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Deutsches
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Stralsund

Conference Abstracts

One Health

EWDA 2024

September 9th-13th, Stralsund, Germany

Challenges and Opportunities for the Surveillance and Management of Wildlife

Understanding the Conference Logo

The conference logo prominently features a cormorant (Phalacrocorax carbo (Linnaeus, 1758)) to highlight the need to reassess the reputation of wildlife species that are often portrayed in a negative light. Cormorants, often involved in human-wildlife conflicts due to their interaction with fisheries, are an integral part of the coastal ecosystems of Mecklenburg-Western Pomerania. They also symbolize the link between wildlife disease and human health, as they are affected by avian influenza. In addition, cormorants face growing challenges from climate change, as shrinking fish populations - key to their survival - are affected by warming waters and environmental degradation. By incorporating the cormorant, the logo emphasizes the need to understand and protect wildlife species in a rapidly changing and anthropocentric environment.

The four tiles symbolise the conference-hosting institutions with a focus on livestock health and marine conservation and establish a link to the One Health Interface.

Cormorant picture © S. Knauf

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Stralsund, September 2024

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09:00 - 13:00 Monday, 9th September, 2024
Ozeaneum, Stralsund

WORKSHOP 2 - Wildlife Student Workshop

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Friedrich-Loeffler-Institut, Riems Island - Loeffler-Haus

WORKSHOP 4 - Surveillance in Wildlife

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Lunch + bus transfer from and to Stralsund

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WORKSHOP 1 - (Part II) Aquatic Mammal Health

14:00 - 18:00 Monday, 9th September, 2024
Nautineum, Stralsund

OPTIONAL, registration required
Be aware that you will not be able to attend the EWDA Network Meeting if you register for the optional practical training on aquatic mammal post-mortem examination.

EWDA Wildlife Health Surveillance Network Meeting

15:00 - 18:00 Monday, 9th September, 2024
Ozeaneum, Stralsund

Be aware that you will not be able to attend the practical training on aquatic mammal post-mortem examination if you register for the EWDA Network Meeting.

Ice-Breaker

19:00 - 22:00 Monday, 9th September, 2024
Ozeaneum, Stralsund

Registration

07:00 - 08:00 Tuesday, 10th September, 2024
Brewery, Stralsund

Opening Session

08:00 - 08:30 Tuesday, 10th September, 2024
Brewery, Stralsund

1. Dr Alexander Badrow (CDU, Mayor Stralsund)
2. Dr Katharina Kluge (CVO Germany, Bundesministerium für Ernährung und Landwirtschaft)
3. Prof Dr Christa Kühn (President Friedrich-Loeffler-Institut)
4. Prof Dr Burkard Baschek (Director Deutsches Meeresmuseum)
5. Prof Dr Carlos das Neves (EFSA Chief Scientist and Co-Chair of the Quadripartite One Health High Level Expert Panel (OHHLEP)) (online)
6. Dr Gudrun Wibbelt (Chair of European Wildlife Disease Association)

Housekeeping Notes

08:30 - 08:35 Tuesday, 10th September, 2024
Sascha Knauf

Group photo

08:35 - 09:00 Tuesday, 10th September, 2024
Brewery, Stralsund

1st Keynote: Unlocking wildlife health data: the case for international information sharing and the challenges.

09:00 - 09:45 Tuesday, 10th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Keith Hamilton[1]
1 World Organisation for Animal Health (WOAH), Paris, France
*Correspondence: k.hamilton@woah.org

Abstract

The World Organisation for Animal Health (WOAH) has responsibility for collecting and disseminating information on animal health at international level. This has benefits for human, animal, and environmental health, as well as for economies. Traditionally, WOAH has focused on livestock disease data but recognizing the importance of wildlife in fulfilling its mandate, WOAH is making efforts to collect and disseminate information about wildlife diseases. Mandatory reporting of diseases in wild hosts is established for more than 60 WOAH-listed diseases. Additionally, information has been collected voluntarily since 1993 on relevant wildlife diseases and the WOAH Wildlife Health Framework has defined WOAH's wildlife health strategy since 2021. Most recently, SARS-CoV-2, Avian Influenza and CWD highlighted the pertinence to One Health of information about diseases in wildlife. Wildlife disease information sharing, in particular, appears to be a critical preparedness aspect enabling prompt response to diseases and larger One Health efforts to preserve human, animal, and environmental health.

Although there are some similarities in the approaches to and the objectives of wildlife and livestock disease surveillance, there are also considerable differences which would challenge a one-size-fits-all model to disease reporting. For instance, wildlife health information sharing requires a One Health, allhazard approach not limited to infectious diseases. The complex stakeholder landscape in wildlife health data calls for inclusive solutions. Finally, the specificity of wildlife health information is generally low compared with livestock health data shared at the Global level to ensure safe international trade. The presentation will aim to explore what we mean by open sharing of wildlife disease information, how open wildlife data can be useful, and what some of the current challenges and opportunities are. It will engage the audience in thinking about the case for international reporting of wildlife health issues.

Session I - Wildlife-Livestock-Human Interface: One Health, Ecosystem Health, Disease Surveillance

09:45 - 10:45 Tuesday, 10th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Keith Hamilton, Christa Kühn

09:45 - 10:00

Early Warning System for highly pathogenic avian influenza in Europe

Rob Robinson[1,2], Stephen Baillie[1], Angus Cameron[3], Jacob Davies[4], Céline Faverjon[3], Gabriel Gargallo[5], Christian Kampichler[6], Henk Sierdsema[6], Julia Stahl[6]
[1]BTO, Thetford, United Kingdom. [2]Euring, Thetford, United Kingdom. [3]Ausvet Europe, Lyon, France. [4]BTO Scotland, Stirling, United Kingdom. [5]Catalan Ornithological Institute, Barcelona, Spain. [6]Sovon, Nijmegen, Netherlands

Abstract

To address the growing threat of highly pathogenic avian influenza (HPAI) in Europe, an early warning system (EWS) was developed and implemented in real time. The system aims at informing stakeholders on the weekly probability of HPAI introduction in the wild bird populations based on the output of a deterministic spatiotemporal risk assessment model.

The EWS estimates the probability of an HPAI outbreak in wild bird by spatiotemporal unit (i.e., week, grid cell of 50x50km) across Europe. The input data are: i) the expected abundance, ii) long-distance movement and iii) local movements of 12 wild bird species known to carry HPAI, and iv) HPAI outbreaks in wild birds. After an initial assessment of the tool's performances based on historical data, the spatiotemporal risk assessment model was implemented in real time. A graphical user interface and automated alert system were developed and integrated into open access online portal (https://app.bto.org/mmt/avian_influenza_map/avian_influenza_map.jsp).

The initial performance assessment of the tool showed that it was able to reach 80% sensitivity and specificity in terms of prediction of HPAI reported outbreaks in wild birds. The model is however associated with limitations due to the quality of the input data (e.g. under-reporting of HPAI outbreaks in wild birds, the quality of movements and abundance data is variable between geographical locations). Current work is focusing on addressing some of these by refining the estimates for the wild bird movements and abundance and expanding the model to incorporate estimates of the risk of introduction and establishment of HPAI in poultry flocks.

Our EWS is the first to provide real time spatial information about the risk of HPAI outbreaks in Europe offering to stakeholders the possibility to make more evidence-based decisions. It is a good example on how research can be translated into a real-time decision-support tool.

10:00 - 10:15

Emergence and spread of high pathogenicity avian influenza (HPAI) H5 in wildlife of South America and Antarctica

Ashley Banyard[1], Lineke Begeman[2], Andrew Breed[3], Meagan Dewar[4], Ruben Fijn[5], Thijs Kuiken[2], Patricia Serafini[6], Marcela Uhart[7], Ralph Vanstreels[7], Michelle Wille[8]
[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]Erasmus University Medical Centre, Rotterdam, Netherlands. [3]Department of Agriculture, Fisheries and Forestry, Canberra, Australia. [4]Federation University Australia, Berwick, Australia. [5]Bureau Waardenburg, Culemborg, Netherlands. [6]Universidade Federal de Santa Catarina, Florianópolis, Brazil. [7]University of California, Davis, USA. [8]University of Melbourne, Melbourne, Australia

Abstract

The continued emergence of high pathogenicity avian influenza (HPAI) virus of the H5 subtype is a major hazard for wildlife health and conservation. This virus was first detected in commercially farmed geese in China in 1996, and has since spread among the rapidly growing poultry populations in Asia, spilled over into migratory wild birds, and caused numerous outbreaks in wild birds in Asia, Europe and Africa. In December 2021, HPAI H5 virus spread to North America, reaching Central and South America by October 2022, and Antarctica by February 2024. Our objective was to provide an overview of the spread and impact of HPAI H5 in wildlife in South America, to evaluate the risk of virus spread in Antarctica, and to assess the relevance for wildlife conservation on these two continents. We did this by reviewing and collating reports of HPAI H5 virus detections in wild animals found ill or dead and of mortality counts of wild animals during HPAI H5 outbreaks. We found that following its introduction into South America, HPAI H5 virus spread southwards along the northwest coast of South America. From there, it spread via two pathways. First, it continued to spread southwards along the southwest coast, and then northwards along the southeast coast. Second, it spread eastward across the Andes. Between November 2022 and December 2023, HPAI H5 virus infected at least 83 wild bird species and 11 wild mammal species, and was the probable cause of death of at least 667,000 wild birds and 53,000 wild mammals in South America. By October 2023, HPAI H5 virus reached the Falkland Islands (Islas Malvinas) and South Georgia (Islas Georgia del Sur) and by February 2024 it reached the Antarctic Peninsula, part of mainland Antarctica. Based on mortality events that have occurred elsewhere, most of the 48 avian species and 27 mammalian species found in the Antarctic region and Subantarctic islands are likely highly susceptible to HPAI H5 virus. The negative impact of HPAI H5 on Antarctic wildlife populations could be immense, given their frequent occurrence in dense colonies of up to thousands of pinnipeds and hundreds of

thousands of birds, allowing efficient virus transmission. The current HPAI H5 outbreak highlights the potential impact of pathogen spillover from farmed animals into wildlife, and the need for human society to assume responsibility for protecting wildlife from anthropogenic diseases, particularly those originating from ever increasing livestock populations.

10:15 - 10:30

One Health: Seals as a bridging host for the adaptation of the avian influenza A virus of wild bird origin to humans

Dr. Elsayed M. Abdelwhab PhD, Dipl.EBVS, Fachtierarzt[1], Diana Palme[1], Maryna Kuryshko[1], Dr. Christine Luttermann Dr.[2], Prof. Thomas C. Mettenleiter Prof. Dr. Dr.[3]
[1]Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany. [2]Institute of Immunology, Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany. [3]Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

Abstract

Introduction

Avian influenza viruses (AIVs) pose a continuing threat as zoonotic and potentially pandemic viruses. Apart from birds and pigs, there is a gap in our knowledge of the risk posed by other mammals for the emergence of human-adapted AIVs. In recent years, there has been an increasing prevalence of (fatal) AIV infections in marine mammals, i.e. seals. In 2014, an H10N7 avian influenza A virus (IAV) was transmitted to grey seals in Europe, killing approximately 10% of the local seal population. Sequence analysis of seal IAV gene segments compared to the avian ancestors revealed amino acid (aa) exchanges in several viral proteins, including the non-structural NS1 and viral polymerase PB2, PB1, PA and NP proteins.

Objectives

We investigated the potential role of seals in the production of AIVs with greater human adaptation. To this end, we examined the effect of aa exchanges found in the NS1 and polymerase segments of seal IAV in avian, human and seal cells.

Materials and methods

We found 3 and 14 aa exchanges in the seal H10N7 NS1 and polymerase proteins, respectively, compared to the avian precursors. We introduced these aa into the corresponding genes and subcloned them into protein expression vectors.

Recombinant avian viruses carrying seal-specific NS1 mutations were rescued and propagated. The replication kinetics and expression levels of NS1 in different cells were investigated. Polymerase activity and interferon (IFN) inhibition were assessed using minigenome reporter assays and/or flow cytometry.

Results

We found that seal NS1 was more efficient at blocking IFN induction in human cells than the avian ancestor. Substitutions in the seal NS1 acted synergistically to balance the expression of NS1 and replication in different cells and modulated the efficiency to inhibit IFN induction, indicating a synergistic effect. Interestingly, although the H10N7 seal polymerase segments do not encode known mammalian adaptation markers such as PB2-627K, we found three aa that were essential for higher polymerase activity in mammalian cells.

Conclusion

Our results support the potential role of seals in the generation of AIVs with higher adaptive potential to human cells. The new mutations described in this study will be useful for predicting AIVs with zoonotic potential.

10:30 - 10:45

Highly pathogenic avian influenza (HPAI) H5N1 in red foxes in Norway 2022-2023

Dr. Malin Rokseth Reiten DVM, PhD[1], Dr. Cathrine Bøe PhD[1], Dr. Line Olsen DVM, PhD[1], Dr. Silje Granstad DVM, PhD[1], Dr. Britt Gjerset DVM, PhD[1], Dr. Maryam Saghafian DVM, PhD[1], Dr. Olav Hungnes PhD[2], Dr. Andreas Rohringer PhD[2], Dr. Bjørnar Ytrehus DVM, PhD[1], Dr. Ragnhild Tønnessen DVM, PhD[1]
[1]Norwegian Veterinary Institute, Ås, Norway. [2]Norwegian Institute of Public Health, Oslo, Norway

Abstract

Highly pathogenic avian influenza (HPAI) viruses belonging to clade 2.3.4.4b continue to spread across continents, causing suffering and death to animals of a wide range of species. Surveillance of HPAI viruses (HPAIVs) is important to understand the evolution of the viruses and the potential for adaptation to new species. Four wild red foxes (*Vulpes vulpes*) were diagnosed with HPAI H5N1 in 2022 and 2023 in Norway. In the current study, we describe pathological changes caused by HPAIV infection and the spatial distribution of virus within tissues. We perform phylogenetic analysis to compare the viruses in the foxes with HPAIVs circulating in birds during the same period. In addition, we screen full genome sequences for mutations associated with mammalian virus adaptation.

All foxes were found in coastal environments and the infections are likely spill-over events from HPAIV-infected wild birds. In 2022, the virus detections in the foxes coincided with an outbreak of HPAI among northern gannets (*Morus bassanus*) in the North Sea region, also affecting the western coast of Norway. In the summer of 2023, a significant HPAI outbreak affected black-legged kittiwakes (*Rissa tridactyla*) and other gull species in Northern Norway, the same region where one fox was found. Three of the foxes were observed alive, displaying clinical signs of nervous system affection, such as circling and reduced shyness towards humans.

Necropsies of the foxes revealed macroscopic changes limited to the respiratory system. The lungs were oedematous and heavy with a marbled surface, exhibiting multifocal to coalescing haemorrhages on cut surface. Microscopic examination showed a granulomatous bronchio- and bronchoinsterstitial pneumonia. In the brain, the pathological changes were consistent with a nonsuppurative encephalitis with perivascular cuffing. Antigen detection and visualization by immunohistochemistry and RNA scope are ongoing and will be presented.

In all samples, HPAI H5N1 clade 2.3.4.4b virus was confirmed by PCR analyses. Two different genotypes were detected (C and BB). The PCR analyses indicated high viral loads in brain and lung, and lower viral loads in other organs such as the liver. Initial mutation analyses showed the occurrence of the PB2-E627K mutation in the viruses from two foxes. This mutation has been associated with increased virulence and replication in mammals. Further phylogenetics and mutation analysis are currently ongoing and will be presented.

Short Coffee Break

10:45 - 11:05 Tuesday, 10th September, 2024
Brewery, Stralsund

Session I - Wildlife-Livestock-Human Interface: One Health, Ecosystem Health, Disease Surveillance (cont.)

11:05 - 12:20 Tuesday, 10th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Gudrun Wibbelt, Christian Gortázar-Schmidt (tbc)

11:05 - 11:20

Citizen science and wildlife disease surveillance: what comes in, who participates and why?

Aleksija Neimanis[1], Maria Nöremark[2], Ellinor Spörndly-Nees[1], Hyeyoung Kim[2], Maria Johansson[3], Åsa Waldo[3], Henrik Uhlhorn[1], Erik Ågren[1]
[1]Department of Pathology and Wildlife Diseases, Swedish Veterinary Agency, Uppsala, Sweden. [2]Department of Epidemiology, Surveillance and Risk Assessment, Swedish Veterinary Agency, Uppsala, Sweden. [3]Environmental Psychology, Faculty of Engineering, Lund University, Lund, Sweden

Abstract

Healthy wildlife populations are integral to biodiversity and resilient ecosystems, and diseases of wildlife can have severe ecological consequences and threaten animal and human health. Wildlife disease surveillance programs therefore provide essential information for management and mitigation of wildlife health issues. Although programs vary in delivery, many rely on voluntary participation of citizens. One example is the Swedish Veterinary Agency's (SVA) general, or passive, wildlife disease surveillance program in which citizens voluntarily report and submit abnormal or dead wildlife. Despite the crucial involvement of volunteers in the field, this human dimension and the factors that influence case submission are poorly understood.

Our study aimed to better understand the material that SVA receives through citizen-science based surveillance and the people behind the submissions. First, we investigated the composition of case material submitted to SVA's general surveillance program from 2010-2020 using descriptive and spatiotemporal analyses. Then, to better understand the human dimension, data was collected prospectively through an anonymous questionnaire offered to people reporting wildlife cases and focus-group interviews with people who had repeatedly submitted material to the program. In addition to gathering other information, both prospective methods were designed to provide insight into motivation of participants and the obstacles they experienced.

Historic cases submitted to SVA as a part of general disease surveillance were highly associated with proximity to SVA. Species composition was skewed with underrepresentation of groups such as rodents, reptiles and amphibians. Additionally, size of the animal played a role in type of material submitted. However, inclusion of case material from other sources and using tools such as legislation helped mitigate these biases.

Questionnaire results revealed that approximately half of all reports of sick or dead wildlife were found on private property and approximately a third of respondents were at home when they discovered the case. Half of respondents

were willing to submit the case for examination and lack of appropriate storage facilities was generally the most important factor hindering submission. Both reporters and repeat submitters were primarily autonomously motivated to participate and expressed this in terms of a genuine interest for nature, curiosity and a desire to contribute. Moreover, the opportunity for building competence was highly valued pointing to the importance of feedback and other strategies tailored to encourage and maintain engagement. Outcomes from this study provide numerous insights and recommendations to help develop wildlife health surveillance in Sweden and elsewhere for improved animal, human and ecosystem health.

11:20 - 11:35

The role of scavenging seabirds in the epidemiology of *Salmonella* in the Southern Ocean and Antarctica

Marta Cerdà-Cuéllar[1,2], Kiara Ortega[1,2,3], Teresa Ayats[1,2], Amandine Gamble[4], Thierry Boulinier[5], Richard A. Phillips[6], Peter G. Ryan[7], Jacob González-Solís[3]
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Abstract

Reports of enteric bacteria, such as zoonotic *Salmonella* serovars, in Antarctic wildlife suggest there are incidences of reverse zoonosis in the region with potential negative impacts on animal health. Animal hosts usually act as asymptomatic carriers, spreading the pathogen in the environment, but mass mortalities in the northern hemisphere have been reported. In the Southern Ocean and Antarctica, terrestrial scavenging and predatory seabirds are likely to be the principal carriers of zoonotic agents, including antimicrobial resistant bacteria, due to these feeding habits. However, our understanding of their

epidemiological role remains limited. To address this gap, we sampled skuas (*Stercorarius* spp.) and striated caracara (*Phalcoboenus australis*) at Amsterdam, Gough, Falkland and Adelaide islands, over one to four years, from 2016 to 2023. All of these islands have small human habitations, typically research stations, except for the Falklands, which has c. 3,600 inhabitants which live in the main town of Stanley, and multiple smaller settlements and farms. We isolated *Salmonella* using standard culture methods. From *Salmonella*-positive birds, we determined the serovars of the isolates and assessed their antimicrobial susceptibility to a panel of 14 antibiotics belonging to 9 different classes. Overall, we sampled 366 seabirds and found 53 bird birds testing positive for *Salmonella*. Among these samples, we isolated *Salmonella enterica* subsp. *enterica* from all localities and identified a diversity of serovars: Havana (n= 14; 26,4%), Newport (n= 13; 24,5%), Typhimurium (n= 12; 22,6%), Enteritidis (n= 8; 15,1%), Chincol (n= 3; 5,7%) and Sandiego (n= 2; 3,8%). In addition, we recovered one *Salmonella enterica* subsp. *salamae* from a brown skua at Gough. Noteworthy, Enteritidis and Typhimurium, and to a lesser extent Newport, are the most common serovars causing enteric disease worldwide, all of which were isolated from skua species at Amsterdam and Gough. Ser. Typhimurium was also recovered from striated caracaras at the Falklands, and only ser. Enteritidis was isolated from south polar skuas *Stercorarius maccormicki* at Adelaide Is. These findings may suggest an anthropogenic origin of these infections from human settlements, although other sources cannot be excluded, particularly as south polar skuas are transequatorial migrants. Nevertheless, all isolates were pansusceptible which indicates a low antibiotic pressure at the sampled sites. Through their annual movements skuas in particular can acquire and spread *Salmonella* and other pathogens, such as avian influenza virus, across the Southern Ocean or even globally, possibly contributing to the spread of zoonotic agents with potentially catastrophic consequences.

11:35 - 11:50

Evidence of endemic circulation of flaviviruses in wild birds in southern Portugal: seroprevalence, host-range, virus isolation and genomic characterization of Bagaza and West Nile viruses

Mr. João Queirós PhD[1,2,3,4], Ms. Catarina Fontoura-Gonçalves Master[1,2,3,5], Mr. David Gonçalves PhD[1,2,3,4], Mr. Luis P. da Silva PhD[1,3], Mr. Alberto Moraga PhD[5], Ms. Marinela Contreras PhD[5], Ms. Tereza Almeida PhD[1,3], Ms. Ana Lopes PhD[1,3,6,7], Ms. Joana Abrantes PhD[1,2,3], Ms. Elisa Pérez-Ramírez PhD[8], Mr. Francisco Llorente PhD[8], Mr. Miguel Jiménez-Clavero PhD[8], Ms. Laura Albentosa PhD[9], Mr. Antonio Mas PhD[9], Mr. João Basso-Costa Bachelor[10], Mr. Gonçalo de Mello Bachelor[10], Mr. Paulo Célio Alves PhD[1,2,3,4], Ms. Ursula Hofler PhD[5]

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Abstract

Flaviviruses are a significant threat to human and animal health worldwide. Among them, West Nile virus (WNV) and Bagaza virus (BAGV) are two emerging flaviviruses in Europe, primarily transmitted by vectors. In Portugal, they present contrasting epidemiological patterns. For WNV, serological evidence of circulation in humans, horses and wild birds has been reported since the 1960s, but only with a few confirmed cases by RT-PCR, all in mosquito pools. BAGV was first detected in wild birds in 2021, associated with a disease outbreak in red-legged partridge (*Alectoris rufa*), but information on its circulation dynamics is scarce. Following the 2021 BAGV outbreak, we have conducted a flavivirus surveillance program on wild birds in southern Portugal. Between September 2021 and December 2023, we captured resident and migratory wild birds using mist nets and walk-in cage traps baited with seeds. Blood and vascular feathers were collected for serology (ELISA and virus neutralization test-VNT), molecular detection (RT-PCR, PCR and sequencing), virus isolation and whole genome sequencing analyses. For >1200 birds from 43 species, we performed a general RT-PCR for flaviviruses, followed by specific RT-PCR for WNV lineage determination and traditional PCR and sequencing for confirmation of the positive samples (BAGV and WNV). We also isolated BAGV in cell cultures. Whole-genome sequencing of BAGV and WNV was performed on a subset of samples, comprising different species, years and geographic localities. BAGV virus was only detected between September and November 2021 in red-legged partridges and in a corn bunting (*Emberiza calandra*), suggesting a sporadic circulation. In contrast, WNV virus was detected every year, with cases confirmed between August and November in live red-legged partridge, Iberian magpie (*Cyanopica cooki*) and Eurasian jay (*Garrulus glandarius*), indicating active circulation and endemicity in resident birds. Phylogenomic analysis of BAGV and WNV and serological assays by ELISA and

VNT are ongoing, but preliminary results of ELISA showed an overall seroprevalence in the bird community of over 30%, and VNT analysis confirmed the presence of specific antibodies against BAGV and WNV in red-legged partridges. Overall, these results provide strong evidence that flaviviruses are actively circulating in wild birds in southern Portugal and reinforce the need to include wild birds in active surveillance programs to better understand the dynamics of these viruses and thus anticipate outbreaks in humans and other animals.

11:50 - 12:05

One Health innovative sampling to understand the ecology and spread of Usutu virusand West Nile virus in the Netherlands, 2016-2022

Reina Sikkema DVM, PhD[1], Emmanuelle Munger MSc[1], Nnomzie Atama DVM[1], Maarten Schrama PhD[2], Chiara Bellegarde MSc[3], Rody Blom MSc[4], Sander Koenraadt PhD[4], Judith van den Brand[5], prof Leo Visser PhD[6], Bas Oude Munnink PhD[1], Henk van derJeugd PhD[7], prof Marion Koopmans[1]
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Abstract

Outbreaks of emerging arbovirus infections are increasing worldwide. In Europe, several arboviruses, including West Nile Virus (WNV) and Usutu virus (USUV) show a growing geographical spread and an increase in cases in recent decades. Extensive molecular and serological surveillance of live wild birds, dead birds, chickens and mosquitoes was therefore set up in 2016 to monitor the introduction and spread of a selection of high-risk arboviruses in the Netherlands. In addition, the risk of arbovirus infections for humans (bird ringers) and predators (wild carnivores) in close contact with wild birds, was investigated.
Mortality in captive and wild birds, as well as wild mammals, is reported through a citizen science-based alerting system. A selection of fresh carcasses is further investigated by the Dutch Wildlife Health Centre. Live wild birds are sampled in collaboration with bird ringers of the Dutch Centre for Avian Migration and Demography. In addition, a network of mosquito trapping sites was set up in July 2020. Serum from chickens from petting zoos around WNV outbreak locations was also collected. A cross-sectional serological screening of bird ringers was conducted between May and September 2021. Samples were tested for USUV

and WNV RNA using RT-PCR and full genome sequencing when positive. Flavivirus antibodies in serum were detected using protein microarray and FRNT.

Endemic Usutu virus circulation was first detected in 2016, with continued enzootic presence in subsequent years. Through phylogenetic analyses, we show co-circulation of USUV lineages Europe 3 and Africa 3, with continued enzootic presence of the USUV lineage Africa 3. Indeed, USUV lineage Africa 3 has also been found during winter, in live wild birds and hibernating mosquitoes. Local West Nile virus (WNV) was found in 2020 in a live Common Whitethroat, followed by detection in other passerine birds, mosquitoes and humans. In 2021, seroconversions in petting zoo chickens showed continued WNV circulation. This was further confirmed in 2022, when West Nile lineage 2 virus was detected in a Grey Heron with a partial sequence closely related to sequences from the 2020 outbreak. Multiple USUV infections in blood donors and in bird ringers, as well as in wild carnivores, show that USUV also infects mammals in the Netherlands, although symptoms appear to be mild or absent.

We show that a One Health framework for research and surveillance of WNV and USUV can serve as early warning for human infections, and provides deeper insights in flavivirus ecology and spread.

12:05 - 12:20

Borna disease virus 1 (BoDV-1), a fatal zoonotic agent transmitted by crocidurine shrews

Dennis Rubbenstroth[1], Effrosyni Michelakaki[2], Viola Haring[1], Philip Starcky[1,3], Andreas Parzefall[2], Rainer Ulrich[1], Martin Beer[1], Kaspar Matiasek[2]

[1]Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany. [2]Ludwig-Maximilians-University Munich, Munich, Germany. [3]Bavarian Health and Food Safety Authority, Munich, Germany

Abstract

Borna disease virus 1 (BoDV-1; species *Orthobornavirus bornaense*) is known as the causative agent of Borna disease, a usually fatal neurologic disorder of domestic mammals for many decades. After more than three decades of controversial debates, BoDV-1 was proven to be transmissible also to humans in 2018, causing severe, usually fatal encephalitis. Up to now, more than 50 laboratory-confirmed cases of human BoDV-1 encephalitis have been reported. The known endemic area covers parts of Germany, Austria, Switzerland and Liechtenstein.

The bicolored white-toothed shrew (*Crocidura leucodon*) is known as its natural wild reservoir host. In addition, BoDV-1 has been found also sporadically in lesser

white-toothed shrews (*Crocidura suaveolens*) and greater white-toothed shrews (*Crocidura russula*), but their capacity to serve as reservoir hosts remains elusive. In contrast to domestic mammals and humans, infected shrews remain apparently healthy and do not develop encephalitis. In shrews, the virus possesses a broad tissue and cell tropism and is excreted via various routes, enabling circulation in the reservoir host populations as well as spill-over transmission to other mammals. In these non-reservoir hosts, BoDV-1 induces lymphohistiocytic encephalitis by T lymphocyte-mediated immunopathogenesis, but viral shedding does not occur due to a strict neurotropism.

While Borna disease has been observed in a broad range of domestic mammals, particularly horses, sheep and alpacas, documented cases in wild non-reservoir hosts are scarce. Recently, we encountered BoDV-1 infection associated with fatal encephalitis in a wild hedgehog (*Erinaceus europaeus*) from Bavaria.

The presentation will summarize the current knowledge on the epidemiology of BoDV-1 in its wild reservoir hosts, its interface to domestic animals and humans and present the first case of Borna disease in a wild hedgehog.

