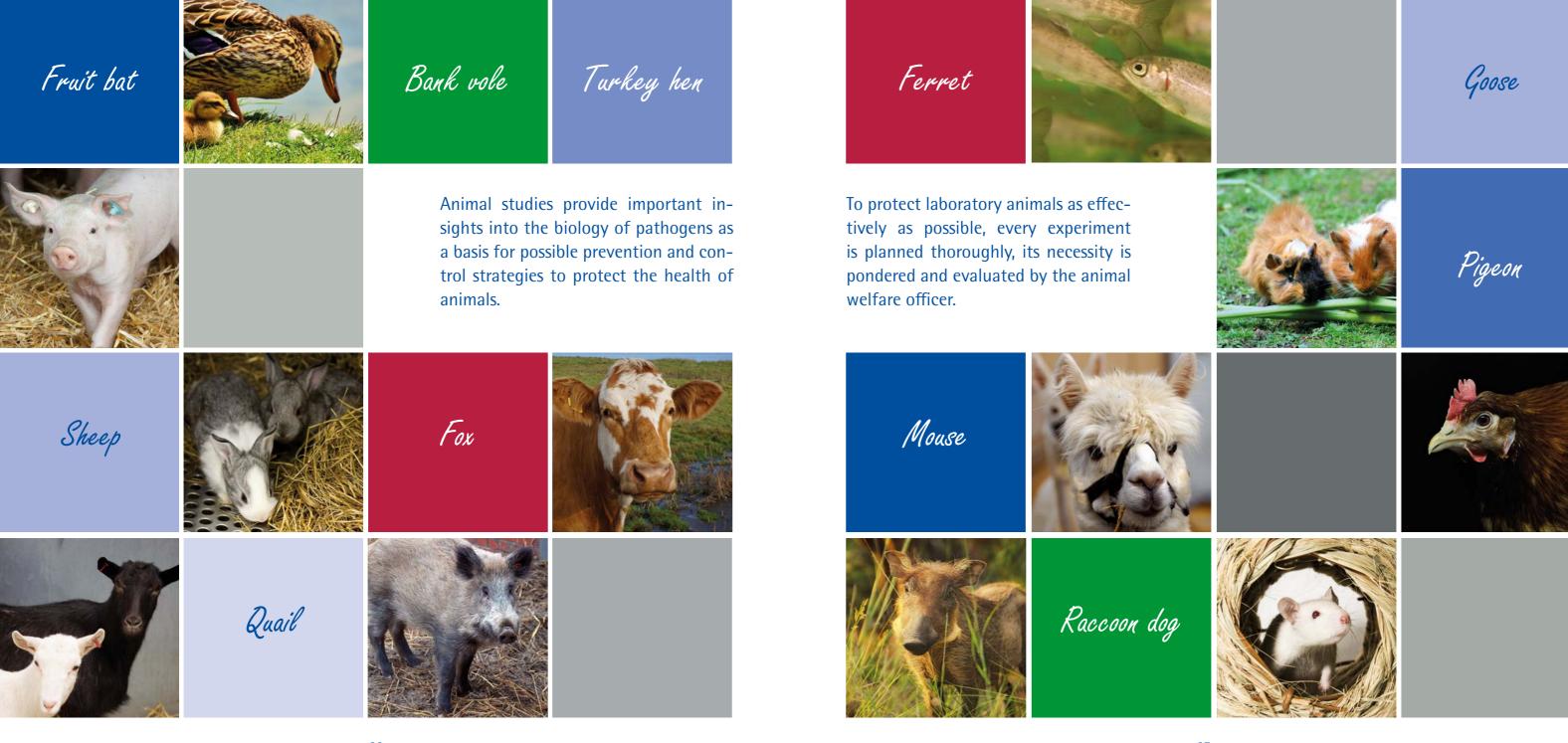
Food products of animal origin are not only subject to veterinary and food law regulations; they should also be of the highest quality possible. The question is under which conditions animals can effectively convert certain feed ingredients into high quality foods. From these points of view, the employees of the Institute of Animal Nutrition of the FLI in Braunschweig investigate and evaluate the effects and efficiency of novel feed additives, the influence of undesirable substances on animal health and the species-appropriate diet of livestock. The identification of alternative protein sources and feedstuffs with beneficial effects on animal health are another subject of research.

If, for example, the effects of a feedstuff are to be investigated, it first is consumed by the approximately 120 cows belonging to the institute. 227 hectares of arable land and grassland as well as a mill owned by the institute are used mainly for cultivation and production of defined feedstuffs and feed mixtures. Thus e.g., the institute investigates which effects a higher concentration of Fusarium toxins in feedstuffs has on ruminants, pigs and poultry, how contaminated grain batches can be detoxified and how critical concentrations in feed can be assessed. The researchers also investigated the effects of feedstuffs produced from grains treated with the controversial herbicide glyphosate on dairy cattle.