One Health Platform Presentation

12:20 - 12:30 Tuesday, 10th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Benjamin Bauer, Member of the Scientific Advisory Board, One Health Platform and Friedrich-Loeffler-Institut

Lunch

12:30 - 13:30 Tuesday, 10th September, 2024
Brewery, Stralsund

Session I - Wildlife-Livestock-Human Interface: Prevention and Pandemic Preparedness (Part I)

13:30 - 15:00 Tuesday, 10th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Becki Lawson, Tony Sainsbury

13:30 - 13:45

Switching the focus of wildlife disease surveillance from pathogens to drivers: benefits and challenges

Prof. Julian Drewe BVetMed PhD MRCVS[1], Dr Janeth George BSc MSc PhD[2,3], Prof. Barbara Häsler DVM PhD[1]
[1]World Organisation for Animal Health Collaborating Centre in Risk Analysis and Modelling, Veterinary Epidemiology, Economics and Public Health Group, Royal Veterinary College, London, United Kingdom. [2]SACIDS Foundation for One Health, Sokoine University of Agriculture, Morogoro, Tanzania, United Republic of. [3]Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania, United Republic of

Abstract

Background: Wildlife disease surveillance usually involves looking for pathogens or the diseases they cause. Such an approach is both resource intensive and often limited by the requirement for some prior knowledge of pathogen or disease likelihood.
Objective: We put forward the case for switching the focus of wildlife surveillance to monitoring drivers of disease emergence (processes that lead to disease outbreaks) rather than on the presence or absence of specific pathogens.
Methods: Drivers relevant to wildlife disease that could become the focus of surveillance include current and future land-use changes, and increasing global interconnectedness. Both of these may profoundly affect interaction patterns between species, for example at the interface between wildlife and humans. Surveillance that focuses on detecting changes in patterns or quantities associated with drivers could include changes in the connectivity of systems that affect the likelihood of disease spread once the pathogen has emerged. This talk will discuss some of these examples, examine what this new form of surveillance may look like, and determine what needs to be done to make the transition from current surveillance practices.
Results: The outcome from this surveillance is likely to be risk-based information on drivers that will allow us to identify areas where additional attention may be needed, and, over time, inform the implementation of prevention efforts. In doing so, the scope of what is detectable would be broadened to include known and unknown causes of disease, since specification of pathogens in advance would no longer be necessary. Surveillance focused on drivers could be expected to bring additional benefits. For example, many drivers are not specific to single diseases so surveillance of these common drivers may inform mitigation against several diseases at once. Further, focusing on drivers rather than pathogens should

enable us to control currently unknown diseases, making this approach particularly timely, given the increasing risk of emergence of new diseases.
Conclusions: Reshaping wildlife disease surveillance away from its current focus on pathogens and instead onto drivers has real potential to deliver wider benefits. This approach is expected to be cost-effective since drivers span multiple diseases, including those as-yet unidentified. In the not-too-distant future we may therefore see a transition in surveillance away from chasing pathogens and towards a new focus on monitoring, and reacting to, the drivers behind disease emergence.

13:45 - 14:00

Wildlife diseases: a framework to prioritise targeted surveillance

Nathan Thenon[1], Emmanuelle Gilot-Fromont[1,2], Julien Hirschinger[3], Thierry Durand[4], Florence Etoré[5,2], Céline Dupuy[6,2], Laurent Georges[7,2], Céline Richomme[8,2], Stéphanie Desvaux[9,2], Anouk Decors[10,2], Nolwenn Le Moal[11], Guillaume Le Loc'h[3], Philippe Gourlay[12], Loïc Palumbo[10], Camille Sandor[10], Yoann Bunz[13], Romain Lacoste[14], Sylvain Larrat[1,2]
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Abstract

Wildlife diseases can have negative impacts on biodiversity conservation, health of domestic animals (diseases shared between domestic and wild species) and human health (zoonoses). As a consequence, opportunistic and targeted wildlife health surveillance is a key part of a "One Health" strategy. However, such surveillance concerns a large number of species and pathogens, with limited human and material resources. Prioritisation of targeted surveillance is therefore an essential step in allocating resources as efficiently as possible in a given territory and context.
Here we propose a prioritisation methodology and an associated online database and tool, "*Priorité Santé Faune*" (PSF), to prioritise wildlife health hazards in a given area. The database includes the rating of 46 prioritisation criteria for each infectious pathogen to be considered. Prioritisation criteria describe pathogen characteristics (e.g., transmission routes), potential impacts on human and animal

health, the status of the pathogen (local presence and regulation) and the feasibility of surveillance and control.

The prioritisation process for a given area involves the participation of stakeholders locally in charge of wildlife, livestock and public health. First, stakeholders are invited to weigh each of the 46 criteria. Weights are then recorded in PSF. Second, this yields three distinct prioritised lists of pathogens to formulate the surveillance priorities, depending on the main goal of the natural area manager (limit the risk to human, livestock or wildlife health). Third, there should be a discussion among stakeholders, to obtain a consensual list of pathogens to be monitored in this specific context of place and time. The prioritisation process can be revised following changes in the sanitary context or on the stakeholders' priorities or resources.

The method and tool are now being tested, and the results compared to pre-existing prioritisations of pathogens. The method has been developed in collaboration among French partners (National Parks, wildlife expertise Pole of the veterinary school VetAgro Sup, Wildlife Group of the national animal health surveillance Platform). The database results from bibliographic synthesis, as part of a broader collaboration including the French veterinary schools, veterinary students and the French Biodiversity Agency. As such, it currently includes species and diseases that are likely to be monitored in terrestrial environments in metropolitan France. The tool will need to be sustained and its information updated periodically to remain relevant. The method and tool could be then made available for use in other countries and contexts.

14:00 - 14:15

Syndromic Surveillance for Wildlife and One Health: Current Practice and Future Directions

Dr. Julie Teresa Shapiro PhD[1], Géraldine Cazeau[1], Romane Di Biagio[2], Dr. Céline Dupuy DVM, PhD[1], Dr. Éric Morignat PhD[1], Dr. Fernanda Dórea DVM, PhD[3], Dr. Viviane Hénaux PhD[1], Dr. Jean-Philippe Amat DVM, PhD[1]
[1]Anses, Lyon, France. [2]GDS France, Paris, France. [3]National Veterinary Institute, Uppsala, Sweden

Abstract

Background:

Syndromic surveillance, the tracking of non-specific indicators to monitor health events, is a powerful tool for the early detection of threats (e.g. disease) or their effects (e.g. climatic events). These indicators range from mortality to Google

searches. While syndromic surveillance was first developed for human public health, it has been adapted for animal health, including wildlife.

Objectives:
We used a systematic review to describe the current state-of-the-art in syndromic surveillance for animal health, including wildlife. Our objective was to investigate the different methods used for syndromic surveillance for different animal sectors across the world to facilitate the improvement of syndromic surveillance and cross-pollination of methods between sectors.

Materials and Methods:
We searched for the terms "syndromic surveillance" AND ("animal*" OR "veterinary") in the titles, abstracts, and keywords of peer-reviewed articles in the Scopus and PubMed databases from 1956 – May 2023, resulting in 196 unique articles. We screened the abstracts and full texts of these articles for relevance. For each system, we identified the sector (wildlife, livestock, companion), geographic location, indicators, and whether it used a OneHealth approach (defined as linking the surveillance of animal and human health).

Results:
After screening, we retained 105 original research papers, describing 27 established syndromic surveillance systems and 67 proof-of-concept studies. Only 14% (n=13) of systems monitored wildlife, while 81% (n=76) were for livestock and 18% (n=17) for companion animals. Four systems monitored wildlife in conjunction with livestock, companion animals, or both. The USA had the greatest number of wildlife syndromic surveillance systems (n=4), followed by Australia, the UK, and France (n=2 each). Wild birds were the most commonly monitored species (n=5); mortality was the most common indicator (n=4). Systems relied on reports from government officials, veterinarians, and members of the public, including hunters (n=3). Data were analyzed at time scales from daily to yearly and using techniques from descriptive statistics to Bayesian hypothesis testing. 69% of systems targeted a specific disease, such as West Nile Virus. Only one system, monitoring rabies in the USA, took a OneHealth approach.

Discussion:
While syndromic surveillance has enormous potential to improve wildlife health monitoring by leveraging non-invasive methods, it is clearly under-utilized in this sector, due to the logistical challenge and lack of resources to regularly collect data on elusive species whose economic value is often considered limited. By providing a world-wide overview of syndromic surveillance for animal health, we can identify commonalities, best practices, and improvements

for optimal adaptation to local contexts and advancement of wildlife- and OneHealth.

14:15 - 14:30

Non-invasive sampling of wild ducks: Proof of Concept for Avian Influenza

MSc Joaquin Neumann Heise Master[1], MVBS Francesca Isabel Rondi Master[2], Prof. Dr. Timm Harder PhD[3], Prof. Sascha Knauf PhD[1,4], Dr. Anja Globig Dr. med. vet[1]
[1]Friedrich-Loeffler-Institut, Institut of International Animal Health/One Health, Greifswald-Insel Riems, Greifswald, Germany. [2]Università degli Studi di Milano, Scienze biotecnologiche veterinarie, Milano, Italia, Milano, Italy. [3]Friedrich-Loeffler-Institut, Institut of Diagnostic Virology, Greifswald-Insel Riems, Greifswald, Germany. [4]Professorship for One Health/International Animal Health, Faculty of Veterinary Medicine, Justus Liebig University, Giessen, Germany, Giessen, Germany

Abstract

Currently, the highly pathogenic avian influenza virus (HPAIV) of subtype H5 (goose Guangdong lineage, gs/Gd) is circulating worldwide at unprecedented levels, causing epizootics in poultry and wild birds. Moreover, associated cases of mass mortality in terrestrial and marine carnivores are reported. The uncontrolled spread in captive and wild birds and the close contact between infected birds and mammals, including humans, is associated with a high pandemic potential. The current spatial spread of HPAI H5 gs/Gd has led to the introduction of HPAIV in Southern America, where it infects a naïve host population. Consequently, globally important biodiversity hotspots, such as the Galapagos Islands or Antarctica, could face a conservation crisis, not only in avian but also marine mammal species. Today, passive surveillance is the primary concept for the early detection of HPAIV. However, because of its limitations in providing comprehensive data, research in live birds is additionally needed to assess the true prevalence of avian influenza. Yet, active surveillance in wild birds is time- and resource-intensive, and a simplified, non-invasive surveillance strategy is needed.

We hypothesize that water, with which wild birds have demonstrably been in contact, is just as suitable for detecting and phylogenetic characterizing influenza viruses as direct bird sampling. The underlying concept is that the wild ducks release viral particles into the water if infected with the virus.

For this purpose, wild ducks present year-round in the rich avi-faunistic shallows of the Greifswalder Bodden, Germany, are attracted by wheat covered with clear shallow water in small boxes. Thus, ducks feeding on the wheat have intensive oropharyngeal contact with the water column above. Virocult® swabs are then

taken three times a week from this water and analyzed by RT-qPCR. To prevent wild mammals from feeding on this wheat, thus affecting the quality of the sample, water and food are added to the box during the morning of each sampling day, and samples are taken in the afternoon before dark, after which the box is emptied.

Until November 1, 2023, 187 water samples have been analyzed. Among these, 6.4% tested positive for avian influenza virus. A higher number of wild ducks feeding on wheat was observed from September to February, showing a positive correlation with the number of AIV-positive samples.

This shows that the presented approach has potential as an active surveillance tool. Still, enrichment techniques of virus from the water column are expected to increase the sensitivity.

14:30 - 14:45

Mixed-method analysis of published national One Health strategic plans

Caroline Wilhelm[1], Amelie Desvars-Larrive[1], Chris Walzer[2,1]

[1]Veterinary University Vienna, Vienna, Austria. [2]Wildlife Conservation Society (WCS), New York, USA

Abstract

Background

A One Health approach offers promise in tackling health threats such as zoonotic-origin pathogens in the face of the climate crisis and ongoing biodiversity loss. The systems-based approach has attracted much attention since the COVID-19 pandemic, but holistic implementation and outcome evaluation remain challenging. While a few countries have formulated national strategic plans for One Health (OH), a comprehensive overview of their content and an analysis of their potential is lacking.

Objectives

We assessed the alignment of existing national OH strategic plans with the One Health definitions of the One Health High-Level Expert Panel (OHHLEP) and the Berlin Principles to provide a framework for the development of future plans.

Materials and Methods

We conducted a comprehensive search to retrieve existing, publicly available, national OH strategic plans. This involved database searches, exploring dedicated websites, and directly contacting governments. The criteria for inclusion were met by eight national OH strategic plans which were subsequently analyzed with a

mixed-method approach consisting of a qualitative content analysis and a quantitative text analysis.

Results

Our results showed that the basic concept of OH, i. e., the interconnections between the health of humans, animals, and the environment as well as the need for collaboration between diverse sectors, disciplines, and public and private institutions, is recognized across the plans. The acknowledgment and consideration of environmental health varied across the individual strategic plans. We also observed that certain OH socio-ecological values, such as animal welfare as well as equity and parity, were overlooked in the strategic plans, with limited recognition of Traditional Knowledge Systems. The quantitative text analysis identified a common semantic and similar pattern in unigram frequencies across the eight documents. However, examination of the 50 most common bigrams revealed great variations in word associations between plans. A more detailed investigation of the subgraphs of terms related to the word "health" suggested varying perceptions of this concept among the different countries.

Conclusion

Publicly available national OH strategic plans represent important groundbreaking efforts, yet there is potential for improvement in institutionalizing OH. This includes integrating additional environmental and multicultural aspects, as well as novel social values such as resilience and solidarity. This work provides a comprehensive and reproducible assessment framework for analyzing documents describing OH strategies and intends to support future OH endeavors.

14:45 - 15:00

Estimating the sensitivity of international reporting system on selected diseases in wildlife

Paolo Tizzani[1], Nathan Layman[2], Noam Ross[2], Lina Awada[1], Claire Cayol[1]
[1]World Organisation for Animal Health, Paris, France. [2]EcoHealth Alliance, New York, USA

Abstract

The World Organisation for Animal Health (WOAH) was founded in 1924 and currently includes 183 Members. Since its foundation, WOAH has been mandated by its Members to collect and disseminate information on diseases affecting animals (including wildlife). Currently data on diseases in wildlife are collected from national authorities through two channels: a) mandatory reporting of a list of

more than 100 diseases (e.g. Avian influenza, African swine fever) and b) voluntary reporting on other around 50 diseases called "non-listed diseases" (e.g. chronic wasting disease, white-nose syndrome) which were prioritized by experts for their high impact on biodiversity and conservation. Information on this second group were collected and disseminated through a system called WAHIS-Wild. The information collected is highly dependent on the surveillance capacities in countries.

The overall aim of this work is to estimate the sensitivity of information reported internationally to WOAH through the WAHIS wildlife reporting system for "non-listed diseases" until 2019.

To estimate the sensitivity of WAHIS-wild, we conducted a 'capture-recapture' analysis comparing information provided to WOAH with information gathered from two scientific literature databases, OpenAlex and Semantic Scholar, each containing abstracts for >200M scientific articles. Both sources leverage machine learning to perform exhaustive literature searches which together identified 100,450 abstracts relevant to the "non-listed diseases". While the data collected in WAHIS-wild is available as an organised dataset, the scientific literature publishes information as free text, requiring extensive manual curation for quantitative analysis. To address this, we applied an innovating approach, leveraging OpenAI's large language model (gpt-4-0125-preview) to automatically classify reports of disease presence between the years 2008 and 2019. Each abstract was evaluated for evidence of disease occurrence, the year(s) of occurrence and the identity of any animal hosts affected. Accuracy of the extraction was good, ranging from 82-97% depending on the context and accuracy measure (i.e. precision and recall) based on comparison against 100 hand-graded outbreaks. Outbreak events were identified for all "non-listed diseases".

The resulting dataset was used to estimate the sensitivity of the WAHIS-Wild reporting system stratified against relevant socio-economic and environmental factors such as geographic region, Member GDP, and disease surveillance capacity. This assessment of WAHIS-Wild's sensitivity has provided valuable information on the robustness of the existing global disease reporting system for non-listed diseases. Additionally, the methodology used offers a novel and complementary method for tracking the spread of these diseases, enhancing our ability to monitor and respond to emerging threats.

Coffee Break

15:00 - 15:30 Tuesday, 10th September, 2024
Brewery, Stralsund

EWDA General Meeting

15:45 - 17:15 Tuesday, 10th September, 2024
Brewery, Stralsund

Poster and Pasta Party

17:30 - 21:00 Tuesday, 10th September, 2024
Brewery, Stralsund

Poster details will follow soon

Registration

07:00 - 08:00 Wednesday, 11th September, 2024
Brewery, Stralsund

Housekeeping Notes

08:00 - 08:05 Wednesday, 11th September, 2024
Brewery, Stralsund
Sascha Knauf

2nd Keynote: A tribute to Marie-Pierre Ryser-Degiorgis

08:05 - 08:50 Wednesday, 11th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Torsten Mörner[1]
[1]Viltvet AB, BRO, Sweden (National Veterinary Institute, Department of Epidemiology and Disease Control, Sweden (retired))

Abstract

This abstract is a summary of my presentation in honor of our dear colleague Marie-Pierre Ryser-Degiorgis who passed away last year after a brave fight against the cancer.

With the loss of Marie-Pierre the field of wildlife diseases has lost a dear friend and colleague characterized by a sharp intellect and a tireless interest in the dynamics in the field of wildlife health. Marie-Pierre´s sharp mind and rare talent for understanding the necessary interdisciplinary approach to wildlife disease investigation made her a leading expert in this field. After graduating in veterinary medicine in Bern, in 1995, she worked 28 years with wildlife management and diseases, often with a focus on the interaction between wildlife, humans, domestic animals and the environment, what we today call One Health.

Marie-Pierre’s scientific work to a large extent focused on infectious diseases of wildlife and especially Swiss wildlife and large carnivores. She presented her doctoral thesis in 1998 and her Habilitation (Venia Docendi) in 2013. Marie-Pierre published 70 peer reviewed articles, a large number of popular articles, and was oauthor of eight books. She attended approximately 100 scientific meetings and published 102 scientific abstracts. In addition to her deep involvement in the study

of wildlife diseases and publication history, she was active in many different meetings and trainings where she willingly shared her great knowledge of wildlife and wildlife diseases.

Marie-Pierre was also very active in numerous associations and scientific groups, such as the Wildlife Disease Association (WDA), EWDA, the Study group for the Ecopathology of mountain wildlife, and she was one of the founders of the Wildlife Health Surveillance Network of the EWDA in 2009.

In 2016 Marie-Pierre became a member of the Wildlife Working Group of the World Animal Health Organization (formerly OIE), in which her conscientious approach to the duties of the group immediately impressed the other members. Marie-Pierre received many awards for her contributions to the wildlife health field and for her outstanding scientific articles. At the WDA's 68th annual conference in 2019 in Lake Tahoe, California, she received WDA's highest award, the Distinguished Service Award, for her tireless work with wildlife diseases and her continuous support for the WDA.

Marie-Pierre had a delightful personality and although she was very serious about her work her beautiful smile and laugh was never far way. Marie-Pierre´s family, friends and colleagues have lost a very special and much loved person and her legacy will live in the hearts and minds of those she impacted.

Session II – In memory of Marie-Pierre Ryser-Degiorgis

08:50 - 10:05 Wednesday, 11th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Ruth Cromie, Torsten Mörner

08:50 - 09:05

Bridging the Gap: Integrating Management Practices into Wildlife Disease Models

Tobias Kürschner[1,2], Elodie Wielgus[1], Maik Henrich[1], Hans Thulke[3], Stephanie Kramer-Schadt[2], Marco Heurich[1,4]
[1]Bavarian Forest National Park, Grafenau, Germany. [2]Leibniz-Institute for Zoo and Wildlife Research, Berlin, Germany. [3]Helmholtz Centre for Environmental Research, Leipzig, Germany. [4]University of Freiburg, Freiburg, Germany

Abstract

Understanding the impact of small-scale management practices on disease control strategies is crucial for implementing ad hoc approaches effectively. Individual movements in reaction to localized disturbances or population control measures are pivotal for devising sustainable strategies against localized outbreaks, such as African Swine Fever (ASF) in wild boar populations. However, incorporating individual responses when modelling management interventions is extremely challenging. Therefore, there is a notable scarcity of literature addressing how ecological models of wildlife diseases can integrate the effects of such management practices on wildlife habitat utilization.

Here, we offer a novel framework for evaluating the impacts of small-scale management interventions on disease control strategies within wildlife populations. We propose modelling algorithms to simulate group movements in response to various external factors, including spatiotemporal variation in habitat quality and local conspecific densities, as well as internal factors, such as resource selection, and a metapopulation structure based on colonization and recolonization dynamics. By integrating these algorithms into our framework, we accurately replicate habitat utilization patterns akin to those observed in telemetry data.

Moreover, by simulating individual and group-level responses to localized disturbances and population control measures, we offer insights into how small-scale interventions can shape disease spread patterns within wildlife populations. This level of detail allows for the identification of key factors driving disease transmission dynamics, thereby facilitating the design of more targeted and effective management strategies.

This study outlines the fundamental principles of wildlife movement modelling and presents a comparative analysis between modelled patterns and telemetry data. By doing so, we provide valuable insights into the efficacy of small-scale management interventions in mitigating disease spread within wildlife populations.

09:05 - 09:20

How can epidemiological monitoring and modelling contribute to management decisions? Lessons learned from ten years of brucellosis in French wildlife

Pr Emmanuelle Gilot-Fromont PhD[1], Mrs Elodie Petit MSc[2], Dr Clément Calenge PhD[3], Dr Pascal Marchand PhD[4], Dr Carole Toïgo PhD[5], Dr Sébastien Gardon PhD[1], Dr Anne Van De Wiele DVM[6], Dr Ariane Payne PhD[7], Dr Isabelle Beloeil DVM[8], Dr Anne Thébault PhD[9], Dr Sébastien Lambert PhD[10]

[1]VetAgro Sup, Marcy l'Etoile, France. [2]Office Français de la Biodiversité, Sévrier, France. [3]Office Français de la Biodiversité, Saint Benoist, France. [4]Office Français de la Biodiversité, Juvignac, France. [5]Office Français de la Biodiversité, Gières, France. [6]Office Français de la Biodiversité, Paris, France. [7]Office Français de la Biodiversité, Orléans, France. [8]Office Français de la Biodiversité, Bron, France. [9]Anses, Maisons-Alfort, France. [10]Ecole Nationale Vétérinaire de Toulouse, Toulouse, France

Abstract

Epidemiological monitoring and modelling are conceived as tools to inform management decisions. However, whether research actually feeds management actions depends on how researchers and decision-makers interact. We use brucellosis in the French Alps to describe the research-policy interaction along the 10-years evolution of the outbreak. In this aim, we identified the main periods in the epidemiological situation and the factors that promoted or limited the science-decision nexus.

In 2012, an outbreak of brucellosis due to *Brucella melitensis* was detected in the French Alps. Although France was officially free of brucellosis since 2001, three persons contracted brucellosis, and one infected cattle herd was identified at the source of these cases. The local population of Alpine ibex *Capra ibex* was discovered to be heavily infected (seroprevalence around 40%), while sporadic cases occurred in the Alpine chamois *Rupicapra rupicapra*. The persistence of *B. melitensis* in an ibex population was an unprecedented situation in Europe, requiring a joint management of the zoonotic, livestock and wildlife health risks while considering the protected status of ibex. A monitoring of brucellosis and of the ibex population was implemented through captures and observations, combined with management actions.

During the first period (2012-2014), management decisions were mostly based on knowledge from domestic species, and massive culling were organized. Between 2015 and 2020, major epidemiological advances emerged: variations with space, time and ibex age were identified. Bacteriological and modelling results permitted to identify main transmission routes and at-risk groups, and management scenarios were compared. In the meantime, management mainly relied on captures, while disease prevalence markedly decreased. However, a new infected cattle herd was discovered in 2021 and infection was detected in an adjacent massif in 2022. Faced with farmers' distress, managers organized large capture and culling operations in 2022.

In general, policy decisions relied more often on epidemiological results over time, but remained related to crisis phases such as cattle infection or militant mobilizations. The research-policy interaction was facilitated by informal interactions between scientists and policy makers, however the

compartmentalization of public services and the lack of discussion limited the consensus among stakeholders. We propose that neglected aspects such as ethical issues or consistency of measures between wildlife and livestock may jeopardize the confidence of some partners. Clarifying these issues may help improving the dialogue among all partners.

09:20 - 09:35

Health Issues in Migratory Species: A Review for the Convention on Migratory Species (CMS)

Katie Beckmann[1], Marja Kipperman[1], Anna Meredith[1], Neil Anderson[1], Ruth Cromie[2]
[1]University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom. [2]CMS COP-Appointed Councillor for Wildlife Health, Bristol, United Kingdom

Abstract

Given increased awareness of the conservation importance of wildlife health and its interdependencies with livestock and human health, the Convention on Migratory Species (CMS) of the United Nations commissioned a substantive review of issues relating to health of migratory species, to inform the work plan of a CMS Working Group on Migratory Species and Health. The review's objectives included a high-level exploration of key health issues affecting migratory species listed on the CMS Appendices I or II, and their drivers. As per the CMS, the (then) 657 listed species were categorised as 'avian' (Class Aves); 'terrestrial', comprising terrestrial mammal (Class Mammalia) and one insect (Class Insecta, Order Lepidoptera) species; and 'aquatic', comprising aquatic mammals, sharks and rays (Class Chondrichthyes), bony fishes (Class Pisces) and reptiles (Class Reptilia).

The listed species were divided into 37 groups, mostly according to their order. A brief, high-level expert consultation exercise was conducted, for which 60 experts familiar with threats to the health of wild-living species within these groups were recruited. They were asked to score the negative impact of infectious and non-infectious causes of disease on the conservation status of species within their group(s) and to identify perceived underlying drivers of these conditions. Using a simple scoring system of 0 (no importance) to 5 (very high importance), a median score of ≥ 3.5 out of 5 across experts was taken to indicate a 'highly important' issue.

Infectious agents were perceived as a highly important conservation issue in 85% (11) of both 13 avian and 13 terrestrial species groups. In avian species, highly pathogenic avian influenza virus was considered highly important in 62% (eight) of 13 groups, whereas a broad range of infectious agents were of concern in

terrestrial species. In avian species, toxins or pollutants were also commonly considered highly important (in 62% of groups). In migratory aquatic species, incidental anthropogenic trauma was considered highly important in 73% (eight) of 11 species groups, and toxins and pollutants in 55% (six) of the groups. 'Habitat loss, degradation or disturbance', climate change and 'agriculture or aquaculture' were considered important drivers of key disease issues.

Infectious disease, poisoning and anthropogenic trauma are evidently conservation concerns for many migratory species. Threats to the conservation status of wildlife are considered to underlie these infectious and non-infectious disease issues, and preventative One Health approaches are therefore needed to address the risks to migratory species from disease.

09:35 - 09:50

Linking demographic rates to disease occurrence explains differential population declines in two common garden bird species in Great Britain

Hugh Hanmer[1], Andrew Cunningham[2], Shinto John[2], Shaheed Macgregor[2], Will Peach[3], Kate Plummer[1], Rob Robinson[1], Katharina Seilern-Moy[2], Gavin Siriwardena[1], Becki Lawson[2]

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Abstract

The extent of supplementary feeding of wildlife is increasing, especially in gardens. This is benefitting numerous species, and even changing bird communities, however there is increasing concern around the disbenefits of such feeding, particularly in terms of increased disease transmission. In Great Britain (and subsequently Europe) the spread of the protozoan parasite, *Trichomonas gallinae*, from columbids to finches, has led to epidemic finch trichomonosis and a rapid, and sustained, decrease in the greenfinch (*Chloris chloris*) population since the initial identification in 2005, as documented by large-scale citizen science bird monitoring. More recently, this monitoring has revealed a similarly rapid decline, since 2012, in chaffinch (*Fringilla coelebs*) populations. Using a range of citizen science data to quantify population changes in different habitats and key demographic rates, we show that both declines were driven primarily by reduced adult survival, with the greatest reductions occurring in peri-domestic habitats, where supplementary food provision is more common.

Post-mortem examinations of carcasses submitted by participants in the Garden Wildlife Health (GWH) project showed a proportional increase in trichomonosis

cases (of the same strain) in chaffinches, near-contemporaneous with its population decline. Furthermore, we combined data from GWH and long running citizen science Garden BirdWatch survey to investigate the local and short term risk factors associated with the occurrence of finch trichomonosis to identify where and what conditions incidents are more likely to occur for the targeting of monitoring and potential mitigation actions. Our results support the hypothesis that supplementary feeding can increase parasite transmission frequency within and between common species. However, the dynamics behind the resultant population change can vary markedly, highlighting the need for integrating disease surveillance with demographic monitoring. Other species susceptible to *T. gallinae* infection may also be at risk, so we suggest that supplementary feeding guidelines for wildlife should include disease mitigation strategies to ensure that benefits to target species outweigh risks.

09:50 - 10:05

Antimicrobial prescribing practices and antimicrobial resistance in wildlife rehabilitation

Irene Bueno[1], Elizabeth A. Miller[2], Rachel Amato[2], Julia B. Ponder[2]
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Abstract

Antimicrobial resistance (AMR) is a global health concern. As such, there have been increased efforts to monitor and standardize antimicrobial prescribing practices in humans and domestic animals. Despite the wide use of antimicrobials to treat rescued wildlife, little is known about specific prescribing practices, AMR emergence in wildlife exposed to microbial interventions, and the potential subsequent dissemination into the wider environment. The goals of this study were: a) to examine antimicrobial and probiotic use (AMPU) in wildlife rehabilitation in the United States (U.S.), and b) to assess the impact of wildlife rehabilitation on the emergence and spread of AMR. For a) we conducted a cross-sectional survey enrolling 105 U.S. permitted wildlife facilities to collect information about AMPU practices, and knowledge and attitudes about AMR. For b) we collected fecal samples from raptors admitted to a rehabilitation facility at two time points: on admission, and before release, along with data on key management interventions while in captivity. Then we performed antibiotic susceptibility testing on *Escherichia coli* and *Enterococcus spp* isolates using Sensititre. Survey response rate was 51.43%. All facilities (100%) used antimicrobials, and larger ones were more likely to consider AMR in their decision-

making when using antimicrobials (adjusted *p*=0.05). Among antibiotics, enrofloxacin and amoxicillin-clavulanic acid were the most commonly used; antifungals were not as widespread, but itraconazole was the most commonly used. For probiotics, 66.67% of facilities used them for a variety of circumstances, ranging from diarrhoea to being used as a calming agent. In terms of knowledge and attitudes, 82.22% of facilities strongly or somewhat agreed to have a good understanding of AMR, 11.11% strongly or somewhat agreed that AMR was a problem at their facility, and 56.67% agreed that probiotics improved clinical outcomes. Data are currently being analysed for b). Results from this work will be a step towards understanding antimicrobial prescribing practices in the treatment of captive wildlife which will help in the development of much needed antimicrobial stewardship programs for wildlife rehabilitation globally. Further, this study will also help elucidate the potential role of wildlife rehabilitation to the contribution of AMR emergence and spread.

Short Coffee Break

10:05 - 10:20 Wednesday, 11th September, 2024
Brewery, Stralsund

Young Scientist Keynote: New threats to carnivores inhabiting human-modified landscapes

10:20 - 10:45 Wednesday, 11th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Ana Balseiro[1]
[1]Universidad de León, Spain

Abstract

In the last years, wild carnivores have been forced to occupy highly anthropized areas due to their growing population trends, the development of urban and suburban areas and the ubiquity of human activities. In that scenario, understanding mortality causes of wild species is crucial because multi-host pathogens emerging and re-emerging at the wildlife-domestic animal interface affect wildlife management and conservation. This is the case of canine distemper virus (CDV) and canine adenovirus type 1 (CAdV-1). Northwestern Atlantic Spain is a hotspot of carnivore diversity, which includes one of the largest wolf (Canis lupus) and Eurasian brown bear (Ursus arctos) populations in southwestern Europe. Since 2020, in this region we have recorded mortality due to distemper in five carnivore species including three mustelids (Eurasian badger Meles meles,

European marten Martes martes, and European polecat Mustela putorius), one canid (red fox, Vulpes vulpes) and one ursid (brown bear). In addition, a molecular study in eight wild carnivore species revealed 19.5% of positivity and a retrospective serological study 43.4% of prevalence in badgers. Since 2014, three free-ranging brown bears have died due to infectious canine hepatitis caused by CAdV-1 and the virus has been detected in 45% of brown bear fecal samples collected in the area. CAdV-1 has also been identified in the sympatric wolf population at 14% and 70% prevalence rates using molecular and serological techniques, respectively. This framework reveals that the new management strategies for the conservation of wild carnivores should consider the emerging and re-emerging pathogens. Ongoing research seeks to elucidate how the high infection prevalence may threaten carnivore populations. For that, it is essential to (i) adopt a collaborative approach within the One Health framework to safeguard the health of livestock, wildlife, humans, and the environment; (ii) establish an enhanced disease surveillance system (e.g., using integrated wildlife monitoring including non-invasive surveillance -feces or sponges-); (iii) implement effective risk mitigation and management strategies (e.g., through vaccination of rural dogs); and (iv) foster coordination and collaboration among national, regional, and global government and non-government agencies and stakeholders.

Session II – In memory of Marie-Pierre Ryser-Degiorgis Student Session

10:45 - 12:30 Wednesday, 11th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Paul Duff, Ana Balseiro

10:45 - 11:00

Water: a reservoir of multidrug-resistant high-risk clonal *Escherichia coli* lineages

Phillip Lübcke[1], Dr. Timo Homeier-Bachmann[2], Dr. Christian Schulze[3], Dr. Elias Eger[4], Stefan E. Heiden[4], Michael Schwabe[4], Prof. Dr. Sebastian Guenther[3], Prof. Dr. Katharina Schaufler PhD[4]
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Abstract

Background: Antimicrobial resistant (AMR) *E*scherichia (*E.*) *coli* are considered to pose a serious threat to human, animal and environmental health. Their spread in large parts of the environment, including aquatic habitats, is a trend of great concern for public and animal health. Aquatic environments are often considered as locations where humans, domestic animals and wildlife get in contact and therefore are a central interface in One Health. Despite these recognized problems, AMR data for the Baltic Sea is scarce.

Hypothesis: The surface water of the Baltic Sea could be a reservoir for multidrug-resistant (MDR) *E. coli*.

Materials and Methods: Here, we investigated the spread of ESBL-producing *E. coli* in the Baltic Sea around Greifswald (Germany), by collecting environmental water samples from August 2021 to August 2022. The samples were screened for ESBL-producing *E. coli*. The strains were characterized pheno- and genotypically in depth, including antibiotic/heavy metal tolerance, carriage of resistance/virulence genes and phylogenetic analyses were performed. Furthermore, we used solid-phase extraction followed by ultra high performance liquid chromatography and mass spectrometry to quantify antibiotic residues in the respective samples.

Results: We detected 30 ESBL-producing *E. coli*, which also showed resistances to some clinically important antibiotics beyond ESBL resistance, such as trimethoprim/sulfamethoxazole, ciprofloxacin and piperacillin/tazobactam. Over 60% could be classified as phenotypically MDR. Whole-genome sequencing revealed the presence of international high-risk *E. coli* sequence types (ST58, ST117, ST131, ST410, ST744). Additionally, the isolates carried a variety of virulence-associated genes, such as genes important for bioflim formation (*cgsDEFG*, *csgABC*), iron uptake (*entABCDEFS*, *fepABCDG*, *fes*), invasion (*Ibes* and *ompA*), and regulation of gene expression (*fur*, *rpoS*, *rcsB*, *phoP*). Phylogenetic analysis showed a close relationship for three ST117 strains, which were sampled over a time span of 5 months (November/December 2021, and March 2022) and depicted a high plasmid load. The highest detected concentrations for ciprofloxacin, sulfamethoxazole, and ampicillin were 553.0 ng/L, 281.8 ng/L, and 164.4 ng/L, respectively, which exceeds most concentrations described for other german water columns (9 – 326 ng/L).

Conclusions: In summary, surface water of the Baltic Sea can be considered as a reservoir for ESBL-producing *E. coli*, while being sporadically contaminated with relatively high antibiotic concentrations. The occurence of closely related isolates indicates persistence of those strains in this area. This raises the possibility of human and wildlife exposure to these specific pathogens via water and underlines the importance of the One Health approach.

11:00 - 11:15

Gulls in southern Spain as carriers and potential spreaders of zoonotic bacteria and their antibiotic-resistant strains

María Pilar González-Navarro[1,2], Teresa Ayats[1,2], Salvador García-Barcelona[3], Jacob González-Solís[4], Marta Cerdà-Cuéllar[1,2]
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Abstract

Zoonotic agents, such as *Campylobacter* and *Salmonella*, can be disseminated and maintained in the environment by wildlife, especially by those species with scavenging feeding habits, such as the yellow-legged gull (*L. michahellis*). These bacteria may develop resistance to antibiotics of veterinary or human relevance. To gain insight into the epidemiology of *Campylobacter* and *Salmonella*, along with their antimicrobial-resistant strains, we sampled 104 yellow-legged gull chicks from 2021 to 2023 in Tarifa, Southern Spain, using cloacal swabs. We assessed the frequency of both pathogens by standard culture methods and the presence of *Salmonella* antibiotic-resistant (AMR) strains using a broth microdilution method using EUSVEC3 plates. Our findings revealed that 34.6% (36/104) of the individuals carried *Campylobacter*, with 47.2% (17/36) being *C. jejuni*, 19.4% (7/36) *C. coli*, 27.7% (10/36) *C. lari* and 5.5% (2/36) were coinfected with *C. jejuni* and *C. lari*. Additionally, *Salmonella* was isolated from 8.6% of the birds, showing diverse serovars, including those commonly associated with human salmonellosis, such as Enteritidis and Kentucky. Over the three years, *Campylobacter* prevalence has remained stable, fluctuating between 33-35%. However, there has been a notable interannual variability regarding *Campylobacter* species, particularly with *C. coli*, which was isolated only in 2023 with a 70% prevalence. For *Salmonella*, a prevalence from 0% to 25% from 2021 to 2023 was observed. Among *Salmonella* isolates 6 out of 9 (66,7%) showed resistance to at least 1 antibiotic, primarily ciprofloxacin and nalidixic acid (66.7%). Two multidrug-resistant serovar Kentucky isolates were identified (AMR profile: SMX-TMP-CIP-NAL-TET-AMP; SMX-CIP-NAL-TET-AMP). Adults within this colony may become infected while foraging in refuse dumps a few km away from the colony or even if covering longer distances to reach the coast of Morocco in

northern Africa. This behaviour has also been observed in other yellow-legged and Audouin's gulls from this region, which transmit the pathogens to their offspring while feeding the chicks. Our findings highlight yellow-legged gulls as reservoirs and potential spreaders of AMR strains, posing risks to the environment and public health, especially if nearby human populated areas, as also shown in other gull colonies in southern Europe.

11:15 - 11:30

Black-headed gulls breeding in Mecklenburg-Western Pomerania as a reservoir for multidrug-resistant high-risk clonal Enterobacterales

Jana Brendecke[1], Angela Schmitz Ornés[2], Michael Schwabe[1], Stefan E. Heiden[1], Elias Eger[1], Katharina Schaufler[1]
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Abstract

Background: Wild animals and the environment are important hotspots for the accumulation of multidrug-resistant Enterobacterales (MDR-E), including extended-spectrum β-lactamase (ESBL)-producing *Escherichia* (*E.*) species and *Klebsiella pneumoniae*. These pathogens often combine resistance with high-level virulence, resulting in a difficult treatment in the clinic. Therefore, these bacteria are categorised as first priority on the WHO Global Priority Pathogens list. The high pathogenic potential for humans, as well as the occurrence in the environment and wild animals that have never been exposed to any antibiotic substance, show the importance of the One Health approach.

Hypothesis: Black-headed gulls breeding in Mecklenburg-Western Pomerania could be potential carriers of MDR-E associated to human infections.

Material and Methods: Overall, we collected 918 faecal samples from two nature conservation islands during the breeding seasons (April – July) from 2021 to 2023. These included adult birds, nestlings, and collective faecal samples. We screened the samples for ESBL-producers on chromogenic agar plates with cefotaxime as antibiotic. Positive *Escherichia* and *Klebsiella* species strains were whole genome-sequenced and bioinformatically analysed for the carriage of additional antibiotic resistance, heavy metal tolerance (HMT), hypermucoviscosity and biofilm formation genes as well as further virulence genes.

Results: We detected twelve MDR-E in 2021 and 29 in 2022. Most strikingly, we found international high-risk clonal lineages of *E. coli* sequence type (ST) 38, ST131 and ST744 to be present in both sampling seasons, which were, however, non-clonal. Of the isolated strains, 39 % are MDR; in addition to 3rd generation cephalosporins, they are also resistant to ciprofloxacin, gentamicin, tetracycline and in one case to colistin (a last resort drug). HMT genes for arsenic and mercury were detected in 29/41 strains, which showed growth at medium and high concentrations of the respective HMs. Due to the lack of literature values, tolerance values were determined by preliminary tests. One strain was identified as *E. marmotae* showing a hypermucoviscous phenotype. Evaluations for 19 strains from 2023 are still ongoing.

Conclusion: In summary, black-headed gulls breeding in Western Pomerania are an important reservoir for MDR-E with clinical relevance (e.g. *E. coli* ST38 and ST131). Due to their migratory behaviour, the gulls are able to carry these pathogens over long distances and thus spread them. Our study highlights the importance of the One Health approach considering the interdependence of human, animal, and environmental health.

11:30 - 11:45

Exploring Oro-pharyngeal Microbiota Diversity of European Bats: A Comprehensive Investigation from Spain.

Carlotta Pasetto[1], Lourdes Lobato[1], Virginia Aragón[2], Nuria Galofré[3], Maria Puig Ribas[1], Johan Espunyes[1], Laura Canós[1], Xavier Fernandez[1], David Guixé[4], Jordi Camprodon[4], Oscar Cabezón[1]
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Abstract

Bats (order Chiroptera) form the most diverse mammalian group after rodents and play a vital role for the function of Earth's ecosystems. Most studies focus on bats as viral reservoirs, but current knowledge on bat-associated bacteria is limited,

and the impact on the health of bats themselves is largely ignored. Previous studies indicate that members of the family Pasteurellaceae can cause severe pulmonary disease and systemic infection in chiropteran species. Precisely, *Pasteurella multocida* is regarded as the causative agent of fatal pneumonia in individuals with predation-associated injuries, as well as mass mortalities in both free-ranging and captive populations. This study aimed to assess the presence of bacteria that can act as potential respiratory pathogens in European free-ranging bats. Oropharyngeal swabs from thirteen bat species (*Hypsugo savii, Miniopterus schreibersii, Myotis blythii, Myotis capaccinii*, *Myotis crypticus, Myotis escalerai*, *Nyctalus leisleri*, *Nyctalus lasiopterus, Pipistrellus pipistrellus, Pipistrellus pygmaeus*, *Pipistrellus kuhlii, Plecotus austriacus, Rhinolophus ferrumequinum*) across Catalonia (NE-Spain) were collected from June to October 2019. All bats were apparently healthy. Samples (n=321) were cultured on chocolate agar and colonies with different morphologies were identified by the partial sequencing of the 16S rRNA gene. The most common bacterial families detected were Pasteurellaceae (35.5%), Bacillaceae (27.4%), Staphylococcaceae (16.2%), Enterobacteriaceae (5.3%), followed by Neisseriaceae (1.9%), Streptococcaceae (1.5%), Yersineaceae (1.5%) and Morganellaceae (1.5%). Overall, the most frequent species was *Vespertiliibacter pulmonis* (21.1%), followed by *Mammaliicoccus* (*Staphylococcus*) *sciuri* (6.5%). Additionally, multi-host opportunistic pathogens (e.g., *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, *Morganella morganii*, *Acinetobacter radioresistens, Klebsiella oxytoca*) were also identified. With the present study, we report for the first time, *V. pulmonis* from other bat species than previously described, and we propose this bacterium being a common member of the oropharyngeal flora of European bats. *Vespertiliibacter pulmonis* has been previously isolated from the lungs of deceased bats. However, its pathogenic role remains largely unknown. Remarkably, *Pasteurella multocida* was not detected and it does not seem to be common in the oral cavity of apparently healthy individuals. The detection of veterinary and medically important emergent organisms, such as *Mammaliicoccus* (*Staphylococcus*) *sciuri* and *Mammaliicoccus* (*Staphylococcus*) *len tus*, requires further investigation.

11:45 - 12:00

Urban habits of the bent-winged bat (*Miniopterus schreibersii*) as possible driver for the spillover of lyssaviruses in Italy

Francesca Festa MSc[1,2], Prof Dino Scaravelli[3], Dr Francesca Cosentino[2], Dr Paola De Benedictis[1], Prof Luigi Maiorano[2], Dr Stefania Leopardi[1]

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Abstract

The bent-winged bat (*Miniopterus schreibersii*) is an endangered species with hypogeal habits and a wide distribution across the southern European Palearctic Region, which has been associated with zoonotic viruses including West Caucasian Bat virus (WCBV) and Lleida Bat Virus (LLEBV), members of the genus *Lyssavirus*. In 2020, we confirmed a rabies case associated with WCBV in a free-roaming cat in an urban centre in Italy and found a stopover population of bent-winged bats in a tombed river beneath the city.

The present study investigated the relevance of *Miniopterus schreibersii* in this roosting site, which raises concerns in conservation and public health. We also studied some urban colonies identified in Emilia Romagna and Tuscany region in Italy to determine the incidence of lyssaviruses and possible interactions with humans and other animals that might act as a bridge for human infection.

We performed a species distribution model with a Bayesian approach, gathering occurrence data from online repositories (GBIF, iNaturalist) and considering bioclimatic variables (WORLDCLIM database), urban settlements and tombed rivers distribution as explanatory variables. We gathered information about human occupancy of the sites and installed camera-traps to monitor the entrance of other animals. We collected blood, swabs and biometric information to assess taxonomical status and to perform virological analyses.

The main output from the habitat-suitability model confirmed that tombed rivers are likely not driving the distribution of these bats, although they do seem suitable roosts within urban settlements. Indeed, we discovered another population roosting in a similar urban site in the country. The installed camera-traps reported several entrances of cats and showed that the local municipality was engaged in some infrastructural works inside the tunnel, confirming that both humans and domestic animals were using this place. WCBV and LLEBV were found to co-circulate in both urban settings, with a prevalence ranging from 2 to 50% for the entire duration of the three-year surveillance.

While *Miniopterus schreibersii* is widely considered cave-dwelling, the identification of at least two populations roosting within urban settings in Italy may indicate changes in its roosting ecology. Ecological models suggest that tombed rivers might provide suitable habitats for this species. Considering the circulation of zoonotic lyssaviruses and the overlap in habitat use with human and domestic animals, it is pivotal to unravel the occupancy of suitable urban roosts to warrant protection of this bat and mitigate risks for spillover.

12:00 - 12:15

Assessment of infection of several pathogens in free-ranging red deer (Cervus elaphus) in a nature reserve in the Netherlands

Jasmin M. Keizer[1], Inês Marcelino DVM MSc[1], Dr. Gustavo Monti DVM PhD[1], Dr. Perry Cornelissen PhD[2], Dr. Inge Santman-Berends PhD[3], Jasper het Lam DVM[4], Prof. Dr. Wim H.M. Van der Poel DVM PhD Dipl ECVM[1,5]
[1]Quantitative Veterinary Epidemiology, Wageningen University and Research, Wageningen, Netherlands. [2]Department Nature & Society, Staatsbosbeheer, Amersfoort, Netherlands. [3]Department of Research and Development, Royal GD, Deventer, Netherlands. [4]Ruminant Health Department, Royal GD, Deventer, Netherlands. [5]Wageningen Bioveterinary Research, Lelystad, Netherlands

Abstract

Wildlife Health Monitoring (WHM) is important for providing information on the presence, emergence and distribution of a wide range of pathogens. These pathogens can be transmissible, and can impact the ecological, economic and health status of not only wild and domestic animals, but also humans in the case of zoonoses. Integrating these different aspects can lead to better health management, a transdisciplinary view known as One Health. WHM is an essential component of the One Health approach because wildlife species play a crucial role in the transmission cycle of zoonotic diseases. The Oostvaardersplassen (OVP) nature reserve in the Netherlands has been actively monitoring the health of free-living red deer and the presence of pathogens since 2000. This active monitoring strategy is exceptional, as wild and free-living species are generally only passively monitored. The aim of our study was to estimate seroprevalence of pathogens in free-living red deer in OVP and evaluate the current monitoring system.

This study analysed data from serological surveys of red deer at OVP between 2000 and 2023. The monitoring system tests serum and faecal samples from several animals obtained through annual population culling for health monitoring and control for 23 pathogens (viruses, bacteria and parasites). Sample size estimation for monitoring assumed a 25% design prevalence. Yearly apparent prevalences (AP) were estimated for each pathogen evaluated. Additionally, probability of freedom from infection was estimated for each pathogen if all the sampled animals tested negative.

Serological tests detected reactions with various AP for several viruses including Bluetongue virus (0.00-40.00%), Bovine Herpesvirus 1 (0.00-93.75%), bacteria: *Mycobacterium avium* subsp. *paratuberculosis* (0.00-53.85%), *Salmonella* Dublin (0.00-25.00%) and *S.* Typhimurium (0.00-28.57%). Finally, parasites like *Fasciola hepatica* (0.00-37.50%), Coccidia (*Eimeria* species), *Strongyloides papillosus*,

and *Moniezia expansa* were observed. We did not detect antibodies against Bovine Leukemia virus, Bovine Viral Diarrhoea virus, *Brucella abortus*, *Mycobacterium bovis*, *Coxiella Burnetii*, *Leptospira interrogans* serovar Hardjo, *Nematodirus spp.*, or Chronic wasting disease. Ongoing freedom of infection analysis will provide more extensive results.

In conclusion, our study provides valuable information on pathogens of significance in free-living red deer in OVP and the current monitoring programme. This generated knowledge can be applied to optimize successful WHM and can serve as a guide for implementing a similar monitoring strategy in different nature reserves in the Netherlands.

12:15 - 12:30

Cases of fatal pneumonia in wild boar in Catalonia: a pathological, microbiological and molecular investigation

Alexandra Kouznetsova[1], Mònica Sagrera[2,3,4], Josep Estruch[1], Marina Sibila[2,3,5], M. Lourdes Abarca[6], Rosa Rosell[2], Florencia Correa-Fiz[2,3,5], María Escobar-González[1], Emmanuel Serrano[1], Joaquim Segalés[3,5,6], Roser Velarde[1]

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Abstract

Background and objectives: Europe's surging wild boar population requires expanded research into the diseases affecting it. The regional passive health surveillance programme of Catalonia reveals that pneumonia is the main natural cause of mortality in wild boar. Pneumonia has been thoroughly researched in domestic pigs, and there are studies concerning farmed and hunted boar; in contrast, we investigate pneumonia as a cause of mortality in free-ranging wild boar, aiming to characterize pulmonary lesions and identify causative and contributing agents.

Materials and methods: Thirty wild boar necropsied between 2018 and 2022 were selected based on gross pulmonary lesions. Lungs were evaluated

histologically across multiple parameters, and analysed using conventional bacterial isolation and polymerase chain reaction (PCR) for African swine fever virus (ASFV), Aujeszky's disease virus (ADV), classical swine fever virus (CSFV), porcine circovirus 2 (PCV-2), PCV-3 and PCV-4, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), *Mycoplasma hyopneumoniae* (Mhp), *Mycoplasma hyorhinis* (Mhr), *Actinobacillus pleuropneumoniae* (App) and *Glaesserella parasuis* (Gp), distinguishing between virulent and non-virulent strains. PCV-2 positive samples underwent genotyping. Additionally, biometric measurements were compared to those of healthy counterparts.

Results: Diseased animals were predominantly juvenile, significantly smaller and lower in weight than healthy animals. Lesions were identified as bronchointerstitial pneumonia, mostly in combination with bronchopneumonia, and approximately half of the lungs displayed chronic inflammation. Lungs tested negative for CSFV, ASFV, ADV, PCV-4 and non-virulent Gp. The most frequently detected agent was Mhp (96.7%, n=29), followed by virulent Gp (70%, n=21), constituting the most common pathogen combination. PCV-3 was found in 26.7% (n=8), PCV-2 and Mhr in 23.3% (n=7), and App in 10% (n=3). PRRSV and SIV were detected in one animal. PCV-2 genotyped samples (n=4) were PCV-2d. Bacterial isolation yielded *Streptococcus* spp (37.9%, n=11), *Pasteurella multocida* (27.6%, n=8), *Trueperella pyogenes* (6.9%, n=2) and *Salmonella* spp (3.4%, n=1). Only one lung was infected with a single pathogen, 24% with two pathogens, 31% with three pathogens, while 41% were co-infected with four pathogens or more.

Conclusions: Given that cases were classified in the range of acute to chronic, it is plausible that the observed size difference may be attributed to both growth retardation resulting from chronic lung inflammation and increased infection susceptibility of weaker animals. Studied cases revealed a polymicrobial aetiology, with *Mycoplasma hyopneumoniae* playing a key role in the pathogenesis. Co-infection combinations closely resembled those observed in clinical cases of porcine respiratory disease complex (PRDC) in domestic pigs.

Lunch Packages and Departure Excursions

12:30 - 12:50 Wednesday, 11th September, 2024
Brewery, Stralsund

Excursions

12:50 - 17:30 Wednesday, 11th September, 2024

Transfer to Isle of Riems

17:30 - 18:30 Wednesday, 11th September, 2024

Guided Tour at Friedrich-Loeffler-Institut

18:30 - 19:15 Wednesday, 11th September, 2024

Barbecue at the Friedrich-Loeffler-Institut & EWDA Silent Auction

19:15 - 20:30 Wednesday, 11th September, 2024

EWDA Live Auction

20:30 - 22:00 Wednesday, 11th September, 2024

Transfer to Stralsund

22:00 - 22:30 Wednesday, 11th September, 2024

Registration

07:00 - 08:00 Thursday, 12th September, 2024
Brewery, Stralsund

Housekeeping Notes

08:00 - 08:05 Thursday, 12th September, 2024
Brewery, Stralsund
Sascha Knauf

3rd Keynote: A new panorama of wildlife disease surveillance in Europe, recent and current initiatives.

08:05 - 08:50 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Dolores Gavier-Widén[1]
[1]Swedish Veterinary Agency (SVA), Uppsala, Sweden

Abstract

Wildlife Disease Surveillance (WDS) in large parts of Europe was in the past restricted to some diseases and/or wildlife hosts. By 2009 only 14 out of 25 European countries had partial or comprehensive general WDS in place (Rev.Sci.Tech, 30:755, 2011). Resources were scarce but expertise and enthusiasm were not lacking and in 1993 the European Wildlife Disease Association was founded.

An escalation of WDS in Europe has taken place in recent years and prospects are very good. Not a minute too late! Accelerating anthropogenic changes have devastating effects on ecosystems, causing climate change and loss of habitat, impacting the health and, in some cases, even the survival of wildlife.

What has brought about the upsurge of WDS? One factor was the boom of the One Health (OH) concept and initiatives. OH recognises the interconnectedness of health across people, the environment and animals, wild animals being an important component, epidemiologically and as sentinels of ecosystem health. The devastating COVID-19 pandemic with the not yet completely understood role of wildlife in the origin and transmission of the SARS-CoV-2 virus was an eye-opening globally. Spread of diseases in which wildlife is important epidemiologically, such as ASF and HPAI, affects livestock and have high socio-

economic impact. Political will and the awareness of citizens recognising the importance of OH and of preserving biodiversity and the health of ecosystems has led to important EU initiatives and OH strategic developments across EU organisations (EFSA, ECDC, institutes, universities).

The very productive EFSA-funded ENETWILD, followed by ENETWILD 2.0, has collected harmonised wildlife population data and created an important instrument for wildlife monitoring, the European Observatory of Wildlife. ENETWILD also collected information about surveillance of zoonoses in the EU, linked with EFSA, contributed to setting up and scaling up coordinated surveillance systems for cross-border priority pathogens, i.e. the OH4Surveillance, funded under EU4Health programme.

The European Partnership Animal Health & Welfare (EUP AH&W) aligns its objectives with the European Green Deal. A joint internal project (Set of activities 6) strengthens wildlife scientists' networks and further develops work on wildlife populations and WDS. It also investigates the impact of human activities and climate change on the spread of diseases.

The strengthened WDS in Europe increases capacity, enlarges networks, expands covering and contributes to wildlife management, protecting the health of wildlife, livestock, people and ecosystems.

Session III – Disease Ecology, Emerging and Re-Emerging Wildlife Diseases and Host-Pathogen-Interactions

08:50 - 10:35 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Peter van Tulden, Dolores Gavier-Widén

08:50 - 09:05

Indispensable surveillance component or superfluous frills: Full genome analyses of outbreaks of highly pathogenic avian influenza in wild birds in Germany

Anne Pohlmann, Ann Kathrin Ahrens, Jacqueline King, Anne Günther, Christin Hennig, Ronja Piesche, Annika Graaf-Rau, Christian Grund, Martin Beer, Timm Harder

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

Abstract

The emergence of avian influenza viruses (AIV) presents a critical challenge to wild bird populations, underscoring the importance of robust surveillance and management efforts. Advances in genomic sequencing technologies have revolutionized our approach to monitor these viruses, offering to making molecular surveillance a cornerstone in the approach to understanding evolution and ecology of AIV.

Avian influenza, characterized by its comparatively small, segmented negative-stranded RNA genome, is an ideal candidate for genetic analysis. Through genome sequencing, researchers can trace the viruses' relationship, identify its potential precursors, and track its transmission pathways. This process also enables the detection of new virus genotypes and mutations that could potentially signal shifts in characteristics, such as virulence, vaccine escape and host specificity.

We developed an efficient sequencing workflow for AIV from field specimens, focusing on cases of highly pathogenic avian influenza (HPAI) in Germany. This workflow includes rigorous data analysis, encompassing quality assessments, genetic differentiation, and mutation screening. Moreover, by incorporating spatiotemporal data into our analysis, we gain insights into the viruses' emergence and dispersion patterns, enhancing our surveillance and containment strategies.

Our findings highlight the recurrent outbreaks of HPAI H5 viruses, specifically the 2.3.4.4b clade of the goose/Guangdong lineage in Germany since 2016 with the characterization of over 40 genotypes. Notably, the outbreaks between 2020 and 2024 surpassed previous records in case numbers, duration, and viral genetic diversity. The summer occurrences of HPAI mark a significant shift towards endemicity.

Our focus on analysis of sequence data from HPAI outbreaks among colony-breeding seabirds in summer 2022 along Germany's North and Baltic Sea coasts revealed species specific co-circulation of two predominant, genetically distinct H5N1 genotypes. Additionally, in 2023, a novel gull-adapted H5N1 genotype emerged, that dominated avian influenza events across various bird species, including poultry and wild carnivores like foxes, in Europe and Germany until September 2023. The 2023/2024 season saw the emergence of new genotypes, each displaying unique spread patterns, and displacing previously dominant genotypes.

In conclusion, genomic sequencing of avian influenza viruses provides invaluable insights for surveillance, offering a detailed view of the virus's evolution, spread, and the emergence of new genotypes, thereby revealing insights into the

interaction of different wild bird populations.

09:05 - 09:20

HPAI on wildlife in France from 2016 to 2022: what did we learn?

Louison Blin[1], Clément Calenge[2], Anouk Decors[1], Anne Van De Wiele[3], Béatrice Grasland[4], Audrey Schmitz[4], Éric Niqueux[4], Claire Guinat[5], Guillaume Le Loc'h[5], Loïc Palumbo[1]
[1]French Biodiversity Agency, Orléans, France. [2]French Biodiversity Agency, Auffargis, France. [3]French Biodiversity Agency, Villeneuve de Rivière, France. [4]ANSES, Ploufragan, France. [5]Ecole nationale vétérinaire, Toulouse, France

Abstract

Highly pathogenic avian influenza (HPAI) is a viral disease with major conservation issues. In France, several outbreaks of HPAI affecting wild birds were identified from 2016 to 2022, through the event-based surveillance of the SAGIR Network. However, the space and time dynamics of the disease during these outbreaks remain unclear.

The aim of the study was to analyze the surveillance data to identify the spatial patterns of the disease in France (including cluster locations and species composition), along with their variability over time.

We use an exploratory analysis of the surveillance data collected by the SAGIR network. We identified the temporal limits of the different outbreaks, and using the approach of Kelsall & Diggle [1995. 'Non-Parametric Estimation of Spatial Variation in Relative Risk'. *Statistics in Medicine* 14. https://doi.org/10.1002/sim.4780142106.] to estimate a risk map for each outbreak period, we could identify probable HPAI clusters (i.e., groups of dead birds diagnosed with HPAI occurring over a short period of time and a small area). The species composition observed within these probable clusters enabled the formulation of hypotheses regarding the epidemiology of HPAI in France.

We distinguished three main types of HPAI clusters depending on their space and time structure:

- The first type of cluster occurred every year (with HPAI cases on wild birds) during winter, in same limited area in eastern France, and affected mainly waterbirds and their predators. We hypothesized that this first type of cluster resulted from wild bird movements (involving particularly migratory birds) introducing the virus into favorable areas for virus survival and maintenance.
- The second type of cluster affected areas unique to each outbreak period, and was essentially composed of synanthropic species. As suggested by

the literature, these species have minimal or limited involvement in virus transmission. These clusters occurred near HPAI poultry farm clusters, which suggests an accidental contamination of wild birds by poultry farms, without the virus being maintained in the wild compartment.
- The last type of clusters corresponded to epizootics involving colonial bird species. The ecology of the involved species and the characteristics of the circulating virus genotypes, suggesting increased fitness to infect some of the bird species, may explain the spatiotemporal dynamics of these clusters.

In conclusion, beside adding to the global understanding of the epidemiology of HPAI, our results will be valuable for updating wild bird surveillance protocols to prevent infections in poultry and safeguarding population of wild species by prioritizing species of concern.