Proper feeding has a decisive impact on the emission of the greenhouse gas methane. The methane emissions resulting from digestive processes are largely due to cattle farming with dairy cows being the most important emitters. Processing of various feeds in the digestive tract of farm animals, especially in the foregut system of the ruminant, is at the focus of research. In particular animals which allow direct access to the foregut system from outside, so-called fistulated cows, are suitable for these investigations. Via the fistula, substances can be introduced or samples can be taken to investigate the causes of methane production in the rumen. The results of these test series have an impact on European legislation, on consumer and environmental protection, and ultimately on the food that lands on our plates.

"The so-called fistula provides direct access to the foregut. This permits closer investigation of the nutrient extraction processes in the foregut. This artificial access does not cause any pain so that the cow can lead a normal and long life."

Julia Hartwiger,
Doctoral researcher at the Institute of Animal Nutrition of
the FLI in Braunschweig



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The numbers refer to the year 2017 and embrace all FLI sites.

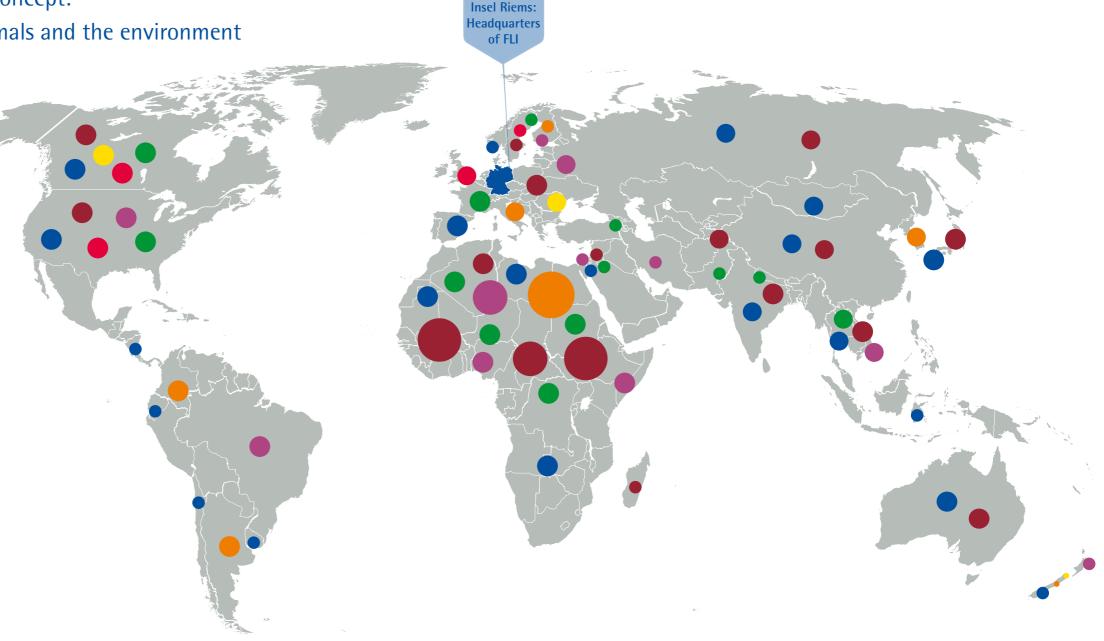
The "One Health" Concept: Worldwide network for humans, animals and the environment

With more than 150 international projects and collaborations in 85 countries worldwide the FLI is part of a global network. This means that information, data, and research results can be bundled and evaluated, risk potentials can be identified more rapidly, and more efficient action strategies can be coordinated and implemented.

"One Health" refers to the comprehensive, interconnected health of humans, animals and the environment. As a federal research institute for animal health, the FLI therefore has a special responsibility beyond national borders and disciplines.

Only in the interdisciplinary cooperation of natural sciences, veterinary medicine, human medicine and environmental research can the global challenge of recurrent classical animal diseases as well as novel and future zoonotic infectious diseases be met successfully. The FLI will continue to address to these special challenges in the future.

- ANIMAL NUTRITION
- ANIMAL WELFARE & ANIMAL HUSBANDRY
- ANIMAL GENETIC RESOURCES
- ANIMAL DISEASES (among others Classical and African swine fever, Lumpy Skin disease, honeybee and fish diseases)
- VIRAL ZOONOSES (among others bird flu, rabies, Ebola, Hantaand Bornaviruses, Crimean-Congo Haemorrhagic Fever)
- BIOSAFETY
- BACTERIAL ANIMAL DISEASES, ZOONOSES & ANTIBIOTIC RESISTANCE (among others Chlamydia, EHEC, Brucellosis and Contagious Bovine Pleuropneumonia)



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ANIMAL HEALTH GENE EXPRESSION BREEDING PROGRAMME BORNAVIRUS MAL WELFARE ZOONOSES CONSULTATION VACCINE **METAGENOMICS** CHLAMYDIA BRUCELLA CRISPR/CAS BIOINFORMATICS BIOBANK LIVE CELL **PATHOGEN** F A O ETONGUE DISEASE BEE DISEASES CROARRAY TSN EVALUATE MODELING