09:20 - 09:35

Long-term Transmission Dynamics of Bat Influenza Virus in Peruvian Vampire Bats

Dr Megan Griffiths PhD, Professor Juan Morales, Ms Alice Broos, Ms I-Ting Tu, Dr Mafalda Viana, Professor Daniel Streicker
University of Glasgow, Glasgow, United Kingdom

Abstract

The discovery of bat influenza viruses (BIV; H17N10 and H18N11) in 2012 has spawned a host of questions surrounding its transmission, maintenance, and zoonotic risk. These BIVs are divergent from conventional Influenza A and currently thought unlikely to infect not-bat species. However, their use of the ubiquitous MHC-II receptors for cell attachment and entry means that the potential for cross-species transmission remains. Common vampire bats (*Desmodus rotundus*), in which H18 antibodies have previously been detected, are of particular concern for potential cross-species transmission due to their blood feeding behaviour. This brings them into frequent and close contact with hosts of canonical Influenza viruses, including humans, pinnipeds, and livestock such as pigs, which are well-established mixing pots for Influenza A virus evolution and reassortment.

In order to assess the spillover risk potential of BIV from *D. rotundus*, we must first understand the ecology and epidemiology of the virus within wild bats. However, extremely low virus detection rates and temporally limited datasets have confounded such studies of within-reservoir transmission. Here, we used both population-level and individual recapture-level longitudinal serology data collected from common vampire bats between 2007-2018, to fit a compartmental model of BIV transmission. We estimated the impacts of seasonality and of large-scale bat culls carried out for rabies control, on the long-term transmission dynamics of BIV in *D. rotundus* and compared the synchronicity in transmission between three environmentally and spatially distinct meta-regions of Peru.

We show that long-term transmission in vampire bats is driven by a combination of immune waning in adults, and seasonal cycles of infection, possibly caused by the births of naïve juvenile bats. The model supported both these annual cycles and longer multi-year cycles in seroprevalence, where the long-term periodicity was largely synchronised across Peru. In comparison, sharp annual peaks in infection were temporally distinct between the meta-regions. The model predicted that these dynamics produce short lived annual peaks in the number of infected bats of up to approximately 10% of the population compared to a baseline infection rate of less than 1%, with these peaks representing high potential spillover risk. Finally, we show that historical bat culling for rabies control had a significant effect of dampening transmission on the unintended target of BIV, and we used the BIV model to predict the previously unknown extent of culling-induced mortality and impact on the total bat population.

09:35 - 09:50

Rustrela virus (RusV): a newly discovered virus causing fatal encephalitis in domestic and wild mammals

Dennis Rubbenstroth[1], Karen Fox[2], Christoph Langner[3], Antonia Klein[1], Angele Breithaupt[1], Rainer Ulrich[1], Martin Beer[1], Florian Pfaff[1]
[1]Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany. [2]Colorado Parks and Wildlife, Fort Collins, USA. [3]Zoo Stralsund, Stralsund, Germany

Abstract

Rustrela virus (RusV; species *Rubivirus strelense*) is a recently discovered relative of the human rubella virus and causes usually fatal non-suppurative meningoencephalomyelitis in a broad range of mammals, including felids, canids, mustelids, rodents and even marsupials. The virus was first identified in zoo animals from Stralsund, Germany, in 2019. Meanwhile, it has been found also in domestic, wild and zoo animals in Germany, Austria, Sweden and the USA. In domestic cats, RusV was demonstrated to be the causative agent of 'staggering disease', a neuronal disorder that had remained of unknown aetiology for almost five decades. The clinical course is characterized by a broad range of neurological signs, with hind leg ataxia being the most prominent. Based on its broad range of susceptible hosts, a zoonotic potential of RusV cannot be excluded.

While encephalitic individuals appear to act as dead ends and do not spread the virus after spill-over transmission, apparently healthy yellow-necked field mice (*Apodemus flavicollis*) and wood mice (*Apodemus sylvaticus*) were identified as potential wild reservoir hosts of RusV. Experimental studies have confirmed the susceptibility of wood mice to RusV infection via intracerebral and oculonasal inoculation, but not via subcutaneous and intramuscular route and demonstrated shedding of viral RNA. The phylogeographic pattern of RusV sequences, with different sequence clusters occurring in separated, non-overlapping parts of the known dispersal areas, further suggests the virus to be bound to a rather non-mobile reservoir host, such as small mammals. However, many questions regarding the biology and epidemiology of RusV in reservoir and spill-over hosts remain elusive, such as course of infection, pathogenesis, and transmission routes. This presentation will summarize the current knowledge on the genetic diversity and distribution of this newly discovered virus, with a focus on the role of the wild natural reservoir. Furthermore, cases of fatal encephalitis in wild animals will be presented, such as a Eurasian otter (*Lutra lutra*) from the city of Stralsund, Germany, and a mountain lion (*Puma concolor*) from Colorado, USA.

09:50 - 10:05

Episystem characteristics drive the maintenance of animal tuberculosis at the wildlife-livestock interface

Alberto Perelló[1], José Sánchez Cesteros[1], Patricia Barroso[2], Nuno Santos[3], Francisco Ruiz-Fons[1], Ana Balseiro[2], Christian Gortázar[1], & the Underworld Team[1]
[1]SaBio IREC Universidad de Castilla La Mancha & CSIC, Ciudad Real, Spain. [2]Department of Animal Health, Faculty of Veterinary Medicine, University of León, León, Spain. [3]CIBIO, Centro de Investigaç˜ao em Biodiversidade e Recursos Gen´eticos, InBIO Laborat´orio Associado, Universidade do Porto, Vairao, Portugal

Abstract

In disease ecology, the reservoir concept has evolved from the "maintenance host – target host" binomial to a richer view which includes the target host among a community of species (the maintenance community) and their environment. Understanding the complex network of interactions among pathogens, hosts and the environment that define these epysistems might help to understand pathogen maintenance and identify suitable interventions. Animal tuberculosis (TB) is caused by *Mycobacterium bovis* and closely related members of the *M. tuberculosis* complex (MTC), a re-emerging and globally relevant multi-host infection shared between wild and domestic mammals. Little is known about the episystems that promote the maintenance of TB-causing bacteria. Thus, we investigated if the variability in maintenance communities and their environment explains differences in TB prevalence.

We studied 18 episystems of the Iberian Peninsula considering all domestic and wild mammals for which there is sufficient evidence of their participation in MTC maintenance, 11 species in total. We deployed camera trap grids to generate data on mammal community characteristics and relative abundance. Cross-species spatial coincidences (two species detected by the same camera in a given time window) were studied using social network analysis and a coincidence index matrix was calculated. We mapped water availability and used CORINE for habitat classification. Data on cattle TB were provided by the Portuguese and Spanish ministries of agriculture and 1080 hunter-harvested wild boar were tested for serum antibodies by P22 ELISA.

Cattle herd prevalence ranged from 0 to 15% and wild boar serum antibody prevalence from 0 to 81%. Host communities with a high TB prevalence had a dominance of wild boar and red deer, with or without livestock presence, and strong wild boar-red deer coincidence. Neither MTC host species richness (range 3 to 8), nor the centrality of a species in the network, explained TB prevalence. Aridity was not the main predictor of high TB prevalence. When wild boar and red deer were dominant, high TB prevalence occurred even in episystems with good water availability.

Host community characteristics, including species dominance and connectivity, were more important than habitat characteristics and host diversity in explaining MTC maintenance. Wild ungulates, rather than livestock and wild carnivores, act as key drivers of TB in our study region. This allows us to infer management implications and represents a showcase of how species interactions can influence ecosystem functioning.

10:05 - 10:20

Camera traps reveal a high prevalence of sarcoptic mange in red foxes from northern Spain

Patricia Barroso Dr[1], Pablo Palencia[2]
[1]University of Leon, Department of Animal Health, León, Spain. [2]1- Biodiversity Research Institute (University of Oviedo – CSIC – Principado de Asturias), Mieres, Spain

Abstract

The mite *Sarcoptes scabiei* affects numerous mammal species causing the sarcoptic mange, a widespread disease with relevance for wildlife conservation, welfare, and management. The red fox (*Vulpes vulpes*) could become infested by direct and indirect routes leading to external skin lesions potentially recognizable by devices such as camera traps (CTs). In the present study, 86 randomly placed CTs were used to investigate the apparent prevalence and severity of *S. scabiei* in a red fox population from northern Spain. Their potential environmental and population-related drivers were also assessed. A total of 341 independent encounters were examined to visually identify mange-compatible lesions. The apparent prevalence was 19.16% (confidence interval (CI) 95%: 15.08-23.80) of which 82.81% (CI95%: 71.33-91.10) were severe. Our results revealed that habitat attributes such as lower altitudes, higher coverage of water-linked habitats and woodland predominance, were significant predictors of the apparent risk of mange. The models also suggested that the apparent prevalence of mange was associated with poor body condition and elevated frequencies of spatial coincidence among fox encounters, which facilitates indirect transmission. Interestingly, we did not observe mange-compatible lesions in other sympatric wild species (more than 15,000 encounters examined). This could be explained by the mite's host specificity and the low probability that these other potential hosts use sites where transmission among foxes usually occurs, such as dens. This study illustrates how camera trapping can be used as an interesting tool for the surveillance of wildlife diseases, thus overcoming the logistic constraints derived from direct sampling and allowing the early detection and better management of pathogens in the riskiest areas.

10:20 - 10:35

Investigating the prevalence and impact of ophidiomycosis in barred grass snake (*Natrix helvetica*) in Great Britain

Steven Allain[1], David Leech[2], Robert Robinson[2], Kevin Hopkins[3], Katharina Seilern-Moy[3], Julia Rodriguez-Ramos Fernandez[4], Richard Griffiths[1], Becki Lawson[3]
[1]Durrell Institute of Ecology and Conservation, School of Anthropology and Conservation, University of Kent, Canterbury, United Kingdom. [2]British Trust for Ornithology, Thetford, United Kingdom. [3]Institute of Zoology, Zoological Society of London, London, United Kingdom. [4]IDEXX Laboratories Limited., Wetherby, United Kingdom

Abstract

Ophidiomycosis is an emerging disease first detected in wild snakes in North America, where it is known to affect a wide range of snake species. The disease, which typically manifests with skin lesions of variable appearance and severity, has more recently been diagnosed in free-living snakes in Europe and Asia. The *Ophidiomyces ophidiicola* (*Oo*) strains found in Great Britain are genetically and

phenotypically distinct from those in North America. Recent whole genome analyses indicate multiple introductions of the fungus into North America, however, whether the pathogen is native or introduced to GB, and its impact on wild snake health in Europe remains undetermined. The aim of this study was to investigate the prevalence and characteristics of skin lesions associated with ophidiomycosis in a study population of barred grass snake (*Natrix helvetica*), and to evaluate any disease impact on survival. Regular field surveys were conducted since 2015 at a wetland site in East England, using artificial cover objects. Snakes were caught by hand over three consecutive field seasons, May 2019-October 2021, and examined for skin lesions which, if present, were swabbed for *Oo* qPCR testing. Biometric data and ventral neck scale pattern photographs were taken to enable individual snake identification, allowing for capture-mark-recapture analyses to estimate survival. In total, 25.5% of individual snakes (191/750) had skin lesions detected, predominantly located on the ventrum, and most commonly characterised as changes in scale colour, crusting and scale margin erosion. The modal score category of skin lesion severity was 'mild' with no indication of generalised ill health observed in affected snakes. The majority of snakes with skin lesion tested qPCR positive, and macroscopic skin lesion appearance was consistent with findings from post-mortem examination of *N. helvetica* from the same site, with diagnosis of ophidiomycosis confirmed through qPCR, mycology and histology. Evidence of trauma was significantly more common in snakes with skin lesions than without, and there was a significant negative association between skin lesions and apparent snake survival. Collectively, our findings suggest an indirect mechanism may be involved in adverse impacts of ophidiomycosis on snake survival. We hypothesise that this may relate to a greater risk of predation due to increased basking behaviour in open areas in response to the disease. The extent to which these findings mirror those for other *Natrix* spp., and the importance of ophidiomycosis as a threat to wild snake conservation in Europe, requires further investigation.

Short Coffee Break

10:35 - 10:50 Thursday, 12th September, 2024
Brewery, Stralsund

Session IV – Student Session

10:50 - 12:20 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Anna Langguth, Julian Dewe

10:50 - 11:05

Serosurveys of wild mammals: an overview of the literature and an Influenza A virus case study

Paule-Émilie Ruy PhD student, Dr Ian O'Connor, Dr Katie O'Dwyer
Marine and Freshwater Research Centre, Galway, Ireland

Abstract

Carcasses of wild mammals can provide important samples for seroinvestigations. Indeed, antibodies can persist in the blood and tissues of animals up to a few days after death and can provide information on exposure to various pathogens. There exist reports of the use of this valuable method to detect antibodies against different microorganisms, including several with zoonotic implications, such as *Toxoplasma gondii*, *Trichinella spp.*, *Brucella spp., and Mycobacterium tuberculosis* among many others. Albeit screening samples from decaying wildlife for antibodies using ELISA is not a new method, to our knowledge, there exists no overview of the literature regarding the wild mammals and pathogens surveyed nor providing a clear outline of best practices to adopt when choosing this technique. Here, we present results from a systematic review of 101 publications representing 274 individual projects to highlight the use of enzyme-linked immunosorbent assay (ELISA) for detecting antibodies in carcasses from wild mammalian groups and the associated benefits and limitations. Our results indicate that artiodactyl ungulates account for 72.2% of the wild mammals examined and that antibodies against *Toxoplasma gondii* are the most commonly targeted. Blood samples were used in 90.5% of the individual projects we reviewed, and, notably, only 15% of those acknowledged the effects that haemolysed serum samples could have on the ELISA results. In addition, 85.4% of projects did not provide details about their sampling size decisions, and only half examined the distribution of seroprevalence in different age classes. Nevertheless, this information is important to allow replicability and accurate interpretation of results. We also report results of a serosurvey targeting Influenza A virus antibodies in red foxes (*Vulpes vulpes*) and American mink (*Neovison vison)* in the Republic of Ireland. For each individual, blood samples and blood on Nobuto filter papers were collected. Using ELISA, positive blood samples were compared with eluate obtained from the Nobuto filter papers to assess the efficiency of this media for seroinvestigation. Wild mammals are reservoirs for diverse pathogens including those with zoonotic potential, and investigating carcasses of wild mammals provides a useful, non-destructive method to better understand the epidemiology of these pathogens and possible interactions between wildlife, livestock, and humans.

11:05 - 11:20

CCHFV serology – lessons learned from a study in wild and domestic swine populations

Caroline Bost[1], Mario Frias[2], Sabrina Castro-Scholten[2], Balal Sadeghi[1], Saúl Jiménez-Ruiz[2], David Cano-Terriza[2], María A. Risalde[2], Javier Caballero-Gómez[2], Pelayo Acevedo[3], Martin H. Groschup[1], Ignacio García-Bocanegra[2], Kerstin Fischer[1]
[1]Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany. [2]Animal Health and Zoonoses Research Group, Competitive Research Unit on Zoonoses and Emerging Diseases (GISAZ-ENZOEM; UCO), University of Cordoba, Cordoba, Spain. [3]Research Group in Health and Biotechnology, Institute for Game and Wildlife Research (SABIO-IREC; UCLM-CSIC-JCCM), University of Castilla-La Mancha and CSIC, Ciudad Real, Spain

Abstract

Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne zoonotic orthonairovirus of public health concern and widespread geographic distribution. In addition to the tick reservoir, wild and domestic animals play a central role in the ecology of this pathogen, either by providing blood meal to questing ticks and/or as virus amplifying host. Interestingly, several animal species seroconvert after CCHFV infection but without showing clinical symptoms. Consequently, they can be used as sentinel for surveillance studies to indicate silent CCHFV circulation among ecosystems, as previously shown in Spain, where serological evidence for the circulation of CCHFV in wildlife was found several years before the first human cases were described.

The commercial availability of a multi-species ELISA has led to an increase in recent CCHFV sero-surveillance studies as well as in the range of species reported to be exposed to the virus in the field, including wild boar. However, the development and validation of confirmatory serological tests for swine based on different CCHFV antigens or test principles are complicated by the lack of defined control sera from infected and non-infected animals. To establish confirmatory tests for the detection of anti-CCHFV antibodies in swine, we used a panel of swine sera from CCHFV-free regions and regions with reported CCHFV circulation and established a swine-specific in-house ELISA. We initially screened more than 700 serum samples from swine and observed a good correlation between the commercial and the in-house test. From these sera, we selected a panel of 60 samples that were further analysed in a newly established indirect immunofluorescence assay (iIFA) and virus neutralization test. ELISA-non-reactive

samples were confirmed as negative. Interestingly, only a subset of samples reactive in both ELISA tests and iIFA displayed CCHFV-neutralizing antibodies.
Overall, our cross-sectional study conducted in wild boar and extensively reared pig populations in CCHFV hotspots in Spain indicates high CCHFV exposure in wild boar populations in endemic areas. Furthermore, it confirms the susceptibility of extensively reared pigs to CCHFV, even though they may only play a limited role in the enzootic cycle. Moreover, we discuss that meaningful CCHFV serology should involve performing multiple tests and careful interpretation of the results obtained.

11:20 - 11:35

To catch a fish: Harnessing Citizen Science and Computer Vision for Salmonidae Disease Surveillance

Ms Agnethe Seim Olsen, Professor Joanne Cable, Dr Sarah E. Perkins
Cardiff University, Cardiff, United Kingdom

Abstract

Traditional methods of wildlife disease surveillance in fish populations can be time-consuming, expensive, and often inadequate in terms of local and global coverage. Here, we explore the potential of computer vision, a form of Artificial Intelligence (AI), to detect and classify diseases in fish based on visible symptoms in still images. This approach has the potential to provide rapid, automatic disease detection at a large scale and with minimal negative impact on the welfare of the animals involved. The focus of this project lies specifically on diseases of fish from the Family *Salmonidae*, particularly the impact of the pathogen *Saprolegnia*, which is responsible for a significant mortality of salmon populations. Given that outbreaks of fish diseases often go undetected, it could be crucial to develop efficient surveillance methods that leverage visible symptoms present in fish. Moreover, angling communities, who actively capture and share images of their catches, present a unique opportunity to gather extensive image datasets and associated metadata such as spatiotemporal information.
Our study objectives are threefold. First, we established a citizen science pilot project, PathoPixel (https://www.pathopixel.co.uk/), engaging with the angling community to expand the pool of Salmonidae images with spatiotemporal metadata available to us. Secondly, we evaluated the availability of online image sources (passive citizen science) and assessed the quantity and quality of images found online, aiming to understand what constitutes high-quality imagery for accurate disease detection. Third, we trained a computer vision model to detect and classify visible signs of disease. Applying scientific and common names of

the *Salmonidae* family as search terms, we acquired fish images and associated metadata from platforms such as Flickr, iNaturalist, WikiMedia, and GBIF. The image datasets were cleaned and labelled based on species identification as well as signs of disease visible on each fish. Using these curated image datasets, we trained a computer vision model to classify fish images based on the presence of visible disease symptoms.
Preliminary results indicate an overall classification accuracy of 92% on the validation dataset, showcasing the effectiveness of computer vision in detecting and classifying fish diseases from images submitted by both citizen scientists and those harvested from the internet. Our proof-of-concept highlights that animal diseases can be detected using AI and shows great promise for the future of infectious disease surveillance.

11:35 - 11:50

One Health Farming: noninvasive monitoring reveals links between farm biodiversity and pathogen markers in outdoor hoofstock

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Abstract

Outdoor farming contributes to biodiversity conservation but raises biosafety concerns. We hypothesized that vertebrate species richness and pathogen marker presence would vary between farmed species and explored the links between farm biodiversity and pathogen detection using logistic regression models. We sampled 15 open-air, grazing-based hoofstock farms (6 cattle, 5 small ruminants, 4 pig), deploying camera traps (CTs) at risk and random points to measure vertebrate richness and conservation value. We utilized sponge-based sampling for environmental nucleic acid detection (ENAD) targeting six bacterial pathogen genetic markers (IS*6110*, IS*900*, IS*1111*, IS*711*, *uidA*, *invA*) and three virulence genetic markers (*stx*1, *stx*2, and *eae* genes). Wild vertebrate richness ranged from 16 to 37 (median 20). The highest mean conservation value was found in pig farms

(7.9, Interquartile range (IQR) = 6.1 – 10), followed by small ruminant farms (3.7, IQR = -0.9 – 3.2) and cattle farms (1.6, IQR = 2.5 – 5). Waterholes and other waterbodies contributed significantly to this biodiversity with 48% of all wild vertebrates detected. All six pathogen markers were detected in small ruminant farms, while only three (IS*6110*, *invA*, *uidA*) were also detected in cattle and pig farms. Marker richness varied between farms (range 2 - 8, median 6), being higher in small ruminant farms (range 6 – 8, median 7) than in cattle farms (range 4 – 7, median 6) or pig farms (range 2 – 5, median 4). Wild vertebrate richness and pathogen marker richness were negatively correlated. Risk points with a higher probability of detecting more pathogen markers had a lower vertebrate richness. While CT-based vertebrate richness and ENAD-based pathogen marker detection are just indicators of true biodiversity and farm health, respectively, we found that farmland vertebrate communities provide ecosystem services and might contribute to limit the circulation of multi-host pathogens.

11:50 - 12:05

Leprosy surveillance of unhabituated western chimpanzees in central Cantanhez National Park, Guinea-Bissau

Marina Ramon[1,2], Harriet Herridge[1,3,4], Livia Patrono[3,4], Elena Bersacola[1], Américo Sanhá[5], Maimuna Jaló[5], Ivo Colmonero-Costeira[2,6,7,8], Isa Aleixo-Pais[2,9], Alexandra Dell[1,4], Michael Brufford[2,10], Sébastien Calvignac-Spencer[3,4], Camille Bonneaud[1], Fabian Leendertz[3,4], Kimberley Hockings[1]

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Abstract

For the first time in any wild primate, we recently identified leprosy, a chronic infectious disease mainly caused by the bacterial pathogen *Mycobacterium* *leprae,* in the critically endangered western chimpanzee (*Pan troglodytes verus*) in Cantanhez National Park (CNP), Guinea-Bissau. To estimate leprosy prevalence in two neighbouring chimpanzee social groups, Caiquene-Cadique and Lautchande, we non-invasively collected a total of 451 chimpanzee faecal samples between May and July 2021. Firstly, we screened each sample for *M. leprae* DNA using two complementary nested PCR systems targeting the repetitive element RLEP and the 18-kDa antigen gene. 27 samples (19 in Caiquene-Cadique and 8 in Lautchande) were positive in one or more assays. Secondly, chimpanzee DNA was amplified at 12 autosomal microsatellite loci and one sexing marker to infer the number and sex of the individuals. 424 samples were successfully genotyped and from these, 100 unique genotypes were identified (47 in Caiquene-Cadique and 53 in Lautchande). When combining the results, 16 individuals (12 in Caiquene-Cadique and 4 in Lautchande) tested positive in one or more samples, with an overall prevalence of *M. leprae* of 16%. To support these findings, molecular data were compared with camera trap footage of individually identified chimpanzees obtained during the same study period. Only two adult females in Caiquene-Cadique, and one adult male and one adult female in Lautchande showed clinical signs of leprosy. We discuss the usefulness and the limitations of combining non-invasive methods to study infectious diseases in unhabituated great apes. This study provides valuable information on leprosy occurrence and suggests an active transmission of *M. leprae* between individuals in CNP.

12:05 - 12:20

Genetic diversity of the Prion Protein Gene in European wild cervids

Sonja Ernst[1], Katayoun Moazami-Goudarzi[2], Agata Piestrzynska-Kajtoch[3], Dolores Gavier-Widén[4], Johann Laubier[2], Fiona Houston[5], Jaana Kekkonen[6], Erik Ågren[4], Gustav Averhed[4], Aurélie Barboiron[7], Vincent Bourret[8], Jörn Gethmann[9], Martin H. Groschup[1], Miroslaw P. Polak[10], Maryline Pellerin[7], Anna Radko[3], Christine Saint-Andrieux[7], Agnieszka Szumiec[3], Michael A. Tranulis[11], Anne Van De Wiele[7], Christine Fast[1]

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Abstract

Susceptibility to transmissible spongiform encephalopathies, including Chronic Wasting Disease (CWD) in cervids, depends on the structure of the cellular prion-protein, encoded in the Prion-Protein Gene (*PRNP*), as *PRNP*-variations influence disease-susceptibility. Since the CWD-emergence in Northern Europen countries in 2016 in novel contagious and 'atypical' forms, its spread within Europe must be considered. We therefore aim to compare *PRNP*-genotypes in cervids to estimate CWD's impact on population dynamics.

Results of five species from seven states were geographically and statistically analysed. Data of red deer (*Cervus elaphus*) from France (n=998), Germany (n=527), UK (n=480), Poland (n=218) and Denmark (n=26), roe deer (*Capreolus capreolus*) from France (n=1116), Germany (n=311), UK (n=297) and Sweden (n=78) as well as 39 German sika deer (*Cervus nippon*), 39 Finnish White-tailed deer (WTD) (*Odocoileus virginianus*) and moose (*Alces alces*) from Poland (n=81) and Sweden (n=151) were compared.

Generally, alleles are designated by a combination of codon and a one-letter amino acid (aa) abbreviation. For haplotypes which involve more codons, all positions and aa are named.

Red deer revealed the broadest *PRNP*-variability: a novel 24 bp deletion and eight polymorphisms (PM) were detected, whereof PMs at codons 21, 78, 136, 185 were synonymous. PMs at codons 98, 168, 226 and 247 lead to aa substitutions resulting in nine haplotypes namely wildtype (wt $T_{98}P_{168}Q_{226}I_{247}$), E_{226}, A_{98}, $A_{98}E_{226}$, $A_{98}L_{247}$, S_{168}, Δ_{69-77}, $\Delta_{69-77}A_{98}$, $\Delta_{69-77}E_{226}$ and 16 genotypes, respectively. Certain haplotypes such as A_{98}, $A_{98}L_{247}$, Δ_{69-77}, $\Delta_{69-77}A_{98}$, $\Delta_{69-77}E_{226}$ and S_{168} occurred geographically clustered, suggesting "*PRNP*-linages" in red deer. Investigation of German sika deer revealed haplotypes wt and E_{226}, and synonymous mutations (SM) at codon 133 (sika-specific) and 136. In Finnish WTD codons 95 and 96 showed variability, leading to haplotypes $Q_{95}G_{96}$, $H_{95}G_{96}$ and $Q_{95}S_{96}$. While Polish moose were monomorphic for wt, in Swedish moose haplotype K_{109} was identified. All European roe deer were homozygous for wt/wt, only SMs at codons 13 (France), 24 (Sweden) and 42 (Germany) were seen.

This is the first geographical and statistical analysis of European deer *PRNP*-variation. Based on data from North American CWD strains and the genotyping results of the Norwegian red deer cases, we would predict that large proportions of the European cervids might be susceptible to CWD. However, for a more detailed risk assessment, further testing of the described PMs is required to determine their impact on prion-protein structure, hence CWD-susceptibility.

Nevertheless, these results contribute to improve EU CWD-surveillance, risk assessment and control measures.

Lunch

12:20 - 13:20 Thursday, 12th September, 2024
Brewery, Stralsund

Young Scientist Keynote: What about One Health outcomes? Can wildlife health outcomes be achieved by bridging food systems thinking with the nexus of biodiversity and infectious diseases?

13:20 - 13:45 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Gebbiena M. Bron[1], PADDLE Consortium[2], Annabella Daburon[3], Marion Kluivers[4], Michelle Verant[5], William R. Easterday[1], Bjørnar Ytrehus[1], Arne Holst-Jensen[1], Joukje Siebenga[6], Katherine L. D. Richgels[7]
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Abstract

The new definition of One Health embraces it as an approach, an outcome, and a moral obligation to optimize the health of people, animals, and the environment. However, how can we navigate the complexities of these interconnected yet diverse systems? What are One Health outcomes, and how do we measure, implement, and optimize them? How can we be transparent about balancing objectives and values in One Health, which requires trade-offs? We explore two concepts to work towards realizing One Health outcomes: 1) Using food systems as a backbone for One Health, and 2) Expanding assessments of the relationships between biodiversity change and disease beyond human diseases.

The relationships observed between food, people, and infectious diseases depend on one's reference framework. Conceptual rethinking of linear food systems to circular bio-economies illustrates, from a veterinarian's perspective, that parasite cycles will be closed. Adding nature and wildlife to this conceptual model

provides a framework in which the interconnected internal and external health threats to people, animals, and the environment can be considered.

Incorporating principles of community ecology as applied to disease systems (e.g., host competence, dilution effect) allows for descriptions of ecosystems, including food production components. This requires the expansion of traditional host-pathogen systems to more complex multi-host and multi-parasite systems. Utilizing these systems to assess One Health outcomes challenges paradigms about biodiversity and disease, expanding them beyond human health.

In exploring these ideas, a challenge emerges when outcomes need to be quantifiable: Human health is expressed in disability-adjusted life years; crop and animal health in production losses, whereas wildlife health may only be captured in a biodiversity index. However, using a food system perspective could highlight the importance of wildlife for food and income, and multi-host and multi-pathogen frameworks could clarify how food production choices may affect wildlife health.

So, what about One Health outcomes? Would embracing a One Health approach with transparent outcomes still lead to an ego-centric versus an eco-centric agenda? How do we shape our moral obligation to optimize the health of people, animals, and the environment? Can entities benefiting from production animal health, like veterinary institutes, take on the moral obligation to invest in all non-human animal health? At a minimum, the new definition of One Health mandates that every project exploring the risks of pathogen spillover from wildlife to domestic animals must also assess the consequences of these food production systems for wildlife health.

Session IV – Student Session (cont.)

13:45 - 15:45 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Bieneke Bron, Damien Higgins (tbc)

13:45 - 14:00

The re-emergence and local persistence of lymphocytic choriomeningitis virus in Germany

Calvin Mehl[1,2], Claudia Wylezich[1], Olayide Abraham Adeyemi[1], Fiona Joan Möhrer[1], Romy Kerber[3], Lisa Oestereich[3,2], Christina Geiger[4], Nicole Schauerte[4], Kerstin Mätz-Rensing[5], Anne Nesseler[6], Katja Schmidt[7], Dirk Höper[1], Miriam Linnenbrink[8], Martin Beer[1], Gerald Heckel[9], Rainer G. Ulrich[1,2]

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Abstract

Lymphocytic choriomeningitis virus (LCMV; species *Mammarenavirus choriomeningitidis*) is a globally distributed zoonotic pathogen transmitted by house mice (*Mus musculus*). In humans, LCMV can cause meningitis and encephalitis in adults and prenatal infections can lead to severe birth defects or miscarriage. In New World primates, LCMV infection causes callitrichid hepatitis.

In this study, we investigate a recent outbreak of LCMV in a zoo in western Germany and the ongoing localized presence. We examined the prevalence and genetic diversity of LCMV in wild rodents and captive monkeys from this zoo through screening by reverse transcription-polymerase chain reaction, high-throughput sequencing and serological investigation. Animals from 2009, 2014 and 2021-2023 were screened and positive samples were analysed with high-throughput sequencing to obtain full genome sequences of LCMV for the reconstruction of phylogenetic relationships.

LCMV was detected continuously in about half of all 280 tested wild mice from the zoo during 2021-2023, but not in any animals from 2009. Two different LCMV lineages (I and II) were identified in mice from 2021-2023, though only lineage II was found in captive monkeys. Serological screening revealed LCMV-reactive antibodies in 5% of tested mice. Full LCMV genome sequences reveal near identical sequences between wild mice and captive monkeys, indicating transmission between these animals. The localized persistence in wild rodent populations may have significant implications for the health of local wildlife and inhabitants. The presence of two different lineages of LCMV in the same host population may allow for reassortment and evolution of novel strains.

14:00 - 14:15

Effects of urbanization on serum proteome and pathogen prevalence in the house sparrow (*Passer domesticus*).

Sara Minayo Martín MSc[1], Margarita Villar PhD[1,2], Alberto Sánchez- Cano MSc[1], Catarina Fontoura- Gonçalves MSc[1,3,4], Ursula Höfle PhD[1]
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Abstract

The house sparrow (*Passer domesticus*) is a ubiquitous species, distributed worldwide in rural, urban and humanized environments. Urbanization has negative effects on wild birds, being for example urban sparrows characteristically smaller and in poorer body condition than rural birds. In Europe, sparrow populations have suffered an alarming decline in recent decades, especially in urbanized areas and disease has been suspected as a cause. Urban sparrows are exposed to pollution and stress factors that could alter their health status, which could increase susceptibility to diseases. In the present study we analyzed the effects of urbanization on body condition, pathogen prevalence and changes in the serum proteome. For this, we captured sparrows in four locations with two different degrees of urbanization. Biometric data, blood samples and oral and cloacal swabs were collected. With serum samples we performed serology to detect avian influenza virus (AIV) and West Nile virus (WNV) antibodies, whilst the oral and cloacal swabs were used to detect AIV and WNV by PCR. For avian malaria detection we performed PCR from blood pellet. The serum was also used to make pools of animals from the same habitat and used for comparative serum proteomics.

Rural sparrows were in significantly better body condition than urban sparrows with no significant differences between sexes. A significantly higher prevalence of avian malaria parasites was found in rural sparrows, 70.16% (87/124), compared to urban sparrows 50.00% (27/54). AIV or WNV was not detected, although one urban sparrow (1/54, 0.58%) had antibodies to AIV. Serum proteomics revealed that in comparison to the normalized mean, rural sparrows had elevated values of certain proteins involved in the carbohydrate metabolism, in contrast to proteins involved in lipid metabolism, the immune system and the coagulation system, which were found to be elevated in urban sparrows. The results obtained confirm that urban sparrows have a lower body condition and an activation of the immune system, above the normalized values. Rural sparrows have a higher prevalence of avian malaria parasites. Furthermore, these results confirm that sparrows are at least occasionally exposed to AIVs and may be involved in virus transmission and

maintenance.

14:15 - 14:30

Vector-borne and zoonotic pathogens in Raccoon Dogs *(Nyctereutes procyonoides*) and Raccoons (*Procyon lotor*) from Schleswig-Holstein, Germany

Jana Christina Klink[1], Dr. Alexandra Rieger Dipl. ECVP, Certified Specialist in Veterinary Pathology[1], Dr. Peter Wohlsein Dipl. ECVP, Certified Specialist in Veterinary Pathology[2], Prof. Prof. h. c. Dr. Ursula Siebert Dipl. ECZM (WPH), Dipl. ECAAH, Certified Specialist in Wild Animals and Species Protection[1], Dr. Anna Obiegala Certified Specialist in Veterinary Epidemiology[3]
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Abstract

Raccoon dogs and raccoons are neozoa in Europe, originating from East Asia and North America, respectively. Both are discussed as predators and competitors of native fauna. Furthermore, they have the potential to act as additional reservoirs and vectors for infectious diseases, including rodent-borne and tick-borne pathogens. Therefore, they are a possible threat for human and domestic animal health and biodiversity and conservation.

An increasing incidence and diversity of vector-borne infections with one-health relevance have been observed in recent years. Leptospirosis, a re-emerging rodent-borne infection is the most common bacterial zoonosis worldwide, while lyme borreliosis is the most prevalent arthropod-borne disease in the northern hemisphere and the emerging and re-emerging spotted fever group rickettsioses are recognised as threats to public health worldwide.
As part of an explorative study on pathogens in raccoon dogs and raccoons originating from Schleswig-Holstein, Germany, 110 raccoon dogs and 30 raccoons were tested via qPCR for the presence of *Leptospira*, *Borreliella* and *Rickettsia* between 2021 and 2022. MLST was carried out on selected *Leptospira* and *Borreliella* positive samples. Warthin-starry silver staining was performed on histological kidney sections of *Leptospira*-positive animals.

Leptospira were found in 21 (19.44%) of the investigated raccoon dogs and in two (6.90%) raccoons. Sequencing allowed the identification of *Leptospira borgpetersenii*, *Leptospira kirschneri* and *Leptospira interrogans* in five raccoon dogs. Warthin-starry silver staining revealed spiral shaped bacteria corresponding to *Leptospira* within the renal tubular epithelium in one raccoon dog. In one raccoon dog (0.93%) and one raccoon (3.45%), the presence of *Borreliella* was confirmed, identified as *Borreliella afzelli* in the raccoon. *Rickettsia* were not detected.

In conclusion, *Leptospira* and *Borreliella* are present in raccoon dogs and raccoons from Schleswig-Holstein, Germany, showing a potential reservoir function for *Leptospira*. Further research, including pathogen detection in vector-ticks is necessary to evaluate their role as reservoirs for tick-borne infections. In general, the assessment of pathological findings in combination with pathogen detection is essential to rule out their reservoir and vector function. A long-term health monitoring, comprising infectious disease surveillance and an extension of the study area is required to assess their potential risk for human and animal health nationwide.

14:30 - 14:45

Investigation of skin mite species infestations of passerine birds in Hungary

Péter Takács[1], Dr. Károly Erdélyi PhD[2], Dr. Jenő Kontschán PhD[3], Dr. Enikő Horváth PhD[2], Dr. Tibor Hadarics PhD[4], Dr. József Gyurácz PhD[1], Dr. Péter Molnár PhD[1]
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Abstract

Parasitic mites *Blankaartia acuscutellaris* and *Harpirhynchus dusbabeki* are regularly found on passerine birds in Hungary. In 2006 a bird ringer was infested by *B. acuscutellaris* showing the symptoms of trombiculiasis. Our knowledge of these species' life cycle, host-range, and potential role in pathogen transmission is extremely limited. Addressing these knowledge gaps, we aimed to perform a survey of skin mite infestations among passerine species during regular ringing activities.

Our field survey and specimen collection efforts were complemented with data gathered from bird ringers of Birdlife Hungary. Meksziópuszta (Fertő-Hanság National Park) served as the sampling area. Using mist nets, we captured 648 birds belonging to 23 species during 2022. Birds were examined for the presence of

skin lesions harbouring mite development stages and parasites were collected for identification and testing for West Nile virus (WNV), Usutu virus (USUV), and Sindbis virus (SINV) by PCR. We have analyzed the seasonal dynamics of mite infections and determined their preference for host age groups.

This study found 12 bird species infected with *B. acuscutellaris, but* only the Bearded Reedling (*Panurus biarmicus*) was affected by *Harpirhynchus dusbabeki*. This passerine exhibited a significantly higher *Blankaartia*-infection rate (63%) than the other affected species. Moreover, differences in infection rates were observed among age groups of Bearded Reedlings, with first-year individuals being more affected than adults (85 and 57% respectively). The PCR assays aimed at the detection of WNV, USUV and SINV yielded negative results.

Our study recorded *Blankaartia* infection for the first time in 4 additional avian species confirming its wide host-range. The infection rates observed in different age groups of Bearded Reedlings are likely to be synchronized with the parasite's life cycle, and this is also supported by the evidence of higher parasite prevalences in June and August (82% and 86%). Only uninfected individuals were captured in October.

As a result of our research, we detected *H. dusbabeki* for the first time in Hungary and provided the first morphological description of its larvae.

Further research is needed to obtain further details on the potential role of these two mite species in pathogen transmission cycles and their impact on the fitness of affected hosts.

This study was supported by the ÚNKP-23-2 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

14:45 - 15:00

Investigation of Haemoparasite Infections at the Wildlife-Livestock Interface to Inform Management of Antelope Conservation Reintroductions in the Sahara-Sahel Region

Stephanie Brien[1], Katherine Mertes[2], Mahamat Fayiz Abakar[3], Bram van Bunnik[1], Tchari Doungous Doungous[3], Jennifer Yu[2], Ahmed Ouled Hatem[4], Mahamat Saboun[3], Erhan Yalcindag[1], Marie Petretto[5], Mahamt Oumar[6], Hassan Abdelkader[3], Melissa Marr[1], Mahamat Hassan Hatcha[7], Mark Bronsvoort[1], Rob Ogden[1]

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Abstract

Background: The reintroduction of antelope, including scimitar-horned oryx (*Oryx dammah*) and addax (*Addax nasomaculatus*), to the Sahara-Sahel region is playing an important role in restoring biodiversity and supporting sustainable ecosystems. However, infectious diseases, including haemoparasite infections, have led to mortality, which threatens the viability of small populations. Bidirectional infection transmission across the wildlife-livestock interface is a particular concern, with risks for human and animal health, conservation of biodiversity, and food security.

Objectives: (i) Investigate haemoparasite infection status of antelope in conservation reintroductions in the Sahara-Sahel region. (ii) Compare infection status of antelope and sympatric livestock in Chad and assess for evidence of inter-species transmission.

Methods: Baseline samples were collected from the source population of antelope (n=150) prior to reintroduction in Chad. Cross-sectional surveys were conducted in livestock (n=393), and reintroduced and wild-born antelope (n=72) in Chad. Opportunistic samples were also collected from semi-free-ranging antelope (n=18) and other wild ungulates (n=14) in Tunisia. DNA was extracted from blood and analysed by PCR of the 16S/18S rRNA genes. Illumina sequencing was used to identify haemoparasite infections, including anaplasma, ehrlichia, piroplasmids and trypanosomes. The amplicon sequence variants (ASVs) generated were compared by host species, location, stage of reintroduction and health parameters.

Results: Livestock were more likely than antelope to be infected with haemoparasites, showed a higher relative abundance of haemoparasites and were more likely to be infected with species known to be pathogenic. The ASVs showed substantial differences between host species, with variation between different livestock species, as well as between livestock and antelope. Some ASVs infected both livestock and antelope. The quality and quantity of DNA extracted varied by location, as did the degree of amplification of non-target bacterial sequences. This may reflect differences in individual health status but also probable sample deterioration and contamination.

Discussion: Some haemoparasites infected both antelope and livestock suggesting niche overlap. Livestock were more likely to be infected with haemoparasites than antelope, with substantially higher read abundance, likely reflecting greater intensity of infection. This suggests that livestock could act as a reservoir of infection for antelope. Different environmental conditions, particularly extreme climatic events, may alter the infection dynamics, so ongoing monitoring is advised. Sub-optimal sample collection and storage of some samples may have impacted the ability to detect target parasites. Whilst opportunistically collected samples are a useful resource in wildlife health research, consideration should be

given to whether collection and storage may affect validity.

15:00 - 15:15

Another one bites the seal: Prevalence, life history and health impact of the seal louse on seals

Insa Herzog PhD Student[1], Dr. med. vet. Peter Wohlsein[2], Prof. Prof. h.c. Dr. Ursula Siebert[1], Anika Preuss PhD Student[3], Prof. Dr. Stanislav N. Gorb[3], Dr. rer. nat. Kristina Lehnert[1]

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Abstract

Grey (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) are parasitized by a unique parasite assembly; the seal louse (*Echinophthirius horridus*) and heartworm (*Acanthocheilonema spirocauda*). *E. horridus* is a permanent, hematophagous ectoparasite, transmitted directly through physical contact between individuals. This marine insect shares a long evolutionary history with its hosts, and is hypothesized to transmit filarial stages of the heartworm during its bloodmeal. Although arthropod parasites are important as indicators for their hosts ecology, precise quantative information about prevalence, intensity and distribution patterns of seal lice is scarce. Growing seal populations in German waters enable rapid transmission of directly transmitted parasites on haul-out sites, shared by both seal species. Therefore parasite dynamics and health impact need to be closely monitored in this coastal ecosystem.

In the present study, a new, personnel intensive *post-mortem* sampling protocol was applied to sample seal lice. Seventy-two harbour seals and nine grey seals were examined from the North and Baltic Sea of the federal state of Schleswig-Holstein (SH), collected within the Stranding Network of SH from April 2022 - April 2023. Histological sectioning and staining of seal lice and seal lice caused lesions were investigated.

58% of harbour seals (n=71) and 70% of grey seals (n=10) in the North and Baltic Sea of Germany were infected with seal lice. A majority of harbour seals displayed a mild infection, while three were infected moderately and two severely. The head of the seal was the preferred predilection site, which indicates that seal lice may prefer body areas with frequent access to atmospheric oxygen for respiration. Different developmental stages and nits were recorded on juvenile and adult grey and harbour seals throughout the year. Severe seal louse infections were associated with alopecia and folliculitis; microbiological investigations isolated bacteria commonly associated with folliculitis. Additionally histology revealed larval nematode stages in the haemocoel, pharynx and intestine of *E. horridus*, supporting its role as vector for the heartworm.

Results of this study are stressing the importance of systematic *post-mortem* investigations and an efficient stranding network for monitoring parasite infections of marine apex predators. This study revealed seal louse prevalence in German waters to be significantly higher than previously recorded and highlights the seal louse as potential vector for other infectious pathogens. Such information is crucial to understand health risks posed to the seal populations and their surrounding ecosystem.

15:15 - 15:30

Tick-borne zoonoses: a One Health survey among humans, dogs, wildlife and environment.

Rachele Vada[1], Andrea Calcagno[2], Stefania Zanet[1], Maria Grazia Carpignano[3], Enrica Fantini[1], Francesco Benatti[1], Enrico Garro[2], Flavia Occhibove[1], Andrea Pepe[2], Anna Trisciuoglio[1], Amir Reza Varzandi[1], Ezio Ferroglio[1]

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Abstract

Tick-borne zoonoses (TBZ) are emerging pathogens that often circulate from wildlife reservoirs to humans and domestic animals. Shifts in climate patterns and changes in land use practices, which also influence the population dynamics of wildlife, have contributed to the increase of ticks' abundance and expanded their geographical distribution. The interface between wildlife, domestic animals and humans has widened, fostering the transmission of TBZ. Considering this intricate ecological interplay, it is of great importance to adopt a holistic approach for comprehensively examining the circulation of these diseases and implementing broad-ranging surveillance strategies for TBZ.

This study aims to carry out, in a rural area, an extensive TBZ survey involving different target hosts (environmental ticks, wildlife, domestic animals and humans), to characterize pathogens' prevalence and gather insights on their circulation in the different groups.

We addressed a hunting district from an alpine valley. 124 pools of questing ticks were collected with dragging transects from 38 points across the valley. 133

spleen samples from culled wildlife (wild boar, red deer, chamois) were collected from the game control centre. Finally, blood samples were taken from 67 volunteer hunters associated with the district and their families, as well as from 38 of their dogs. All samples were tested by end-point PCR for *Babesia divergens/B. capreoli*, *Babesia microti-like*, *Borrelia burgdorferi sensu latu*, *Rickettsia Spotted Fever Group*, *Anaplasma phagocytophilum*. An additional questionnaire was submitted to the volunteers to explore outdoor habits which may influence exposure to tick bites.

The most prevalent pathogen in all groups was *Babesia* spp, reaching up to 45% (c.i. 37-54%) in wildlife. In wildlife and environmental ticks, *A. phagocytophilum* reached a lower prevalence (1%, c.i. 0.8-2%) than in humans and dogs (up to 11%, c.i. 3-18%). *B. burgdorferi* and *Rickcettsia* SFG prevalences were consistent between humans and questing ticks (2%, c.i. 0-4%), but much higher in dogs (up to 16%, c.i. 4-27%). Answers from the survey suggested that awareness has risen in people spending long hours in the natural environment.

Our results remark on the close link between different tick hosts for the circulation of tick-borne zoonoses and the importance of broadening the view when it comes to disease surveillance, potentially also expanding to other important reservoirs such as birds or micromammals. The positive attitude of the hunting sector to participate in the study highlights its potential for data collection.

15:30 - 15:45

Limited occurrence of *Borrelia* spp. in European hedgehog (*Erinaceus europaeus*) and *Ixodes hexagonus* in Great Britain

Marco Vecchiato[1,2], Becki Lawson[1], Katharina Seilern-Moy[1], Mia White[3], Nicola Jones[3], Faye Brown[3], Dylan Yaffy[2], Jolyon Medlock[3], Kayleigh Hansford[3]
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Abstract

Borrelia spp. are multi-host bacteria and the causative agents of the zoonotic disease, Lyme borreliosis, for which *Ixodes* spp. are the vectors. In Great Britain (GB), research to date has primarily focussed on *I. ricinus* as the main tick transmitting this pathogen, while the role that the European hedgehog (*Erinaceus europaeus*) and the hedgehog tick, *I. hexagonus*, might have in the transmission cycle requires investigation. This study aimed to examine the occurrence of *Borrelia* spp. infection in hedgehogs and *I. hexagonus* in GB; to characterise the genospecies if present, and to better inform our understanding of these species as

potential hosts or vectors. Post-mortem examinations have been conducted on hedgehogs found dead from across GB over the period 2013-2022 inclusive, as part of the Garden Wildlife Health project (www.gardenwildlifehealth.org). We collated the available convenience sample archive from 96 hedgehogs from which both frozen ear tissue and *Ixodes* spp. ticks (comprising 563 *I. hexagonus*, 18 *I. ricinus* and one *I. frontalis*), stored in 70% ethanol, were available. Supplementary tissue samples were analysed from the hedgehogs where either ear tissue or ticks tested Borrelia DNA-positive, to investigate whether the infection was localised or disseminated. An additional 86 *I. hexagonus* collected from 14 hedgehogs from which ear tissue was not available were included to increase the sample size. DNA was extracted from these tissues and tick samples, and tested using a pan-*Borrelia* qPCR assay. Only 4.2% (4/96) of hedgehogs and 1.2% (4/335 total: 0.6%, 2/329 *I. hexagonus*; 40%, 2/5 *I. ricinus*) of tick pools were qPCR-positive, suggesting that *Borrelia* spp. infrequently circulate in hedgehog and *I. hexagonus* in GB and, therefore, both species may have a limited role in wider transmission cycles here. *B. afzelii* was the sole genospecies characterised by subsequent sequence analysis, providing some evidence of host-vector interaction at larval and nymph life stages, as all the positive ticks were collected from similarly *Borrelia* DNA-positive hedgehogs. Histopathological examination found no evidence of borreliosis suggesting no clinical significance of *B. afzelii* infection to hedgehog health. The low occurrence of *B. afzelli* detected in *I. hexagonus* in GB indicates that the public health risk of infection from bites from this tick species is lower than for *I. ricinus*. Notably, our dataset found minimal co-feeding of *I. hexagonus* and *I. ricinus* on hedgehog hosts in contrast to studies in mainland Europe, which could influence pathogen dynamics.

Short Coffee Break

15:45 - 16:00 Thursday, 12th September, 2024
Brewery, Stralsund

Session V.I – Sponsor Session: GIZ "Reducing Health Risks in Wildlife Trade: A Multi-Stakeholder - One Health Approach to Primary Pandemic Prevention"

16:00 - 18:00 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Kim Grützmacher, Michael Nagel

16:00 - 16:10: Introduction
16:10 - 17:36: Presentations

17:36 - 17:55: Open Q&A and Discussion
17:55 - 18:00: Wrap-up

16:10 - 16:20

Operating in the fields of environmental policy, biodiversity and forests at GIZ, a service provider for international cooperation

Claudia Mayer[1], Doerte Segebart[2]
[1]Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, Division for Environmental Policy, Biodiversity and Forests, Bonn, Germany. [2]Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, Bonn, Germany

Abstract

GIZ is a service provider for international cooperation and works to shape a future worth living around the world. We work with businesses, civil society actors and research institutions, fostering successful interaction between development policy and other policy fields and areas of activity. Under the overarching themes of climate change, environment and infrastructure, our department operates in the fields of environmental policy, biodiversity, forests and the integrated protection of the ozone layer and climate. Striving to safeguard our livelihoods we support our partners in managing natural resources, preserving ecosystems, building a circular economy and an inclusive green economy. Together with our commissioning parties and local partners our department carries out more than 25 customised projects and extensive programmes worldwide. One of these global programmes strives to reduce health risks in wildlife trade as a measure of primary pandemic prevention.

Wildlife trade, consumption of wildlife products and biodiversity loss due to human activities are major risk factors for emerging infectious diseases that mostly originate in non-human animals, primarily wildlife. Spillover risks arise along the human-wildlife interface, driven by ecological disruptions like land use shifts and unsustainable consumption – while growing population densities and mobility exacerbate disease spread. Yet, human behaviour offers critical control points to mitigate these risks, prevent spillover of emerging pathogens with pandemic potential and safeguard wildlife for the future. To date, there has been little international exchange and coordination to reduce health risks in the wildlife trade. In 2021, the German Federal Ministries BMZ and BMUV initiated the 'International Alliance against Health Risks in Wildlife Trade' as an international multistakeholder platform to address health risks through primary prevention and a one health approach.

The International Alliance focuses on providing and communicating evidence, supporting interventions, connecting its members and catalysing actions to reduce the risks of future disease outbreaks, epidemics, and pandemics deriving from human-wildlife contact along the wildlife trade chain. Operating through a One Health approach, the Alliance aims to concurrently enhance health, equity, and well-being for all species and the environment. By co-creating and infusing collective knowledge, the Alliance strives to bridge the gap between science and implementation as well as science and policy**.** The Alliance takes action by providing tailored government consultation and policy recommendations, contributing to international processes and forums, and piloting a broad range of approaches: including identification of pathogens, behavioural change, and value-based interventions.

16:20 - 16:39

Wildlife Diseases at the Human-Wildlife Interface

Alex D. Greenwood
Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany. School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Abstract

Humans and wildlife interact in many contexts in ways that increasingly promote transmission of disease from one to the other. Activities that impact wildlife population health may, in some cases, promote emergence of pathogens into other wildlife, domesticated animals, humans or all of the above. I study wildlife diseases and the activities of humans often play an important if not defining role in the health of wildlife populations on which I focus. The mission of the International Alliance Against Health Risks in Wildlife Trade overlaps with my research in that at some point in the trade process, humans have to enter areas where wildlife are present and remove them from their environment, usually a process that is disruptive not just to the animals caught. In addition, many societies rely on or come in contact with wildlife and wildlife products for their livelihoods and may be the first to suffer adverse effects of landscape change. As an example, in a current Volkswagen Stiftung supported project I lead together with WCS New York, WCS Colombia and WCS Bolivia, we are investigating how landscape change such as commercial agricultural expansion affects wildlife pathogen prevalence at the new edges between undisturbed forest and moderately to intensively altered land. In many of these altered landscapes Indigenous or low income farming communities are nearby and may face greater exposure to wildlife affected by recent landscape

changes. We are trying to uncover potential drivers of disease emergence and general animal health within the different environments in order to provide evidence for science based policy that could lower infectious disease risk in Indigenous communities and provide information that may be relevant in other countries undergoing similar landscape change.

Another form of wildlife trade is the acquisition and trading of animals, mainly for breeding purposes, in zoological gardens. This may alter wildlife health. In our research on the koala (*Phascoloarctos cinereus*), as a consequence of the small breeding pool and relatively close genetic relatedness among captive koalas, health problems caused by a common koala retrovirus have been exacerbated with koalas suffering extremely high levels of neoplasia. It is not currently known whether or not the virus has zoonotic potential. Our work together with the company Illumina and the San Diego Zoo Wildlife Alliance is helping to guide breeding practices to mediate the retroviral threat to lower health risks. In both examples, healthier wildlife should promote lower health risk overall.

16:39 - 16:58

Evolution and epidemiology of emerging viruses in the Maya Biosphere Reserve

Wendy K. Jo[1], Lincoln Carranza[2], Carlo Fischer[1], Fernando Martinez[2], Alejandro Morales[2], Jan Felix Drexler[1,3]

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Abstract

The virus epidemiology laboratory at the Institute of Virology at the Charité – Universitätsmedizin Berlin focus on the evolution and epidemiology of emerging viruses at the animal-human interface. We investigate the genetic alterations, evolutionary pathways, and ecological factors that lead to the emergence of viruses in a novel host or their increased incidence and expansion in a geographical range. Our research has a strong public health component. Early viral detection, investigation of transmission routes, and monitoring the spread of emerging viruses are crucial to implementing effective public health measures or targeted interventions to control or mitigate outbreaks. We investigate the genetic alterations, evolutionary pathways, and ecological factors that lead to the emergence of viruses in a novel host or their increased incidence and expansion in a geographical range. The illegal wildlife trade threatens biodiversity and ecosystems, and poses significant risks to public health due to close interactions between humans, wildlife, and domestic animals in illicit markets, facilitating the spillover and transmission of zoonotic diseases. Within the scope of the International Alliance against Health Risks in Wildlife Trade, we investigate the potential zoonotic pathogens circulating in wildlife in the biodiversity hotspot Maya Biosphere Reserve in Guatemala and in animals consumed as bushmeat in western Africa, both of which are wildlife trafficking hotspots. In the Maya Biosphere Reserve, samples were taken between 2016-2023 from live animals that are residing in Wildlife Rescue and Conservation Association (ARCAS). We collected plasma, nasal swabs, and rectal swabs samples from 26 mammalian, 37 bird, and 7 reptile species, most of which are protected species protected. We detected and isolated from spider monkey material the arbovirus Venezuela equine encephalitis virus (VEEV), which can cause febrile illness and severe disease in mainly horses and humans. Preliminary results indicate that the virus belongs to the subtype 1E, previously associated with equine epizootics in Mexico. The host range of this subtype includes also humans, rodents, and mosquitoes. However, the capacity of the subtype 1E to cause disease in humans and non-human primates is unknown. Those findings have substantial conservation medicine as well as for human health. Mitigation strategies include adequate diagnostics in humans and non-human primates as well as targeted vector control.

16:58 - 17:17

Transformative change in One-Health: The Elephant in the Room at the Science-Policy Interface

Barbara Maas[1,2], Thukten Jamtsho[3], Batzorig Tsend-ayush[4], Pham Ngoc Van Hien[5]
[1]NABU International, Berlin, Germany. [2]International Buddhist Confederation, New Dehli, India. [3]Central Monastic Body of Bhutan, Thimphu, Bhutan. [4]Association of Mongolian Buddhist Devotees, Ulaanbaatar, Mongolia. [5]Conservation Foundation, Ho Chi Minh City, Vietnam

Abstract

Human actions have eroded the Earth's life-sustaining ecosystem functions for decades. About three quarters of the planet's land surface and about two thirds of the world's oceans have already been damaged. Humans and livestock constitute more than 98% of the Earth's mammalian biomass. Six of nine planetary boundaries have been exceeded as a result of human appropriation of the Earth's net primary production, and all of the United Nation's Sustainability Goals (SDGs)

are once again significantly off track. This proliferation of red flags signals an intensifying compromise of the Earth’s live sustaining biosphere functions.
Human activities that cause biodiversity loss and climate change also fuel more frequent zoonoses and pandemics. Direct exploitation specifically constitutes the second largest of five principal drivers of progressive global environmental deterioration (IPBES 2019). Legal and illegal wildlife trade for food, status symbols, fashion, as medicines or pets remains a significant element of this dynamic and also contributes to the development and spread of zoonotic diseases.
In recognition of this deepening polycrisis, calls for profound systemwide transformative change have moved into the scientific and political mainstream. Yet, despite a growing public profile and the threat of catastrophic, irreversible global impacts, prevailing approaches have so far failed to catalyse action at the speed, scale or depth required to effect change. One of the underlying causes for our failure to address these challenges lies their habitual framing as primarily technical problems external to humanity and human nature. Cognitive and behavioural predispositions and biases, ingrained cultural practices, vested interests, the inability to process and act in accordance of high levels of complexity, as well as psychological and social factors frequently converge to maintain maladaptive behaviour on every level. We argue that a value-based approach towards a genuine shift in our relationship with nature and other species offers a promising route towards making the necessary adjustments.
Southeast Asia is a major hub for national and international wildlife trade and plays a key role in the accelerating destruction of local, regional, and global biodiversity. Our pioneering programme attempts to address this problem in three key Asian countries - Vietnam, Mongolia, and Bhutan - by drawing on the fundamental Buddhist principles of compassion towards all life, universal responsibility, and interdependence. This approach mirrors many aspects of the One Health concept, which embraces the inextricable and multifaceted interconnections between the health of humans, animals, and plants, and their shared environments.

17:17 - 17:36

A framework to implement evidence-based interventions to reduce health risks in the wildlife trade.

Professor Craig Stephen DVM, PhD[1], Professor Sascha Knauf PhD habil.[2], Dr Colmán Ó Críodáin PhD[3], Professor Chris Walzer Dip Tzt, Dr, vet. med,Dr. Habil[4,5]
[1]McEachran Institute, Nanoose Bay, Canada. [2]Friedrich-Loeffler-Institu, Greifswald, Germany. [3]WWF International, Driebergseweg, Netherlands. [4]Wildlife Conservation Society, New York, USA. [5]University of Vienna, Vienna, Austria

Abstract

A framework designed for assessing health interventions in the wildlife trade is urgently needed. There is insufficient evaluation of the effectiveness of wildlife trade interventions, let alone the conditions favouring an intervention's sustainability and uptake. We developed a conceptual framework to support the implementation of science and program evaluation based on a narrative literature review and consultation with experts and stakeholders. The former was used to identify transdisciplinary principles of implementing science in complex settings. The later method was used to obtain feedback on the framework's logic, consistency, relevance, and potential utility and feasibility of its application. Integrating seven implementation science tools yielded five guiding questions: (1) Is there evidence that the intervention could work? (2) What are the necessary processes and steps to tailor an implementation strategy to the implementation context? (3) Was the intervention successful and sustainable? (4) Who needs to do what to make the changes leading to the targeted risk reduction? And (5) what individual, organisational or systems processes need to be in place to allow necessary changes? Each guiding question gave rise to subsidiary questions that can help inform implementation, evaluation planning, and program assessments. Cross-cutting issues consideration included: (1) the need to understand the political, cultural and economic dimensions of implementation; (2) how success is defined and measured; (3) the role of social and ecological context; (4) the implementation process; and (5) how evidence is assessed.

Session V.II - Wildlife-Livestock-Human Interface: Prevention and Pandemic Preparedness (Part II)

18:00 - 19:30 Thursday, 12th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Károly Erdélyi, Christiane Herden

18:00 - 18:15

Evaluating the success of a citizen science approach to wildlife disease surveillance

Sarah Fowkes[1], Katharina Seilern-Moy[1], Andrew A. Cunningham[1], Emily Jones[1], Mike Toms[2], Will Peach[3], Kathy Wormald[4], Becki Lawson[1]

[1]Institute of Zoology, London, United Kingdom. [2]British Trust for Ornithology, Thetford, United Kingdom. [3]Royal Society for the Protection of Birds, Sandy, United Kingdom. [4]Froglife, Peterborough, United Kingdom

Abstract

Adoption of wildlife-friendly practice in domestic gardens supports biodiversity through resource provision, and improves human well-being through connection with nature. While often used to attract wildlife, supplementary feeding may inadvertently alter pathogen transmission dynamics, and this close human–wildlife interface potentially risks zoonotic infection. Consequently, there is a need for wildlife health surveillance (WHS) in peri-domestic habitats to safeguard the health of wildlife and people. Garden Wildlife Health (GWH) was launched in 2013 to create a general WHS scheme for garden wildlife across Great Britain (GB), integrated with population monitoring for target species to understand disease threats and evaluate their impact. This collaborative project uses a combination of opportunistic (via www.gardenwildlifehealth.org) and systematic (via the BTO's weekly Garden BirdWatch scheme) approaches to solicit observations of morbidity and mortality in garden birds, amphibians, reptiles and hedgehogs from members of the public. Post-mortem examinations are conducted to reach a definitive diagnosis, and to generate a tissue archive. Having reached the 10-year milestone, here our objectives are to 1. appraise the success of GWH in delivering WHS in terms of spatiotemporal and taxonomic coverage; 2. review key findings in a One Health context and 3. identify lessons learned for information dissemination to inform development of similar citizen science projects. Over the last decade, GWH has received ~36,500 disease incident reports with excellent spatiotemporal coverage across GB, with only minor gaps in areas of low human population density. ~2,500 post-mortem examinations have been conducted, with a skew towards diurnal, highly visible garden birds (~48%), followed by amphibians (~26%), hedgehogs (~22%) and a small number of reptiles (~4%). Our understanding of disease threats to biodiversity and wild animal welfare (e.g. finch trichomonosis, ophidiomycosis), and public health (e.g. *Salmonella* spp., *Escherichia albertii*) has been advanced through the holistic approach taken. Findings are disseminated in a variety of formats tailored to different audiences, including science and policy (e.g. >40 peer-reviewed publications and open access reports to government) and used to inform disease mitigation and best practice guidance for habitat management and supplementary feeding of wildlife. Hosting biannual meetings with invited stakeholders (e.g. government, academia, wildlife rehabilitators, NGOs) has increased awareness of disease threats to wildlife conservation and promoted collaboration. To encourage long-term participation by citizen scientists, results are communicated to the public through social media, e-newsletters, outreach events and >30 disease factsheets. Overall,

citizen science offers a cost-effective and far-reaching tool for WHS of peri-domestic species.

18:15 - 18:30

Evaluating the potential of virus exchange between people and bats in the Netherlands

Dr. Lineke Begeman PhD, DVM, Dipl of ECVP[1], Marco van de Bildt[1], Dr Fred de Boer PhD[2], Dr Judith van den Brand PhD[3], Dr Phaedra Eble Dr[4], Dr Herman Egberink Dr[3], Msc Mirjam Geschiere Master of Science[3], Vita Hommersen[5], Ingrid Keur DVM[6], Prof. Marion Koopmans Dr, DVM[1], Herman Limpens MSC[5], Peter Lina[7], Vera Mols[1], Dr Bas Oude Munnink Dr[1], Dr Chantal Reusken Dr[8], Mauro de Rosa DVM[6], Marcel Schillemans Msc[5], Dr Corien Swaan PhD, MD[8], Dr Kim van Zoonen PhD, Msc[8], Prof Thijs Kuiken PhD, DVM, Dipl of ACVP[1]
[1]Viroscience, Erasmus University Medical Centre, Rotterdam, Netherlands. [2]Wildlife Ecology and Conservation, Wageningen University, Wageningen, Netherlands. [3]Faculty of Veterinary Medicine, University of Utrecht, Utrecht, Netherlands. [4]Virology and Molecular Biology, Wageningen University, Lelystad, Netherlands. [5]Dutch Mammal Society, Nijmegen, Netherlands. [6]Netherlands Food and Consumer, Utrecht, Netherlands. [7]Naturalis Biodiversity Centre, Leiden, Netherlands. [8]National Institute for Public Health and the Environment, Bilthoven, Netherlands

Abstract

Bats belong to the order Chiroptera, which has the second-highest species diversity of all mammalian orders, and is therefore expected to carry a proportionally large viral diversity. Eighteen species of bats occur in the Netherlands, of which the most common species live close to people, using buildings as roost sites. Contacts between humans and bats can be expected and this provides opportunities for virus exchange. Novel virus infections in immunologically naive populations (bats as well as humans) can cause disease epidemics. We aimed to evaluate the potential for virus exchange between bats and humans by investigating virus diversity in bats, human-bat contacts and evidence for human exposure to bat viruses.

Viral diversity of bats was studied by testing fresh bat faeces for viral RNA of five viral families and one genus (*Paramyxoviridae*, *Coronaviridae*, *Filoviridae*, *Orthohantaviridae*, *Flaviviridae* and *Lyssavirus*). Human-bat contacts were studied via questionnaires distributed to people at risk of having bat contacts (n=227), as well as people representing a cross-section of the general population (n=265). Human exposure to bat viruses was investigated by testing for antibodies to bat

viruses in sera of humans in risk groups (n=187) and representatives of the general population as controls (n=300). Cats were considered as potential intermediate hosts. Therefore, sera of stray cats (n=162) and indoor cats as controls (n=132) were investigated.
Faeces from almost 800 roosts across the Netherlands, sampled between 2017-2019, were investigated. Seemingly species-specific viruses were found commonly: coronaviruses in 11 bat species, paramyxoviruses in five bat species. In addition, European bat lyssavirus type 1 (EBLV-1) in *Eptesicus serotinus*, and Usutu virus in *Pipistrellus pipistrellus* were detected. No other known human or domestic animal viruses were detected. Direct contacts with bats or their products were indicated by 75% of people in the bat contact risk group and 4% in the general population group. Neutralizing antibodies against EBLV-1 were detected in one person (1/187) who was not vaccinated for rabies, and in 5% of stray cats.
Based on the limited percentage of people of the general public who had direct contact with bats, the potential for virus exchange between bats and people in the Netherlands seems small. However, close contacts occur in specific risk groups. This could be taken into account in surveillance and prevention. The risk for transmission of EBLV-1 from bats to cats and people, although not observed in the Netherlands, is realistic, and vigilance for bat-human risk contacts should continue.

18:30 - 18:45

Raptors in the focus of pathogen surveillance: Implications of enzootic circulation of highly pathogenic avian influenza virus of subtype H5 (2.3.4.4b) in European wild birds

Anne Günther[1], Oliver Krone[2], Anja Globig[3], Anne Pohlmann[1], Jacqueline King[1], Christine Fast[4], Christian Grund[1], Christin Hennig[1], Christof Herrmann[5], Simon Piro[6], Dennis Rubbenstroth[1], Jana Schulz[7], Christoph Staubach[7], Lina Stacker[1], Lorenz Ulrich[1], Timm Harder[1], Martin Beer[1]
[1]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany. [2]Leibniz Institute for Zoo and Wildlife Research, Dept. Wildlife Diseases, Berlin, Germany. [3]Institute of International Animal Health/One Health, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany. [4]Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany. [5]Agency for Environment, Nature Conservation, and Geology Mecklenburg-Western Pomerania, Hiddensee Bird Ringing Scheme, Güstrow, Germany. [6]Agency for Environment, Nature Conservation, and Geology Mecklenburg-Western Pomerania, Nature Conservation Department, Güstrow, Germany. [7]Institute of

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

Abstract

Highly pathogenic (HP) avian influenza viruses (AIV) of the hemagglutinin subtype H5 (HPAIV H5), clade 2.3.4.4b, have established enzootic status in wild birds in northern Europe since 2022. Infections with HPAIV H5 demanded year-round mortality in a broad variety of avian species – many of which are predators or scavengers. In previous HPAI epizootics that were strictly seasonal to autumn and winter months, juvenile to adult raptors were infected while e.g. hunting for primary avian hosts. The enzootic status now might have increased potentially infected prey exposing secondary hosts. The analysis of HPAIV H5-positive samples from these species at the end of the food chain offers valuable benefits, at least from a virological point of view: HPAIV H5-positive samples can i) confirm the circulation of the pathogen, ii) reveal its presence even in previously unaffected regions and thus iii) support the understanding of its genetic diversity and its geographic expansion.

The shift towards enzootic virus activity including late spring and summer now not only exposes and threatens endangered species, but also so far unaffected age cohorts during the imminent hatching season, e.g. when parental birds may feed infected prey to their nestlings. Investigating potential impacts on the population stability of these species requires laborious and time-consuming sampling approaches.

Our retrospective screenings revealed the first cases of breeding failure in white-tailed sea eagles [*Haliaeetus albicilla*] in northern Germany caused by HPAIV H5 infection when all nestlings (n=2) of one hatch died in 2021. In the period March to July 2022, we confirmed additional cases in nestlings (n=8, two of them sampled alive) from five locations during active surveillance in the monitored area (Mecklenburg-Western Pomerania (MWP), Germany), coinciding with massive die-offs in seabird breeding colonies in this region. Low seropositivity rates in nestlings (5% in 2021; 7.9% in 2022) suggested a low level of protection in this age cohort (not yet fledged and depending on parental care); alternatively, timing of sampling with bird ringing activities is suboptimal due to advanced decay of maternally derived antibodies. The HPAIV H5-positive findings for nestlings sampled while still alive suggested possible virus shedding, raising the question of the need for hygiene standards when handling wildlife and self-protection measures to prevent zoonotic spill-over. We will present our sampling strategy since 2021 and corresponding findings, including population data on a so far stable breeding success for white-tailed sea eagles in MWP.

18:45 - 19:00

Quantitative risk analysis of the release of African swine fever from Eastern to Western Europe by movements of live wild boar between 2014 and 2018.

Dr. Ferran Jori PhD[1], Dr. David Pleydell PhD[2], Elsa Burnichon MSc[1], Dr. Jordi Casal Professor[3], Dr. Jose Angel Barasona PhD[4]
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Abstract

Captive breeding of wild boars (*Sus scrofa*) is practiced in several EU countries, primarily aimed at repopulating hunting estates for the sport hunting industry. Wildlife farming activities often have a particular legal status and do not consistently adhere to the same control standards as conventional livestock farms. This situation can potentially increase the risk of disease dissemination and spread into new territories when wildlife farms trade internationally. However, the volume of trade related to this activity and its associated risk have been rarely quantified to date. Between 2010 and 2017, a total 93 live wild boar movements entering to France and 34 to Spain, encompassing a total of 5567 animals moved were registered and retrieved from the European TRACES. Hungary was the main exporting country to thee countries, with a total of 55 trade movements and 3286 animals, while Poland was the main exporter to France (48 movements and 1617 animals). These data were subsequently used to develop a probabilistic model for quantifying the risk of African swine fever virus (ASFV) introduction arising from movements of live wild boars occurring between Eastern (Hungary and Poland) and Western Europe (France and Spain), respectively. The model considered two risk release scenarios in the trade process at the country of origin: i) the scenario of loading asymptomatic infected wild boar for transport and ii) the scenario of loading of healthy wild boar into a contaminated transport vehicle. A sensitivity analysis was performed to identify the parameters exerting the greatest influence on risk of introduction into the country of destination based on the volume and frequency of trade movements. The highest risk was detected when considering the number wild boar imported to France from Poland between 2015 and 2017 quantified at $7.8*10^{-3}$. The annual risk for ASFV introduction into France from Hungary was virtually negligible before 2015 but increased progressively to $9*10^{-4}$ between 2015 and 2017, coinciding with outbreaks in the adjacent Ukraine border. Such risk of introduction increased three-fold ($2.7 * 10^{-3}$) in the case of the movements to Spain from Hungary during the same period. While these risk estimation rates are currently hypothetical and without tangible consequences, they allow to quantify past registered levels of legal trade of wild boars between

EU countries and estimate potential animal health risks arising from this activity the last decades which is very often subject to lower levels of controls and regulations than conventional livestock animals.

19:00 - 19:15

White-toothed shrews (genus *Crocidura*) – neglected reservoirs in European ecosystems?

Viola C. Haring[1], Florian Pfaff[2], Anna Obiegala[3], Jens Jacob[4], Martin Pfeffer[3], Sandra Diederich[1], Rainer G. Ulrich[1], Martin Beer[2]
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Abstract

Small body size, synanthropic behaviour and ubiquity are characteristics of small mammals, that may facilitate spillover events. While rodents are known carriers of multiple pathogens with pandemic potential, less is known about insectivores, such as shrews.

We have investigated the pathogen presence in white-toothed shrews (Crocidurinae) from Central Europe. Almost 400 individuals of bicolored white-toothed shrews (*Crocidura leucodon*), lesser white-toothed shrews (*Crocidura suaveolens*) and greater white-toothed shrews (*Crocidura russula*) were tested for the presence of *Leptospira* spp., *Coxiella burnetii*, *Brucella* spp. *Anaplasma* spp., *Babesia* spp., *Neoehrlichia mikurensis* and *Bartonella* spp. with different conventional and real-time PCR methods. Prevalence of *Leptospira* spp. DNA was 12% in *C. russula* with *L. kirschneri* sequence type 100. Additionally, two *C. russula* were positive for *N. mikurensis*, which is the first description in shrews. Both pathogens cause febrile illnesses with varying symptoms, especially in immunocompromised patients. All other pathogens were not detected in the samples investigated.

Based on the identification of *C. leucodon* as reservoir for Borna Disease Virus 1 (*Orthobornavirus bornaense)*, which causes lethal encephalitis in humans and domestic animals, we further investigated the virosphere of white-toothed shrews in Central Europe by implementing a metagenomic next-generation sequencing approach. RNA from individual organ pools of *C. leucodon*, *C. suaveolens*, *C.*

russula and *Suncus etruscus* (Etruscan shrew), the latter from a husbandry, were extracted, processed and finally sequenced. Whole-genomes were obtained for several novel viruses belonging to the families *Nairoviridae*, *Paramyxoviridae* and *Hepeviridae*. Some of them form phylogenetic clusters closely related to highly pathogenic viruses such as Crimean-Congo haemorrhagic fever virus and henipaviruses. Most interestingly, one novel paramyxovirus is next of kin to the zoonotic Langya virus, which has been found to cause febrile infections in humans in China. The prevalence of these novel viruses within the white-toothed shrew populations was assessed and attempts on virus isolation and characterization were performed.
Our study indicates an important role of white-toothed shrews as pathogen reservoirs in the European ecosystem. These findings will guide biosafety assessments for exposed working groups such as agricultural workers and field biologists. In a holistic One Health approach, future studies should evaluate the potential influence of anthropogenic land use, biodiversity and climate change on the range of these neglected reservoir species and their potential as reservoirs for zoonotic agents, while acknowledging the need for conservation of white-toothed shrews.

19:15 - 19:30

Oral immunization of European wolves (*Canis lupus*) against rabies: Non-invasive evaluation of the suitability of a novel soft-blister bait via color-coded blister packs

Dr Anna Langguth DVM, MSc (Wild Animal Health)[1,2], Dr Ad Vos[3], Mr Steffen Ortmann[3], Prof. Dr. Michael Böer apl.Prof., Dr. med. vet.[4,5]
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Abstract

Background

In recent years, the European wolf (*Canis lupus*) has returned to central Europe, including countries like Germany. With it, the wolf brings the potential risk of rabies reintroduction, a lethal zoonotic viral disease. While still occasionally reported in neighbouring countries such as Poland, the widespread use of oral rabies vaccination among native fox populations successfully eradicated rabies in Germany by 2008.

However, currently available fox bait systems are unsuitable for immunising larger canids. Oral vaccination requires liquid vaccine to come into contact with oral mucosa (e.g. through the animal chewing the bait) in order to trigger an immune response. Fox baits, due to their small size, are easily swallowed whole by wolves, preventing effective vaccine delivery.

The potential reintroduction of rabies by wolves poses significant public health concerns and could negatively impact public perception of the species. As apex predators, wolves play a crucial role in maintaining healthy ecosystems. However, in urbanised central Europe, conflicts between humans and returning wolves are frequent and shape policy decisions.

It is therefore imperative to develop alternatives to currently available bait systems which can be readily available for potential future immunisation of the growing German wolf population.

Objectives

The purpose of this study was to assess the suitability of a novel softblister bait for efficient oral delivery of liquid rabies vaccine to European wolves.

Materials and Methods

Using a topical colour marker that stains the oral cavity if blister contents are spilled, we non-invasively assessed bait efficacy. Between 2020 and 2021, individual observations of captive European wolves consuming baits were undertaken to assess bait handling and 'vaccination' success.

Results

Our findings revealed that in 73.5 % of trials where wolves consumed the bait, distinct staining of the oral cavity indicated successful delivery of the colour marker contained in the baits, with no adverse effects recorded.

Conclusion

This study presents a novel softblister bait that is safe and palatable for European wolves and demonstrates its applicability for administering oral rabies vaccine to this species.

Additionally, the topical marker used provides a non-invasive means of evaluating bait candidates for oral vaccination of other wildlife species.

Although further evaluation under field conditions is required for prospective integration into rabies vaccination programs for free-ranging wolves, the success rate of this novel softblister bait surpassed 70 %, meeting the threshold required for achieving herd immunity in a given population and establishing it as a promising candidate.

PARALLEL PUBLIC EVENT (German Language only)

High-Level Panel Discussion "Der One-Health-Ansatz – Wie die Gesundheit von Umwelt und Tier auch unser Wohl beeinflusst"
Moderation Prof. Dr. Chris Walzer (Wildlife Conservation Society)

19:00 - 20:35 Thursday, 12th September, 2024
Ozeaneum, Stralsund
Chris Walzer

Join us: https://www.deutsches-meeresmuseum.de/news/post/der-one-health-ansatz-wie-die-gesundheit-von-umwelt-und-tier-auch-unser-wohl-beeinflusst

Student-Mentor Mixer

20:00 - 22:00 Thursday, 12th September, 2024
Ozeaneum, Stralsund

Registration

07:00 - 08:00 Friday, 13th September, 2024
Brewery, Stralsund

Housekeeping Notes

08:00 - 08:05 Friday, 13th September, 2024
Brewery, Stralsund
Sascha Knauf

4th Keynote: Aquatic mammals in a changing environment: impacts on their health.

08:05 - 08:50 Friday, 13th September, 2024
Brewery, Stralsund
Presentation type Conference Session

Ursula Siebert[1]
[1]University of Veterinary Medicine Hannover, Institute for Terrestrial and Aquatic Wildlife Research, Büsum, Germany

Abstract

Aquatic mammals are sentinel species for marine and freshwater environments. Knowledge on aquatic mammals has been increased worldwide through numerous projects over the last years. National and international programs have been started and international guidelines agreed on, especially in the area of the European Union. While in some countries data have been collected over three and more decades, in other areas infrastructure and know-how have been established over the last years only because of funding restrictions. The collection of data in sensible environments such as the polar regions is possible through an increasing number of projects.

At the same time, the pressure from anthropogenic activities is increasing, and their effects are becoming more complex. Chemical and pharmaceutical pollution and microplastics in marine and aquatic ecosystems and their effects require advanced international efforts. The increase of offshore windfarms driven by the wish to increase renewable energies has the potential to cause behavioral changes, habitat loss, and health impacts.

Large amounts of ammunition from the Second World War impact the hearing ability of marine mammals in case of explosions but also have the potential to increase chemical pollution through corrosion of the material after 70 years in the

sea. Activities such as fishing, aquaculture, and military activities create conflicts with aquatic mammals.

Investigations on the health of aquatic mammals have become more important to assess the single and cumulative effects of anthropogenic activities. The identification of direct causes of death, such as road e.g., kill, by-catch, explosions, is still a challenge. But the understanding of chronic stress through habitat loss, food depletion, and chemical pollution on reproduction and susceptibility requires large interdisciplinary studies. Infectious diseases in aquatic mammals are important indicators for our changing environment. Increasing attention has been dedicated to the occurrence of infectious agents in wildlife as the pressure of anthropogenic activities has a boomerang effect on humans.

Agreements such as ASCOBANS, ACCOBAMS, OSPAR, HELCOM, Natura 2000, WSFD, and MSFD oblige the states to protect and preserve aquatic mammals and their ecosystems. But in order to approach the concept of load/burden limits of those very mobile aquatic species, interdisciplinary and international approaches are needed. Thus, decisions on the protection, habitat, and health preservation of aquatic mammals need to be achieved with the best international data and knowledge.

Session VI – Aquatic Animal Health

08:50 - 10:35 Friday, 13th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Burkard Baschek, Ursula Siebert

08:50 - 09:05

30 Years Post-Mortem Health Monitoring: Development of a scoring system to collate lung pathology data of Harbour Porpoises (Phocoena Phocoena) from the German North Sea

Dr. Luca Schick[1], Dr. Peter Wohlsein[2], Dr. Joseph Schnitzler[1], Dr. Ellen Prenger-Berninghoff[3], Prof. Christa Ewers[3], Prof. Ursula Siebert[1]
[1]Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, Foundation, Buesum, Germany. [2]Department of Pathology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany. [3]Institute of Hygiene and Infectious Diseases of Animals, Justus-Liebig-Universität Giessen, Giessen, Germany

Abstract

Since 1990, stranded and bycaught harbour porpoises (*Phocoena phocoena*) have been collected in Schleswig-Holstein, Germany, through the stranding network for health investigations. The health situation of the harbour porpoise population in the North Sea is considered as abnormal based on pathological investigations in different countries. Especially the lung shows a higher level of changes compared to remote areas like the Arctic. Therefore, a scoring system for the respiratory tract was developed to provide a health parameter of the local population for an assessment of cumulative effects of environmental factors and anthropogenic activities.

The scoring system aims to summarize different findings of the lung into a single score. 377 animals, stranded along the North Sea coast, were included in this first approach. The scoring system consists of four criteria: 1) Pulmonary inflammation was rated based on macroscopic findings and standardized histological parameter, multiplying the extent and severity of inflammatory changes, 2) parasite infection was rated semiquantitatively during necropsy and upon histological evaluation; the infection intensity was combined and included in the final score; 3) additional reactive/reparative lesions including vascular thrombosis, vasculitis, fibrosis were rated histologically and the occurrence of such lesions was included as extra points in the total score; 4) microbiological results were assessed for potentially pathogenic bacteria and the severity of infection was included in the total score.

Of the 377 porpoises 189 were male and 187 female. In 33 animals bycatch was suspected based on pathological findings. 284 animals exhibited pathological findings in the lung. Solitary or simultaneously occurring findings included parasitic infections (n = 196) and pneumonia (n = 174), reactive/reparative lesions (n = 82) and potentially pathogenic bacteria (n = 181). First applications of the data to the scoring system showed that the lung score, hence pulmonary health, correlate with the age of the animals, with adults showing the highest overall scores. Sex and origin do not have significant effects.

The development of a scoring system to sum up different pathological, parasitological and microbiological findings in the lower respiratory tract is an attempt to provide a correction factor about the population health, which needs be taken into account when the effects of multiple stressors are assessed.

09:05 - 09:20

Casting a wide net reveals obligate intermediate hosts of the seal lungworm *Parafilaroides gymnurus*

Dr Jocelyn Elson-Riggins PhD[1,2,3], Dr Lynda Gibbons PhD[4], Mr Sander van Dijk MSc[2], Dr Anique Kappe PhD[5], Dr Damer Blake PhD[1], Dr Carlos Hermosilla PhD[4], Dr Helias Udo de Haes PhD[6], Dr Nynke Osinga PhD[6]

[1]Royal Veterinary College, North Mymms, United Kingdom. [2]The Seal Centre, Pieterburen, Netherlands. [3]Dengrove Scientific Services, Shaftesbury, United Kingdom. [4]Justus Liebig University Giessen, Giessen, Germany. [5]Gendika, Veendam, Netherlands. [6]Leiden University, Leiden, Netherlands

Abstract

Lungworms are responsible for the principal infectious disease of eastern Atlantic common seals, *Phoca vitulina vitulina*, in the Dutch North Sea. Parasitic bronchopneumonia is caused by two nematode species, *Parafilaroides gymnurus* and *Otostrongylus circumlitus*. Juvenile seals become infected after weaning when they feed on prey harbouring lungworm larvae. A sharp increase in lungworm infections of juvenile common seals from this area prompted us to ask which marine organisms serve as obligate intermediate hosts (IH) for these species and whether host-parasite interactions are being affected by ecosystem change. We hypothesized that shrimp and small-bodied fish could be involved since they have been recorded as important food sources post-weaning. We used artificial digestion as a high throughput method to release parasites from a wide range of potential host species caught in three seasons (winter, spring and summer of 2013) by commercial fishermen in the Dutch Wadden Sea. This method allowed us to examine not only fish as potential IH, but also crustaceans and polychaetes. We first screened the digests by stereomicroscopy. Then, using the compound microscope, we photographed and measured each nematode larva retrieved before extracting their DNA. Using a combination of morphology and DNA sequencing, we found *P. gymnurus* larvae in three demersal fish species: two flatfish species (common dab, *Limanda limanda* and European plaice, *Pleuronectes platessa*) and one ray-finned fish species (hooknose, *Agonus cataphractus*). The latter was the only species to harbour *P. gymnurus* larvae in all three seasons and this constitutes a new host record. The body lengths of all three of these infected fish species were smaller than has been reported for adults and they have all been recorded as prey for juvenile common seals. We did not find *P. gymnurus* in the other 17 fish species or the invertebrates we examined, including shrimp. Furthermore, in common with other studies, we did not detect *O. circumlitus* in any of the species examined. We conclude that artificial digestion is an effective high throughput method for retrieving nematode larvae from marine organisms. Small-bodied demersal fish are IH for *P. gymnurus* and we report a new host record. We will discuss the implications of our findings, including how ecosystem change may affect these host-parasite interactions.

09:20 - 09:35

Three decades of anisakid nematode infections in harbour porpoises of the North- and Baltic Sea: trends and health effects.

Dr. rer. nat. Kristina Lehnert Dr. rer. nat., Ms. Aylin Öztürk, Ms. Insa Herzog, Dr. Juan Felipe Escobar Calderon PhD, Prof. Prof. hc. Dr Ursula Siebert Prof. Prof. hc. Dr.
University of Veterinary Medicine Hannover, Hannover, Germany

Abstract

Harbour porpoises are infected by anisakid nematodes, which become mature in the stomach of marine mammals after completing a multi-stage life cycle including free-living larvae and crustacean intermediate and fish paratenic hosts. They cause gastritis and ulcerations in their definite hosts and anisakidosis in humans when accidentally ingested with undercooked fish. A stranding network collected harbour porpoises along the coasts of Germany since 1990 and investigated their health status. Necropsy reports and samples from ~1500 harbour porpoises were analysed for prevalence, intensity (none, mild, moderate, severe) and lesions associated with anisakids. A GLM with logistic regression analysed sex and age class differences as well as time trends from 2004-2023.

Anisakis simplex s.l. is the most common gastric nematode in porpoises in the North- and Baltic Sea and anisakid nematodes were prevalent from 1990 onwards. Prevalence of stomach nematodes increased slightly in the period from 2004-2023 in porpoises from the North- and Baltic Sea. Prevalence was significantly lower in the Baltic Sea (11%) compared to the North Sea (19%). No differences in infection patterns between male and female porpoises were found. In adults prevalence (23%) was significantly higher than in juveniles (11%) while neonates were almost never infected. Intensity of infection did not change over time nor showed differences between North- and Baltic Sea, age and sex of porpoises. Pathological changes associated with stomach nematode infections were correlated to intensity of infection and included chronic ulcerative gastritis of varying severity and characteristics. The striking differences in infection patterns observed between North- and Baltic Sea and between age classes reflect ecosystem characteristics, diet as well as life history of porpoises. Environmental conditions may drive prey species composition and the viability of larval stages and intermediate hosts. The epidemiology of this generalist, trophically transmitted nematode with zoonotic implications needs to be monitored closely.

09:35 - 09:50

Herpesviruses survey in migrating procellariforms, northeastern Brazil

Dr. PhD Carlos Sacristán PhD[1], Dr. PhD Aricia Duarte-Benvenuto PhD[2], Dr. PhD Pedro Enrique Navas PhD[2], Dr. PhD Roberta Zamana-Ramblas[2], BS Laura Baes[3], Dr. Larissa Pavanelli[4], Dr. Joana Ikeda[5], Dr. PhD Professor José Luiz Catão-Dias[2], Dr. PhD Ana Carolina Ewbank[1]
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Abstract

Procellariiformes are amongst the most endangered threatened taxonomic groups; thus, understanding the infectious agents affecting this group is crucial to their conservation. Herpesviruses (order *Herpesvirales*, family *Orthoherpesviridae*) are linear, enveloped, double-stranded DNA viruses subdivided into subfamilies *Alpha-*, *Beta-* and *Gammaherpesvirinae*. Due to a suggested co-evolution with their host species, herpesviruses generally show low pathogenicity and establish latent infections in their natural hosts. Less commonly, immune challenges (e.g., concomitant disease, captivity) may trigger viral reactivation, leading to disease and even death of the natural hosts. Additionally, cross-species infection ("spillovers") may cause severe disease and high mortality, even leading to severe outbreaks. In seabirds, several alphaherpesviruses have been described in Charadriiformes, Gaviiformes, Procellariiformes, Phaethontiformes, Sphenisciformes, and Suliformes; however, little is known about herpesviruses in Procellariiformes, aside from the description of a herpesvirus in a Yellow-nosed Albatross (*Thalassarche chlororynchos*) undergoing rehabilitation in Brazil. Stranding events of pelagic seabirds provide an opportunity to assess population threats, infectious agents and involved stressors. Thus, our goal was to molecularly survey and characterize herpesviruses in migrating Procellariiformes stranded alive in northeastern Brazilian coast and admitted into a rehabilitation center, between June and July of 2021. We analyzed 12 Cory's shearwaters (*Calonectris borealis*), two Great Shearwaters (*Ardenna gravis*, syn. *Puffinus gravis*) and one Yellow-nosed Albatross (*Thalassarche chlororynchos*) found in an unusual mortality event in Bahía state. Following necropsy, selected tissue samples (i.e., lungs, brain, liver, spleen, and kidneys) were frozen at -20/-80 °C and tested against herpesvirus using a broad-spectrum nested PCR. Overall, 20% (3/15) of the birds were herpesvirus-positive, i.e., two Cory's shearwaters and one Great

Shearwater, that according to our phylogenetic tree, were classified within the genus *Mardivirus*, which includes relevant avian herpesviruses, such as *Gallid alphaherpesvirus 2* (Marek's disease). In all three herpesvirus-PCR-positive cases, the same tissue samples (fixed in 10% buffered formalin) were also evaluated by histopathology. No associated lesions were observed, most likely due to autolysis. To our knowledge, these are the first herpesvirus descriptions in shearwaters and only the second in Procellariiformes worldwide. This study contributes to the currently very scarce data regarding infectious agents in Procellariiformes. Further studies are necessary to evaluate the presence and characteristics of herpesvirus in Procellariiformes and the presence (or not) of related disease in order to understanding the epidemiology of this infectious agent and contribute to the conservation of this endangered seabird group.

CS and ACE receive a Juan de la Cierva Incorporación (#IJC2020–046019-I) and a Juan de la Cierva formación (#JDC2022-048632-I) fellowships, respectively, granted by AEI/MICIN.

09:50 - 10:05

Physiological responses to capture, handling and tagging in flapper skate (*Dipturus intermedius*)

Dr Georgina Cole BVetMed MSc[1], Dr Edward Lavender PhD[2], Dr Adam Naylor BVetMed MScWAH CertAVP DipACZM DipECZM[3], Prof Simon Girling BVMS (Hons) PhD DZooMed DipECZM[1], Dr Steffen Oppel PhD[4], Dr Jane Dodd PhD[5], Dr James Thorburn PhD[6]
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Abstract

Catch-and-release angling is a popular pastime and an essential component of many fish research programmes. Marked physiological disturbances have been documented in elasmobranchs in response to capture and handling, however skates and rays remain understudied. Physiological responses of the Critically Endangered flapper skate (*Dipturus intermedius*) to capture, handling and tagging were investigated. It was hypothesised that captured skate would experience metabolic and/or respiratory acidosis, with the degree of acidosis increasing with fight time, water temperature and time on deck. Sixty-one skate were captured from angling vessels as part of an acoustic tagging programme. Blood biochemistry was analysed at two points: post-capture and pre-release. Heart and respiratory rates were recorded during handling and the surgical insertion of acoustic tags. Skate generally experienced a mild metabolic acidosis characterised by decreases in blood pH and HCO_3^- and increases in lactate and glucose. Respiratory acidosis characterised by limited increases in PCO_2 was also observed. The degree of acidosis was greater with warmer sea temperatures and longer fight times, and worsened during the time that skate were handled on deck. Associations between the variables of fight time and temperature were found for heart rate, and both heart and respiratory rates were negatively associated with skate size. These results suggest that higher sea temperatures and longer fight times may increase the physiological stress experienced by flapper skate during capture. Efforts to reduce fight times and minimise heat exposure (such as shading and irrigation) may be beneficial for skate.

10:05 - 10:20

Population dynamics of mussels and their invasive parasites in a warming climate

Elli Rosa Jolma DipECZM (Wildlife Population Health)[1,2], Prof. Hans Heesterbeek[2], Prof. Mick Roberts[3], Prof. David Thieltges[1,4], Dr. Mathias Wegner[5], Dr. Anieke van Leeuwen[1]
[1]Department of Coastal Systems, NIOZ Royal Netherlands Institute for Sea Research, Den Burg, Netherlands. [2]Department of Population Health Sciences, Utrecht University, Utrecht, Netherlands. [3]New Zealand Institute for Advanced Study and the Infectious Disease Research Centre, Massey University, Auckland, New Zealand. [4]Groningen Institute for Evolutionary Life-Sciences (GELIFES), University of Groningen, Groeningen, Netherlands. [5]Alfred Wegener Institute – Helmholtz Centre for Polar and Marine Research, Coastal Ecology, List, Germany

Abstract

Climate change may affect the spread of emerging infectious diseases and the impact they have on ecosystems. The speed of global warming is especially high in temperate coastal ecosystems. Here, we explore if and how the warming of their collective environment affects the impact of invasive parasites on mussel populations in the Dutch Wadden Sea. We study a system consisting of a marine native host, the blue mussel (*Mytilus edulis*), and two invasive parasitic copepods (*Mytilicola intestinalis* and *Mytilicola orientalis*). We combined controlled laboratory experiments in a range of past and predicted future temperatures with population dynamical modelling. In the experiments, we learned that an increase in temperature in the range of 10°C-26°C accelerates the life cycles of both parasites and that only the highest temperature (26°C) limits parasite development success. Parasites therefore complete more lifecycles per year in the predicted future temperature regimes. At the highest temperature (26°C), the survival of both infected and uninfected hosts decreased. Infection with *M. intestinalis* lowered host condition and infection with *M. orientalis* decreased host growth. In our models, the decrease in growth led to an increase in the duration of pre-reproductive juvenile stages, and the decrease in condition led to a lower reproductive output of adult mussels. The model then showed cyclicity in host and parasite population levels for a range of parameter values. These effects, combined with the increase in infection pressure resulting from more life cycles being completed per year, can exacerbate the known negative consequences of climate change on the reproductive success of blue mussels. Our results indicate that invasive parasites can shape and increase the impact of climate change on the population of a native species even in the absence of lethal effects at the individual level.

10:20 - 10:35

Systemic amyloidosis and chronic entanglement in a grey seal (*Halichoerus grypus*)

Stephanie Gross[1], Bianca Kühl[2], Alexandra Rieger[1], Peter Wohlsein[2], Ursula Siebert[1]
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Abstract

Amyloidosis belongs to the protein misfolding diseases and is characterized by extracellular deposition of insoluble protein fibrils. With increasing severity, amyloid deposits can disrupt the structure and function of affected organs. Deposits can occur locally in a single organ or systemically. Amyloidosis emerges primarily or secondarily with chronic inflammatory processes being the most common cause for secondary amyloidosis in animals. This disease has been reported in humans, various domestic animals and wildlife. Here, we report on a moribund, four-year-old, female grey seal (*Halichoerus grypus*) that stranded on the German Baltic Sea coast and was subsequently euthanized for animal welfare reasons. In the following, the animal was dissected at the Institute for Terrestrial and Aquatic Wildlife Research. Further investigations included histopathology, parasitology, and microbiology. Additionally, the marine mammal post-mortem database of the institute was reviewed for amyloidosis cases over the last 30 years.

Externally, a moderate body condition was noted as well as an extensive chronic, ulcerative and purulent dermatitis with granulation tissue around the neck caused by entanglement with fishing gear. On internal examination, the main finding was a markedly enlarged and altered liver. Histopathology revealed almost complete destruction of the regular liver architecture due to chronic inflammation, fibrosis, necrosis and severe amyloid deposition. Amyloid deposition was also seen in the thyroid gland, the stomach, the pancreas, the kidney, and the tunica media of small- and medium-sized blood vessels, hence the diagnosis of systemic amyloidosis was made. It was not possible to distinctly determine the cause of the liver alterations but a causative link to a bacterial infection is conceivable. How far the systemic amyloidosis was caused by the alterations of the liver and/or the chronic inflammatory process associated with the entanglement stays also residual. Interestingly, over the last 30 years only in six out of 293 dissected grey seals amyloid deposition was seen. None of the other five amyloid cases showed entanglements. All six animals originated from the Baltic Sea but none from the North Sea. Furthermore, none of the necropsied harbour seals (n=2,404) nor of the harbour porpoises (n=925) were affected.

Here, we present a case of systemic amyloidosis in a grey seal from the German Baltic Sea. The animal also showed severe hepatic alterations that were consistent with a macronodular cirrhosis. Further research is needed to identify the reason for the detected species-associated and spatial distribution of amyloidosis in the resident marine mammals of Germany.

Short Coffee Break

10:35 - 10:50 Friday, 13th September, 2024
Brewery, Stralsund

Session VI (cont.) – Impact of Climate Change and Urbanisation

10:50 - 12:20 Friday, 13th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Claire Cayol, Craig Stephen (tbc)

10:50 - 11:05

Morbidity and mortality in a free-tailed bat (Tadarida teniotis) colony in an urban environment linked to lead toxicity: investigations in a One Health context.

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Abstract

Bats can be found in a variety of ecosystems, including urban environments where chemical pressure may include toxic agents impacting their conservation but also human or animal health. France's chiropterans are protected, and certain species are threatened so early detection of any emerging health problem is essential. For this purpose, the Chiroptera Unusual Mortality Surveillance (SMAC) network was created in 2014. It is an event-based participatory network facilitated by the French Biodiversity Agency, with the collaboration of chiropterologists, regional veterinary laboratories and specialized laboratories.

Between August and November 2022, an unusual morbidity/mortality event was reported in a colony of free-tailed bats (*Tadarida teniotis*) which were nesting in an expansion joint in between residential buildings in Nice (French Riviera). Juveniles were found on the ground either dead, incapable of flying or seen falling at a rhythm of approximately 10 per night, with more than 200 in between August and September. Around one hundred individuals were transferred to rehabilitation centers in the area. Retrospective investigations (2014-2021) highlighted similar clinical cases within colonies of the region during previous years.

Clinical examination revealed animals in good nutritional condition with symmetrical wing or toe fractures. Radiographs showed variable degrees of fractures with suspicion of a metabolic or nutritional cause. Gross examination performed on 29 individuals identified occasional abdominal filariasis and wing fractures. Ancillary testing ruled out classic pathogens (rabies). Histologic examination mainly revealed complex osseous abnormalities comprised of various degrees of bone fractures with callus formation, irregular periosteum, and prominent deep cartilage core retention. A similar clinical presentation was reported in a colony of free-tailed bats in Rome in 2019 with confirmed lead exposure, without histopathology. The combination of the clinical presentation,

histologic lesions, and history of similar situations in bat colonies in the region resulted in lead testing and revealed concentrations at over 360.000 µg/kg. Another mortality event occurred in August 2023 at the same location with similar results.

It was concluded that lead intoxication of juveniles via their environment resulted in abnormal bone development and ensuing fractures with incapacity to fly in most severely affected individuals.

In 2024, investigation of the expansion joint where the colony was located confirmed heavy lead toxicity. A procedure to close the site was initiated with the local authorities. This case series highlights the importance of interdisciplinary collaboration when faced with an issue that concerns the conservation of a species but also public health.

11:05 - 11:20

The relationships between urban green space and the abundance of rats and their zoonotic pathogens

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Abstract

Urban greening has become an increasingly popular strategy to improve human health in cities. However, urban greening may also influence wildlife living in cities. The effects of urban greening on the abundance of wildlife and the prevalence and diversity of their zoonotic infectious pathogens are not thoroughly investigated. From a public health perspective, the brown rat (*Rattus norvegicus*) is an important wild urban species.

Therefore, the aim of our study was to examine the relationships between the amount of urban green space and the abundance of wild brown rats and their pathogens.

We examined 222 systematically trapped brown rats and 185 rats retrieved from pest controllers from three cities in the Netherlands, collected in 2020-2021. We tested them for 18 zoonotic pathogens (*Bartonella* spp., *Leptospira* spp., *Borrelia* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, *Spiroplasma* spp., *Streptobacillus moniliformis*, *Coxiella burnetii*, *Salmonella* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta- lactamase (ESBL)/AmpC-producing *Escherichia coli*, rat hepatitis E virus (ratHEV), *Seoul orthohantavirus*, *Cowpox virus*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), *Toxoplasma gondii* and *Babesia* spp.) with methods consisting of (q)PCR, ELISA, IFA and bacterial culture. Using generalized linear mixed models, we modelled the relationships between pathogen prevalence and diversity and urban greenness. We included both rat-specific and location-specific variables in these pathogen models. The amount of green space per location was calculated using the Normalized Difference Vegetation Index (NDVI).

We observed positive relationships between the relative abundance of rats and both greenness (NDVI) and different proxies for food resources (restaurants, waste items, and petting zoos). We detected 13 different zoonotic pathogens. Rats from greener urban areas had a significantly higher prevalence of *Bartonella* spp. and *Borrelia* spp., and a significantly lower prevalence of ESBL/AmpC-producing *E. coli* and ratHEV. Rat age was positively correlated with pathogen diversity while greenness was not related to pathogen diversity. Additionally, *Bartonella* spp. occurrence was positively correlated with that of *Leptospira* spp., *Borrelia* spp. and *Rickettsia* spp., and *Borrelia* spp. occurrence was also positively correlated with that of *Rickettsia* spp.

Our results show an increased rat-borne zoonotic disease hazard in greener urban areas, which for most pathogens was driven by the increase in rat abundance rather than pathogen prevalence. This highlights the importance of keeping rat densities low and investigating the effects of urban greening on the exposure to zoonotic pathogens in order to make informed decisions and to take appropriate countermeasures to prevent zoonotic diseases.

11:20 - 11:35

Investigating pesticide and anticoagulant rodenticide exposure in two non-target British mammals

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Abstract

Anticoagulant rodenticides (ARs) and other pesticides are widely used chemicals that may pose an inadvertent risk to non-target wildlife health. Although most studies to date have focused on obligate predatory species, omnivores such as the European hedgehog (*Erinaceus europaeus*) and red fox (*Vulpes vulpes*) may also be at risk of exposure. We aimed to 1. investigate the occurrence of ARs, molluscicides, and neonicotinoids in hedgehogs and foxes from England; 2. analyse the relationship between exposure and potential risk factors; and 3. evaluate evidence of acute toxicity. We screened 100 hedgehog and 84 fox liver samples collected post mortem through passive surveillance of animals found dead across England, 2012-2020, and 99 liver samples from foxes shot during winter 2020/2021. Following a QuEChERS extraction method, chemical residues were analysed by liquid chromatography-tandem mass spectrometry. Binomial regression models were applied to investigate the likelihood of residue detection in relation to various host and environmental factors. Only hedgehogs were systematically examined post-mortem and findings were reviewed for evidence of acute haemorrhage consistent with AR toxicity and for association of residue detection and cause of death category. AR residues were detected in 62% of hedgehogs and 89% of foxes, with more than one AR found in 83% of all animals examined. Additionally, 25% of hedgehog and 56% of fox samples contained AR concentrations above the commonly used reference value for poisoning or regulatory concern (>100 ng/g). Despite these high levels, no evidence of internal haemorrhage consistent with acute AR intoxication was observed in hedgehogs. We also found no indication of reduced exposure following the introduction of a national AR stewardship scheme in 2016. Except for a single fox with low clothianidin, no neonicotinoids or molluscicides were detected. The liver half-life of these pesticides, however, is short and therefore samples such as stomach content may be more suitable for detection. Overall, our findings suggest that current AR usage practices in England lead to extensive exposure of hedgehogs and foxes and potentially other non-target mammals. While no evidence of acute AR toxicity was detected in hedgehogs, investigation of possible subclinical impacts of AR exposure is required, particularly as this is a species of conservation concern. In conclusion, this study demonstrates that hedgehogs and foxes, as synanthropic species, may serve as sentinels to monitor AR usage in both agricultural and amenity settings, and to evaluate the success of changes in regulations designed to mitigate non-target wildlife exposure.

11:35 - 11:50

Per- and Polyfluoroalkyl Substances (PFAS) in Icelandic Arctic foxes (*Vulpes lagopus*)

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals with various industrial and commercial applications. PFAS entering the environment are globally distributed through atmospheric and oceanic currents through which they can reach remote habitats, such as the polar regions where they accumulate in both marine and terrestrial carnivores (e.g. polar bears (*Ursus maritimus*) and Arctic foxes (*Vulpes lagopus*)).

Although studies on the presence of PFAS in Arctic foxes exist, relatively little is known about the degree of contamination in Iceland and the potential negative effects of PFAS accumulation. In this study, we determined the PFAS concentrations in several tissues (liver, kidney, and muscle) of hunted Icelandic Arctic foxes by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) and correlated these with various fitness and health measures (body condition, immune and stress markers, current reproductive effort).

Overall, 26 PFAS were identified in the fox tissues. Most quantified substances were long-chain PFCAs and PFSAs (C6 to C14) with PFOS being the most abundant. PFAS concentrations were highest in the liver, followed by kidney and muscle samples. Foxes feeding from marine environment in coastal habitats showed higher ΣPFCAs (factor 10) and higher ΣPFSAs (factor 40) concentrations compared to inland foxes. There were no differences between age groups, while females showed higher ΣPFCAs concentrations compared to males, although without statistical significance. During our preliminary statistical analysis, we could not find a correlation between PFAS concentration, body condition, number of fetal scars, three immune markers (total immunoglobulin levels, lysozyme, and neopterin concentrations), and hair cortisol levels.

Because of the relatively low biodiversity within Arctic ecosystems and the involvement of the species in both marine and terrestrial ecosystems, the Icelandic Arctic fox population proved to be a sentinel for the overall ecosystem health of Iceland. In corroboration with previous results on higher levels of mercury,

helminth burden and richness, and pathogen exposure on coastal foxes compared to the inland populations, our results on PFAS not only support that the two ecotypes are quite separate and distinct but also raise conservation concerns. Further work on the potential fitness and health effects of these pollutants on wildlife is warranted.

11:50 - 12:05

Immunological perspectives on disease risk in the koala

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Abstract

Chlamydial (*Chlamydia pecorum*) infertility contributes to the koala's (*Phascolarctos cinereus*) Endangered status. Disease is chronic and expression varies among and within populations and over time. Predicting risk associated with coinfections (particularly koala retrovirus, KoRV), emerging climatic extremes, and associated nutritional and physiological stressors is critical to long-term management, as highlighted by a recent National Koala Disease Risk Analysis (Vitali et al 2023). However, in chronic persistent diseases, pathogen load or disease outcome can be problematic as indicators of disease susceptibility, as they rarely coincide with the co-infection or environmental drivers that induced them.

We first applied RT-qPCR and Nanostring^{TM} to quantify expression of 12 immune genes and additional KoRV, chlamydial and stress-associated genes in buffy coats of 108 koalas across two koala populations in differing climatic and co-infection contexts. Principle components generated were consistent with innate/adaptive immune systems. ANOVA or repeated measures MANOVA of principle components indicated that, relative to a comparatively healthy population south of Sydney, the declining and drought-affected population in western New South Wales had greater and more variable innate immune gene expression (e.g. IL-1β, IL-6, IL-8), and retroviral transcription. Additionally, among three sampling events within the western population, an extreme heat event (30d mean Max 37°C) was associated with lower expression of adaptive immune genes (e.g. CD3, CD4, CD8, IFNg, CD79b), upregulation of a stress pathway gene (FKBP5), and lower haematocrit and plasma protein (Fernandez et al 2024).

To further investigate relevance, similar data, collected from 95 rescued koalas on clinical admission, was subjected to PCA and unsupervised k-means clustering with Wards method. Clusters, arranged along the dominant dimension (from high to low expression of adaptive immune genes), aligned with increasing odds of poor outcomes at admission (regardless of the presenting disease), greater transcription of KoRV, FKBP5, Resistin and innate immune genes and, among *C. pecorum* infected koalas, lesser transcription of a chlamydial gene associated with suppression of replication (hsp60) (Muir et al, unpublished).

Together, these studies support further development of this approach to predict thresholds at which coinfection, climatic extremes and habitat degradation are likely to impact disease risk.

Vitali SD et al. (2023) University of Sydney. 194 pp. doi: 10.25910/xkkv-nk38.

Fernandez CM et al. (2024). Scientific Reports, 14(1), 7260.

12:05 - 12:20

Fatal *Sarcoptes scabei* and *Demodex* sp. co-infestation in wolves (Canis lupus) at Białowieża National Park, Poland as a consequence of climate change?

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Abstract

In winter 2021/2022, a wolf population in Białowieża Primeval Forest, an UNESCO Natural Heritage site, Poland was struck by an outbreak of severe cases of mange related to mixed infestation of mites: *Sarcoptes* and *Demodex*. Hereby, we present epidemiological analysis of mange causing significant morbidity and mortality of Białowieża's wolves. This is also the first characterization of demodicosis in wolves. We monitored potential factors that could have led to disease caused by these commensal skin parasites with limited pathogenicity. Climate alterations such as increase of air and ground temperatures, decrease of humidity, thinning of the snow cower and shortening of the period with snow cover in the recent years may have contributed to the emergence of mange by influencing ecology of wolves and their prey. Other potential drivers included the increase of the wolf population and migration crisis on the border between Poland and Belarus, which have started in 2021 with influx of thousands of refugees from Asia and Africa

through BPF, which may have affected general health status of wolves by stress-induced immunosuppression.

Lunch

12:20 - 13:20 Friday, 13th September, 2024
Brewery, Stralsund

Session VII – The Big Picture in Wildlife Diseases

13:20 - 15:05 Friday, 13th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Xavier Fernández Aguilar, Anja Globig

13:20 - 13:35

Avian Air Accident Investigations: why do Starlings crash land?

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Abstract

The Starling (*Sturnus vulgaris*) is a declining species in its native range. Understanding any factors that may be contributing to this decline is important.

Sudden occurrences of large-scale deaths are described in this species and reports often speculate associations with a variety of factors including buildings, water, vehicles, light conditions, poisons, predators, and adverse weather. Events are seldom witnessed but an incident in Wales in December 2019, led to the opportunity to thoroughly investigate the circumstances with the aim of learning as much as possible about the fatalities and likely causes.

It was hoped that through a site visit, collection of environmental data and information from local people, and detailed carcase examination and laboratory tests, the probable cause of the mortality could be determined.

A member of the public found over 200 starlings dead in a rural location. In the UK the public and other bodies are encouraged to report wild bird deaths to the Animal Health authorities. A proportion of these are collected for avian influenza surveillance and disease investigation at APHA. In this case the initial examination

of carcases was inconclusive, and a site visit was arranged. At the visit it was found that all the carcases on the rural road had been removed and disposed of by the Local Authorities. Searching the surrounding area including hedgerows found 34 birds suitable for examination.

The local area was examined with the local police, and details collated from witnesses. The police were contacted by multiple members of the public with information. Metrological data was obtained from the National Meteorological Office. Absence of aircraft activity at the time was described by the local Royal Air Force. The mass mortality event immediately received extensive media coverage with mounting speculation and theories put forward online.

The carcases were examined in detail for evidence of underlying disease. Laboratory testing included histopathology, bacteriology, toxicology, virology and bacteriology. In conclusion the proximate cause of death was blunt trauma, but the combined evidence from the investigation indicated the ultimate cause was fatal flight error by the murmuration associated with poor and deteriorating weather and light conditions, while attempting to roost 100m away from their natural wetland roost site. These cases are unlikely to be a significant conservation issue for the species, but the events are often associated with man-made structures and can result in widespread media coverage, public concern and significant welfare issues for the birds.

13:35 - 13:50

Lead (Pb) concentrations and associated pathology in tissues of Scandinavian brown bears (*Ursus arctos*) and One Health concerns

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Abstract

Lead (Pb) is toxic to wild mammals and other vertebrates. Previous studies using blood have shown that the Scandinavian brown bear population is exposed to Pb.

This study aimed to examine different tissue Pb concentrations and potential associated pathologies in Scandinavian free-ranging and captive brown bears (*Ursus arctos*).

Liver, kidney, bone, skeletal muscle, subcutaneous fat and spinal cord samples were collected from 56 free-ranging hunted brown bears and 26 captive brown

bears in Sweden. To avoid potential Pb nanoparticle contamination from the ammunition used in the hunted bears, the samples were collected as far from the wound channel as possible, and no organ surfaces were sampled. The samples were freeze-dried and analysed for Pb concentration using sector field inductively coupled plasma mass spectrometry. Liver and kidney tissues from 38 free-ranging bears, and liver, kidney and spinal cord tissues from 8 captive bears were stained with Mayer's haematoxylin and eosin and analysed histologically.

All 363 analysed tissue samples contained Pb, confirming that both the free-ranging and the captive brown bear populations in Sweden are exposed to Pb. The different tissue concentrations found in this study support that the Pb is likely distributed in the body in a three-compartment model, as seen in humans. However, no histopathological changes were identified on routine microscopic examination of liver, kidney and spinal cord tissues. Consequently, other ways to assess brown bear health effects from Pb exposure are needed.

Tissues from hunted Scandinavian brown bears are an easy source to access for ongoing Pb monitoring of environmental health. However, this study also showed that tissues from hunted Scandinavian bears pose a risk to human health if consumed. The majority of the tissue samples exceeded the maximum allowable Pb concentrations in meat and offal from domestic livestock, according to the current EU regulation. Additionally, 95% of the hunted bears were shot with Pb-based ammunition which then further increases the contamination of bear tissues with Pb. According to the World Health Organization, there is no safe level of Pb exposure. Pb continues to be a One Health issue and this study supports the need for regulations on Pb in wildlife/game tissues used for human consumption.

13:50 - 14:05

Ethical reflections around lynx in distress management in France

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Abstract

Despite its status of protected species since 1981, the Eurasian lynx (*Lynx lynx*) is still threatened with extinction in France. As part of a public action of conservation

in France, a multidisciplinary collective expertise was brought together. One of the tasks of the group was to improve the protocols of management of lynxes in distress. To this aim, the group first identified an ethical framework, to guide decision-making and act as consistently as possible from one context to another.

In general, conservation plans are mostly based on environmental ethics, which values the entire ecosystem ("ecocentric" system of values), whereas in animal ethics, the aim is to avoid or minimize the suffering of individuals ("pathocentric" system of values). With the growing consideration of animal welfare, our theoretical framework is based on the two emerging fields, "compassionate conservation" and "conservation welfare", which both represent the intersection of environmental and animal ethics and advocate better consideration of individuals in conservation plans. In practice, in addition to this theoretical framework, it is also necessary to take into account the logistical and regulatory constraints to make decisions.

For example, in conservation, decisions of intervening are often restricted to the compensation or repair of the damages caused to nature by human activities, to minimize interference with the functioning of natural ecosystems and natural selection processes. Thus only individuals which distress is caused by anthropogenic actions should be considered for interventions. For French lynx populations, this postulate suffers from several limits:

- As contemporary lynx populations in France result from reintroduction events following an extinction caused by human activities, their evolution cannot be described as natural.
- One of the objectives of the national conservation plan for this species is to increase population size and not just to maintain it.
- In practice, it is generally difficult to determine whether the cause of distress is of anthropogenic or natural origin.

Our ethical reflections therefore lead us to propose that it seems justified to intervene on any lynx in distress in France, breaking with previous intervention plans. Other issues have been discussed such as the management of lynxes that cannot be re-introduced, live prey distribution, etc. These considerations will thus contribute to future actions for the conservation of the Eurasian lynx and could be extended to other wildlife species with different conservation statuses.

14:05 - 14:20

Survey on viral pathogens and parasites of the respiratory tract of European wildcats (*Felis silvestris*) from south-western Germany

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Abstract

Background or context

The recent expansion of the European wildcat (*Felis silvestris*) in Germany is leading to more interactions between domestic cats and wildcats. Not only hybridisation but also the reciprocal transmission of pathogens may occur. This could lead to a threat to wildcat populations or they may act as a reservoir.

Hypothesis or objectives

The influence of viral pathogens on the health status of the European wildcat is currently almost unclear. Since the wildcat as a predator carries a large number of parasites, including lungworms, which may have a pathogenic effect, it is of interest to examine the population for those parasites as well. For the evaluation of both pathogen groups a larger number as a representation of the wild population is necessary.

Materials and Methods

The best method currently available for obtaining sample material from endangered species is to analyse dead-found animals. In this context, 138 wildcats were dissected. In the following105 effusion fluid samples and 20 Lung Tissue Extract samples as comparison were serologically analysed for viral pathogens typically found in domestic cats (Feline Herpesvirus (FHV), Feline Calcivirus (FCV), Feline Parvovirus (FPV), Feline Leukosis Virus (FeLV), Feline Coronavirus (FCoV), Feline Immundeficiency Virus (FIV)). In addition, 134 lung samples were analysed for the genome *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* using a new combination of methods.

Results

Antibodies or antigen of at least one virus were detected in 59% of the wildcats. The prevalences of the antibodies against FHV, FCV, FPV, FCoV and FIV were between 0% and 21.9%. The prevalence of antigen of FeLV was 27.6%.

The prevalence for *A. abstrusus* was 13.4% and for *T. brevior* 1.5%.

Conclusions

All viruses except FIV are present in the wildcat population studied. 59% of the wildcats studied had contact with at least one virus.

The prevalences for *A. abstrusus* and *T. brevior* are lower than in previous studies. This could be due to the arbitrary selection of the sampling localisation.

14:20 - 14:35

Rapid RNA and DNA nanopore sequencing for non-invasive avian influenza monitoring.

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Abstract

Avian influenza virus (AIV) currently causes a panzootic with extensive mortality in wild birds, poultry, and wild mammals, thus posing a major threat to global health. The migration of wild birds along long-distance flyways has led to substantial AIV transmission, also between wild and captive birds, and viral evolution. To efficiently monitor and control the spread of AIV and the emergence of new mutations, we established a genomics-based viral surveillance protocol that (*i*) relies on portable nanopore sequencing technology for rapid, in situ AIV profiling, even at remote locations; and (*ii*) allows for AIV monitoring from non-invasively collected environmental samples.

We first utilized a well-defined AIV strain to systematically investigate AIV characterization through nanopore sequencing by (*i*) benchmarking the performance of fully portable RNA extraction and viral detection approaches; (*ii*) comparing the latest DNA- and RNA-nanopores for in-depth AIV profiling; and (*iii*) evaluating the performance of various computational pipelines for viral consensus sequence creation and phylogenetic analysis at different levels of viral genome coverage. We finally validated our portable AIV surveillance set-up through

applications to real-world environmental samples, such as dust samples collected from a duck farm in France, passive and active air samples collected from a "vulture restaurant", and passive water samples collected from a wetland area in Spain.

Our results show for the first time that the latest RNA-nanopores can accurately genomically profile AIV from sequencing native RNA. We established that converting viral RNA to cDNA and employing a reference-based approach for consensus building is the most effective method for sequencing AIV in low-concentration samples, such as those from the environment. Additionally, our study introduces non-invasive monitoring techniques, including passive and active air sampling via liquid impingement and water sampling with torpedo-shaped devices, as superior alternatives to traditional AIV surveillance methods. These innovative approaches successfully detected AIV in wetlands and vulture restaurants, where conventional fecal sampling failed.

Overall, our study emphasizes the utility of portable nanopore sequencing technology for non-invasively and rapidly detecting and sequencing AIV in wild bird populations all around the world. We foresee the huge potential of this fast and automatable pipeline to monitor and profile various pathogens in wildlife to better understand their evolution and transmission patterns, and inform One Health risks at the intersection of human, animal, and environmental health systems.

14:35 - 14:50

Risk of rabies reintroduction into the European Union as a result of the Russo-Ukrainian war: a quantitative disease risk analysis

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University of Bristol, Bristol, United Kingdom

Abstract

Canine vaccination and the successful oral vaccination of wildlife against rabies has resulted in the eradication of terrestrial rabies from much of the European Union (EU). However, terrestrial rabies freedom in the EU is continually threatened by the importation of rabid animals from endemic areas, such as parts of Eastern Europe. Following the Russian invasion of Ukraine in 2022, the European Commission eased the rules on companion animal importations into the EU through derogations to the EU Pet Travel Scheme (EU PETS). This was to allow Ukrainian refugees to bring their pets as they fled the war. As rabies is endemic in Ukraine, this study aimed to quantitatively assess if changes in regulations affected

the risk of rabies introduction to the EU. Transmission pathways for both EU PETS and derogations to this scheme were considered, as well as the scenarios of 100% compliance and reduced compliance within both schemes. Stochastic modelling was performed via a Monte Carlo simulation to calculate the median and 95% confidence intervals of the annual risk of rabies entry and the years between rabies entries into the EU. The derogation scheme posed a lower risk of annual rabies entries in comparison to EU PETS across both compliance scenarios. Following 100% compliance, the risk was significantly lower at a value of 3.63 x 10^{-3} (CI95% 1.18 x 10^{-3} - 9.34 x 10^{-3}) rabies entries per year compared to 4.25 x 10^{-2} (CI95% 1.44 x 10^{-2} - 9.81 x 10^{-2}) with EU PETS. However, the derogation scenario was more sensitive to a reduction in compliance which resulted in a 74-fold increase in risk. The modelled derogation scheme, which included a 4-month period of quarantine, reduced the annual risk of rabies entry into the EU and may present a viable solution for the management of companion animal influxes in future crises. This is important for the protection of hard-earned freedom from terrestrial rabies across most of the EU, whilst at the same time providing a compassionate solution to refugees.

14:50 - 15:05

Experimental Borna disease virus 1 infection of greater white-toothed shrews (Crocidura russula) – a new insectivore and reservoir model

Dr Daniel Nobach[1,2], Leif Raeder[1], Jana Müller[1], Dr. Sibylle Herzog[3], Dr. Markus Eickmann[4], Prof. Dr. Christiane Herden[1]
[1]Institute for Veterinary Pathology, Gießen, Germany. [2]2Chemical and Veterinary Analysis Agency Stuttgart (CVUS), Stuttgart, Germany. [3]Institute for Virology, Gießen, Germany. [4]Institute for Virology, Marburg, Germany

Abstract

Background:
Borna disease virus 1 (BoDV1) infection causes a fatal, severe neurological disease accompanied by a nonpurulent meningoencephalitis in dead end host such as horses and humans. Numbers of human encephalitis cases caused by infection with BoDV1 are increasing continously. The reservoir host of BoDV1 is the bicoloured white-toothed shrew (*Crocidura leucodon*), in which a symptomless infection with viral persistence, widespread organ distribution, and viral shedding typically occurs. However, the route of transmission and virus-host interactions in the reservoir have not been analyzed so far.

Objective:

To establish an experimental reservoir model, 15 greater white-toothed shrews (*Crocidura russula*) were infected with a shrew-derived BoDV1 isolate by different inoculation routes.

Material and methods:
Adult male and female shrews were infected via five different inoculation routes (oral, intranasal, subcutaneous, intraperitoneal, intracerebral) with a group size of three animals each. Animals were monitored over 41 days.
At necropsy, a widespread organ panel was collected for RNA isolation, virus isolation, and detection of BoDV1 by immunohistochemistry (IHC) and BoDV1-RT-PCR. Blood was collected postmortem for detection of BoDV1-reactive serum antibodies by indirect immunofluorescence assay. Tissues were fixed in 4% neutral-buffered formalin, embedded in paraffin and processed for histopathology and IHC. RNA from oral swabs, skin swabs, and faeces was also extracted. Tissues were examined histologically for inflammatory and degenerative lesions. IHC was performed applying the monoclonal antibody Bo18 detecting the BoDV-1 nucleoprotein. Antigen distribution and number of infected cells was graded according to a semiquantitative score.

Results:
11/15 shrews were successfully infected via all routes except for the oral route. Seven animals displayed temporary reduced food intake and loss of body weight from day 21 on. All infected animals developed virus specific serum antibodies with titres between 1:20480 – 1:40960. There were no histological lesions in any organ investigated. Viral RNA was demonstrated by RT-qPCR in the central nervous system (CNS) and the majority of organs. Highest viral load was found in the CNS of intracerebrally infected animals. Immunohistochemistry demonstrated BoDV1 antigen in neurons and astrocytes in the CNS and salivary gland, skin and lung. Infectious virus was detected in the saliva at 41 dpi.

Conclusion:
In general, successful experimental BoDV1 infection of shrews indicates their usefulness as animal model, enabling further studies on maintenance, transmission, pathogenesis, and risk assessment for human spill-over infections.

Session VIII – Sustainability in Wildlife Health/One Health

15:05 - 15:20 Friday, 13th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Ana Vale, Thijs Kuiken (tbc)

15:05 - 15:20

The Sorrow of Empty Mountains

Dr. Bjørnar Ytrehus dr.med.vet. [PhD]
Norwegian Veterinary Institute, Ås, Norway

Abstract

The finding of a wild reindeer with chronic wasting disease (CWD) in Norway in March 2016 was totally unexpected and no one were prepared for a wildlife disease that may have such dramatic consequences. We, the wildlife disease scientists, responded as we are trained to and worked persistently to contain and if possible eradicate the disease. One of the solutions was to eradicate the affected wild reindeer population. This was completed in 2018. In 2020, however, a CWD-infected reindeer was found in another population. The management of CWD in Norway is currently in a "locked position" where different stakeholders and groups of scientists have diverging opinions about the measurements taken and which way to go forward, and the public debate partly is characterized by rigid and irreconcilable attitudes.

The objective of the current presentation is to reflect about what we, the scientists, have done during this period, which values that lie behind our actions, and which effects they may have caused.

First, we believe in the truth of natural science and the dissemination of knowledge in order to promote action. We tend to think that as long as we explain what happens and what a rational response is, people will act adequately. Here, we may have failed in not recognizing that people were emotionally disturbed - that they actually were in a state of sorrow and grieve, not motivated to comply and adhere to the authorities' measures.

Second, we believe that it is important to gather and publish new knowledge simultaneously as we perform disease management, in order to facilitate good management. However, scientific competition may be an obstacle for efficient and vital cooperation and mutual understanding, and the people in sorrow may feel that we exploit their situation for our own benefit.

Third, we believe that rapid action is necessary and emphasize the need for immediate implementation of measures. We fail, however, in communicating the deep time aspects of a disease as CWD: That the benefits of both our successful disease management and the eventual burdens of our failures, perhaps not will be seen in our life time. People's motivation for continued disease management consequently disappears after a few years.

In my opinion, empathy, decency and deep time thinking are qualities that should be encouraged in a situation of complex and long-lasting wildlife disease

management. This require continuous discussion and reflections over our own values and modes of action.

Coffee Break

15:20 - 15:50 Friday, 13th September, 2024
Brewery, Stralsund

Session VIII – Sustainability in Wildlife Health/One Health (cont.)

15:50 - 16:50 Friday, 13th September, 2024
Brewery, Stralsund
Presentation type Conference Session
Ana Vale, Thijs Kuiken (tbc)

15:50 - 16:05

Introduction to transformative change of wildlife health research

Thijs Kuiken
Erasmus MC, Rotterdam, Netherlands

Abstract

In its comprehensive global biodiversity assessment, completed in 2019, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) of the United Nations concluded that “the biosphere, upon which humanity as a whole depends, is being altered to an unparalleled degree across all spatial scales” and that “biodiversity is declining faster than at any time in human history”. Their main recommendation, ratified by over 100 national governments, stated that “goals for conserving and sustainably using nature and achieving sustainability cannot be met by current trajectories, and goals for 2030 and beyond may only be achieved through transformative changes, (…) a fundamental, system-wide reorganization across technological, economic and social factors, including paradigms, goals and values”. Given the above, the members of the EWDA Sustainability Committee thought of ways to stimulate transformative changes in wildlife health research. In discussion with other members of the WDA and EWDA, we thought a good starting point for making transformative changes might be to gauge what are the paradigms, goals and values we aspire to, that promote a sustainable way of living and conserve nature.

After looking through the IPBES report, we realized that our own WDA Charter of Values, which represent the basic goals and values that WDA members hold in common, provides suitable guidance. However, these goals and values only become meaningful if we act accordingly. To enable transformative changes in our research on wildlife health, we devised a number of guidelines. First, the formulation of research problems addressed in response to a wildlife health issue might be broader than is usually done in the current situation, giving as much attention to inorganic nature, ecosystems, wildlife, and, if applicable, to domestic animals and humans. Also, when formulating the research problem, one should consider ecological and social costs to society as well as financial costs. Second, the choice of methods employed to conduct scientific research should be determined not only by financial costs, but more importantly by their environmental impact. Third, solutions for addressing wildlife health issues should include not only short-term measures like the development of treatments, but also long-term measures that address underlying causes of wildlife health problems. Such transformative changes in wildlife health research should help to make the transition to a sustainable society and to improve health according to a One Health approach.

16:05 - 16:20

Brief guide for researchers to enable transformative changes

Josie Chambers
Urban Futures Studio, University of Utrecht, Utrecht, Netherlands

Abstract

There is an urgent need for more transformative approaches to addressing persistent societal problems such as hunger, climate change, biodiversity loss, unsustainable growth, poverty and social inequality. While there is growing consensus that transformative change is needed, there is less agreement about what it entails and how it can be achieved. It is also not clear what the role of research is in informing and fostering transformative change. Our research—based on interviews and dialogues with 200+ diverse researchers across Wageningen University & Research—shows that achieving an outcome of transformative change fundamentally depends on the process of working together in different, more transformative ways. We identified four ways of working that were consistently seen to enhance the transformative potential of research were: (1) Pluralizing – finding common ground in ways that foster respect and learning across diverse beliefs, values and goals; (2) Empowering – building individual capacity to act, and collective momentum to move towards transformative visions; (3) Politicizing –

becoming politically aware and engaging with power in ways that accelerate rather than block systems change; (4) Embedding – integrating research and learning into every day decision making without compromising its moral and intellectual independence. By increasing your knowledge, confidence and ability to apply transformative processes and methods in your own research, it is better possible to navigate emerging challenges and to maximize the beneficial impacts of your current and future research.

16:20 - 16:35

Pathogen spillover driven by rapid changes in bat ecology

Dr Peggy Eby PhD[1], Dr. Alison Peel PhD[2], Dr. Andrew Hoegh PhD[3], Msc. Wyatt Madden Msc[4], Dr. John Giles BSc, MSc, PhD[5], Prof Peter Hudson Phd[6], Dr Raina Plowright PhD, MS, BVSc[7]
[1]Centre for Ecosystem Science at the University of New South Wales, Sydney, Australia. [2]Griffith University, Brisbane, Australia. [3]Department of Mathematical Sciences, Montana State University, Bozeman, USA. [4]Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, USA. [5]Institute for Health Metrics and Evaluation, University of Washington, Washington, USA. [6]Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, USA. [7]Cornell University college of Veterinary Medicine, Ithaca, USA

Abstract

During recent decades, pathogens that originated in bats have become an increasing public health concern. A major challenge is to identify how those pathogens spill over into human populations to generate a pandemic threat. Many correlational studies associate spillover with changes in land use or other anthropogenic stressors, although the mechanisms underlying the observed correlations have not been identified. One limitation is the lack of spatially and temporally explicit data on multiple spillovers, and on the connections among spillovers, reservoir host ecology and behaviour and viral dynamics. We present 25 years of data on land-use change, bat behaviour and spillover of Hendra virus from Pteropodid bats to horses in subtropical Australia. These data show that bats are responding to environmental change by persistently adopting behaviours that were previously transient responses to nutritional stress. Interactions between land-use change and climate now lead to persistent bat residency in agricultural areas, where periodic food shortages drive clusters of spillovers. Pulses of winter flowering of trees in remnant forests appeared to prevent spillover. We developed integrative Bayesian network models based on these phenomena that accurately

predicted the presence or absence of clusters of spillovers in each of the 25 years. Our long-term study identifies the mechanistic connections between habitat loss, climate and increased spillover risk. It provides a framework for examining causes of bat virus spillover and for developing ecological countermeasures to prevent pandemics.

16:35 - 16:50

Effects of sea level rise on vector borne disease in Delta regions

Dr. Reina Sikkema DVM, PhD[1], Jordy van Beek MSc[2], Maarten Boonekamp MSc[3], Tijmen Hartung MSc[1], Dr. Stephan de Roode PhD[3], Thom Bogaard PhD[3], Eldar Rakhimberdiev PhD[4], Henk van der Jeugd PhD[5], Prof Pier Siebesma PhD[3], prof Marion Koopmans DVM, PhD[1], Maarten Schrama PhD[2]
[1]ErasmusMC, Rotterdam, Netherlands. [2]Leiden University, Leiden, Netherlands. [3]TU Delft, Delft, Netherlands. [4]University of Amsterdam, Amsterdam, Netherlands. [5]NIOO-KNAW, Wageningen, Netherlands

Abstract

One of the fast-expanding global health threats is the risk of arboviral infections. Arboviruses are transmitted by mosquitoes, ticks or other vectors, and the potential for outbreaks results from a complex interplay of factors. Hitherto, there has been a major focus on factors directly related to climate change (temperature, precipitation), but much less is known about the indirect effects of climate change such as sea level rise and salinization, which can cause the upwelling of salt water from the subsoil, as well as the, intrusion of salt water in river deltas. This is is projected to increase sharply in the coming decades, which is of particular concern in low lying, densely populated delta areas. As a consequence of salinization and changing precipitation patterns, the surface area with water storage and buffer areas will increase. Both the salinization as well as wetting of lands are expected to have large impacts on birds and mosquitoes, both of major importance to disease transmission of arboviruses.

In this transdisciplinary frontrunner project we aim to answer the following question: **How will climate change, subsequent mitigation measures and increase in salinization affect mosquito and bird populations and their role in direct or indirect transmission of zoonotic pathogens to humans?** This will be done in a by connecting the fields of climate change modelling, water systems modelling, landscape ecology, animal ecology, insect ecology, virology and public health, using the Meuse/Rhine delta as a model system. Via historical datasets, changes of mosquito distributions, their predators and bird populations as a consequence of salinization and wetland development will be studied. Effects

of salinization on mosquito development rate, mortality, longevity and dispersal capacity will be assessed in mesocosms, and vector competence for WNV and USUV will be assessed in laboratory infection experiments. The role of birds as bridge vector between newly developed wetlands and urban areas will be further assessed by tracking studies and live bird sampling is will be used to further understand the role of different species as amplifying host. This data will be combined with fine scale climatic data to understand and predict future and present arbovirus risks. Lessons learned in this PDPC frontrunner project in the Rhine/Meuse delta are likely of great use for future developments in the other (densely populated) delta regions.

Session VIII – Panel Discussion: Sustainability in Wildlife Health/One Health

16:50 - 17:20 Friday, 13th September, 2024
Brewery, Stralsund

Panel Discussion
Josie Chambers, Peggy Eby, Reina Sikkema, Graham Smith, Baetriz Rubio Alonso, Sascha Knauf

Closing Session

17:20 - 17:45 Friday, 13th September, 2024
Brewery, Stralsund

Ozeaneum Open Museum

18:00 - 19:00 Friday, 13th September, 2024

Conference Banquet Dinner

19:00 - 23:00 Friday, 13th September, 2024
Ozeaneum, Stralsund

Post-Conference Tour

10:00 - 23:00 Saturday, 14th September, 2024

POSTER

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Vaccinating wildlife in a post-cull landscape

Dr Clare Benton BSc, MSc, PhD[1], Dr Jess Phoenix BSc, MA, PhD[2], Dr Andrew Robertson BSc, PhD[1], Professor Dez Delahay BSc, PhD[1]

[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Context

Where wildlife populations are implicated in the transmission of disease to humans or livestock, management interventions may include culling or vaccination of wild hosts. This is the case in several European countries for the management of bovine TB (caused by infection with *Mycobacterium bovis*), where a range of wildlife species may contribute to transmission to livestock populations.

In the UK, the European badger is widely considered to be the primary wildlife reservoir of *M. bovis* and populations have been managed via various culling interventions, most recently led by the farming industry. A licenced injectable vaccine for use in badgers (BadgerBCG) has been available for over a decade and as industry culls run their course, a transition to badger vaccination has been proposed, with the aim of reducing transmission amongst badgers and hence risks for cattle during population recovery. However, considerable scepticism exists within the farming industry that trapping a low density, highly mobile badger population immediately following a cull will be practically achievable.

Objectives and Methods

In this talk we present practical insights from the first three years of vaccinating badger populations immediately following the cessation of culling. We describe adaptations made to field practices to achieve rates of trapping success comparable to those in unculled populations. Using an established method, based on capture-mark-recapture, we estimate the size of badger populations in vaccination areas and from that derive the estimated proportion that is accessible to vaccination over two nights of consecutive trapping.

Results

Encouragingly, estimates of the proportion of badgers vaccinated in recently culled populations were similar to those expected for unculled populations. Densities of badgers vaccinated varied widely amongst previously culled areas which may be attributable to differences in pre-cull starting densities and culling duration as well as seasonal effects and inter-year differences in breeding success within badger populations.

Conclusions

Our results demonstrate that trapping and vaccinating badger populations is feasible even closely following the cessation of culling. We highlight subtle but important adaptations to field practices to ensure trapping success is maintained when working in low density, more mobile populations. Our findings provide important evidence to counter industry scepticism surrounding the feasibility and practicality of rolling out badger vaccination over large areas as a post-culling intervention.

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Uncovering the contribution of environmental and ecological factors to predicted highly pathogenic avian influenza risks in wild birds across Europe

Sarah Hayes[1], Joe Hilton[2], Joaquin Mould-Quevedo[3], Christl A. Donnelly[1], Matthew Baylis[2], Liam Brierley[2]

[1]University of Oxford, Oxford, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]CSL Seqirus, Summit, USA

Abstract

Background

The current epidemic of highly pathogenic avian influenza (HPAI) is the worst ever recorded with devastating effects on the poultry industry and wild bird populations. Wild bird hosts are natural reservoirs for avian influenza and an important route of transmission to livestock and wildlife. Despite this, the dynamics of HPAI within wild bird hosts remains poorly understood.

Objectives

The aims of this study are to use species distribution models to estimate the spatial risk of HPAI in wild birds across Europe, identify high-risk areas, and

explore the importance of environmental and ecological traits of potential wild bird hosts in predicting the risk of HPAI in wild birds.

Methods

Machine learning methods (Bayesian additive regression trees), are used to model the presence of HPAI at a 10km resolution. Models are trained using a dataset of over 10,000 geospatial records of HPAI from 53 wild bird families, adjusting for national sampling strategies. In addition to incorporating the environmental variables previously reported as predictors of avian influenza, we include variables capturing the dynamics of wild bird ecology, such as phylogenetic diversity, species richness, and habitat and diet preference. Four separate models are used to reflect the seasonality within bird population movements and models are validated on outbreaks excluded from model training.

Results

Risk predictions, along with their associated uncertainty, of HPAI in wild birds across Europe are generated. Model outputs align with current understanding in highlighting coastal regions and inland waterways as high-risk areas. Of the environmental variables, altitude and temperature are important predictors in all models, consistent with previously published studies. Adding ecological variables, including species richness and feeding behaviours, improves model predictions, although the importance of individual factors varies by season.

Conclusions

As well as having serious impacts on wildlife and the livestock industry, HPAI has important public health implications as a potential source of human pandemic influenza. Improving our understanding of HPAI in wild bird hosts is a key component of the One Health strategy that is vital in controlling this disease. The models presented here align well with real-world HPAI reports and offer a framework that can be utilised to uncover the spatial determinants of HPAI. These models could be used as an aid in planning effective surveillance strategies for this and future avian influenza epidemics.

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Insights on highly pathogenic avian influenza H5N1 epidemiology in newly affected species: understanding the large-scale contamination of southwestern European Griffon Vultures

Julien Hirschinger[1], Ursula Höfle[2], Alberto Sánchez-Cano[2], Claire Guinat[1], Guillaume Croville[1], Marta Barral[3], José Antonio Donázar[4], Chloé Le Gall-Ladevèze[1], Mathilda Walch[1], Vega Alvarez[3], Xeider Gerrikagoitia[3], Louis Du Plessis[5,6], Simon Dellicour[7], Eneko Arrondo[8], José Antonio Sánchez-Zapata[8], Ainara Cortés-Avizanda[4,9], Sara Minayo[2], Jérémy Tornos[10], Samuel Perret[10], Thierry Boulinier[10], Anne Van De Wiele[11], Jean Luc Guerin[1], Olivier Duriez[10], Guillaume Le Loc'h[1]

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Abstract

Since 2021, an unprecedented epizootic of HPAI is under way on a global scale, following the emergence of a H5N1 HPAI virus variant from clade 2.3.4.4b. This epizootic is marked by a profound change in epidemiology with a loss of seasonality and an increase in geographic range and host species. While millions of domestic birds have died or have been culled, the number and diversity of wild bird species affected in often devastating outbreaks warrant a significant impact on avian biodiversity.

Southwestern Europe serves as a stronghold for several vulture species, including Griffon vultures (*Gyps fulvus*) whose southwestern European distribution represents approximately 75% of the global population.

Due to their ecology, vultures have been considered less likely exposed and less susceptible to HPAIV. However, abnormal mortality of nestling and adult vultures was detected in France and Spain in spring 2022, and confirmed to be due to

H5N1 HPAIV. Leveraging long-term monitoring of populations in both countries, we studied the epidemiology of HPAIV in a hitherto unaffected species.

Specifically, we endeavored to determine the origin of contamination in the southwestern European Griffon vulture population and to assess the magnitude of viral spread by combining virological, serological and ecological data obtained from field samples of individuals from France and Spain.

While we did not detect any virus in live birds from late summer 2022 to autumn 2023, indicating that the virus was no longer circulating in Griffon vulture populations at the time of sampling, antibodies were detected in all sampled colonies, with local variations in seroprevalence. The analysis of sequences obtained from dead vultures, as well as from other wild and domestic birds, revealed different introduction events into the vulture populations, with one being the origin of most of the cases in France and Spain, potentially originating from wildlife in Spain. None of the vulture sequences were related to poultry.

Overall, we highlighted a short but intense viral circulation in Griffon vulture. We hypothesize one major introduction event, probably from Spanish wildlife, followed by widespread dissemination to the entire population due to the ecology of the species that is characterized by large range movements connecting France and Iberia. Moreover, we confirmed that H5N1 HPAIV should be considered a severe threat to endangered bird species, especially those with colonial and scavenging behavior.

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Genomic-based health-screening of reintroduced *Emys orbicularis*

Carolin Eichert[1], Johannes Meka[2], Damian Baranski[2], Jean-Yves Georges[3], Benoît Quintard[4], Kathrin Theissinger[2]

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Abstract

1. Background

Species reintroductions are considered promising strategies for limiting biodiversity erosion. Yet, translocations of captive-bred individuals into the wild rely on their capabilities to adapt to new conditions. Genomic-based health-screening of gut microbiome can provide important insights into eco-evolutionary adaptations. Indeed, the communities of microorganisms coinhabiting an animal are highly driven by environmental factors and ultimately influence individual fitness.

1. Objectives

Within our project Emys-R (www.emysr.cnrs.fr), where we investigate best practices for European pond turtle (*Emys orbicularis*) reintroductions, we establish guidelines for health screenings in this species. We infer whether changes in diet between captivity and after translocation affect the turtles´ microbiome. Further, we hypothesize that turtles with most diversified microbiomes are less infected with pathogens and have better fitness.

1. Methods

We screen the gut microbial community of *E. orbicularis* from different breeding facilities via fecal samples, cloacal and combined oral-cloacal swabs, using a shotgun metagenomic approach.

1. Results

We aim at defining 1) an original index of individual health based on the microbial composition, complementary to biometric and genetics indices; 2) guidelines for sampling techniques in the field and 3) the most appropriate diet prior translocation for ensuring best adaptive capacities of the individuals after translocation.

1. Conclusion

As the connection of the microbiome and host health are of high importance, equipping hosts with set of beneficial bacteria and creating conditions to maintain that flora can be integrated in continuous health screenings of translocated species as an efficient tool to ensure long-term reintroduction success of captive-bred individuals.

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Exotic Pet Owner's Perceptions of Veterinarian's Communication on Zoonoses and AMR

Amelie Lisa Arnecke[1], Dr. Katharina Charlotte Jensen[2,3], Dr. Antina Lübke-Becke[4,5], Univ.-Prof. Dr. Stefan Schwarz[4,5], Univ.-Prof. Dr. Mahtab Bahramsoltani[1]

[1]Institute of Veterinary Anatomy, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany. [2]Institute for Veterinary Epidemiology and Biostatistics, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany. [3]Clinic for Cattle of the University of Veterinary Medicine Hannover, Hannover, Germany. [4]Institute of Microbiology and Epizootics, School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany. [5]Veterinary Centre for Resistance Research (TZR), School of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Abstract

The wildlife trade, including exotic pet trade, was identified as one of five major contributors to the spread of pathogens. In addressing the challenges posed by zoonotic pathogen transmission, a critical imperative emerges - the necessity for preventive measures and behavioural changes. Veterinarians hold the expertise in animal health but also bear a distinct responsibility to the public. Hence, they could potentially serve as allies in curbing the exotic pet trade by combating disinformation and misinformation.

In our study, exotic pet owners in Germany were surveyed on animal husbandry, veterinary consultation and risk communication. To evaluate the perception of communication, we used questions related to Schulz von Thun's 4-sides model and derived a score from the mean value of the model. The maximum of four represents the optimal score and stands for the perception of excellent communication quality. The survey, conducted via LimeSurvey from December 2022 to April 2023, was promoted on social media.

A total of 344 exotic pet owners who kept reptiles, exotic mammals, birds, insects, arachnids, fishes, amphibians and other exotic animals, completed the survey. In total, 20% stated that at least one of their animals was imported from abroad and 3.5% reported a wild catch. Even though 36% of the participants reported the presence of vulnerable groups in their household, 49% fed raw meat and 46% permitted access to shared living spaces. During veterinary consultation within the last 12 months, 8% of participants reported the detection of pathogens with zoonotic potential. Among participants who consulted the veterinarian for a routine check-up, 9% received information on zoonotic pathogens and 7% on

AMR. The communication during these check-ups was rated with 3.8. Interestingly, the communication was perceived significantly worse during visits due to specific symptoms with pathogen detection. While the presence of vulnerable groups did not result in a higher communication score, longer duration of veterinary-patient relationship correlated with better communication ratings.

In conclusion, there is a need for enhanced information on zoonoses and AMR in veterinary communication. The duration of the relationship positively correlates with better communication perception, but pathogen detection and other risk factors pose challenges, indicating the importance of targeted risk communication. Thus, veterinarians can empower clients, promoting responsible pet ownership and potentially reducing the demand for exotic pets. Consequently, this research offers insights into leveraging communication for enhanced preventive measures within the One Health framework, fostering behavioural change through risk awareness.

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Processes for strategic development of a national wildlife health program in Thailand

Dr Sarin Suwanpakdee DVM, PhD[1], Witthawat Wiriyarat DVM, PhD[2], Dr Walasinee Sakcamduang DVM, PhD[2], Dr Anuwat Wiratsudakul DVM MVPH PhD[1], Dr Nareerat Sangkachai DVM MSc[1], Dr Serena Elise George DVM[3], Moniek Smink BSc[3], Dr Craig Stephen DVM PhD[4], Dr Jonathan Sleeman B Vet Med, MA[5]

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Abstract

In response to the growing needs and expectations for coordinated wildlife health programs, a collaboration between Mahidol University, the US Geological Survey National Wildlife Health Centre and Canadian Wildlife Health Cooperative, set out to facilitate the creation of the Thailand Wildlife Health Network. This work was undertaken as part of a World Organisation for Animal Health twinning program. Development of the Network went through 5 steps. (1) Understand needs and obstacles to action as perceived by potential network members and stakeholders. (2) Create a value proposition that explained how the Network could meet the needs identified in step 1. (3) Fill gaps in network capacity through training. (4)

Develop network leadership skills and perspectives needed to sustain the network. (5) Collective strategic planning and outreach to secure collaborative agreements and support. Relationships and trust between organizations was developed through inclusive engagement in training and planning to ensure mutual benefit. The project benefitted from strategic planning methods including key informant interviews, surveys, gap assessments and logic models. A participatory approach was used to develop a theory of change that helped identify core inputs and activity to achieve the desired outcomes and to help explain the Network to potential partners and funders. Despite much of this work being conducted virtually due to the COVID-19 pandemic, periodic project evaluations revealed participant satisfaction with the training and planning efforts.

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Causes and temporal trends on admissions of wild nocturnal raptors to a rehabilitation centre in Portugal (2005-2023)

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Abstract

Seven species of nocturnal birds of prey can be found in Portugal. Although the majority prefer rural environments, some species can often be found on the outskirts of cities. They face numerous threats such as direct/indirect poisoning, habitat destruction, electrocussion, road collision or human persecution. *Asio flammeus* is considered Vulnerable and *Bubo bubo* is considered Near-threatened according to the Portugal Red Book of Vertebrates. Due to their high trophic position, they can be excellent bioindicators of ecosystem health. This study investigates the admission causes, and outcomes of nocturnal birds of prey admitted to a wildlife rehabilitation centre in Northern Portugal. The data were obtained from the archives of the Wildlife Rehabilitation Centre (WRC) in North Portugal. For 18 years (2005-2023), 757 animals were admitted: 53.8%(n=408) *Strix aluco,* 20.5%(n=155) *Athene noctua,* 19.2% (n=145) *Tyto alba*, 3.3% (n=25) *Bubo bubo,* 2.8% (n=21) *Asio otus*, and 0.4% (n=3) *Asio flammeus.* In 2016 (n= 71) was the year with more admissions. Spring (36%, n=273) and Summer (34%, n=261) were the seasons with more admission. Data show that *A. noctua* and *S. aluco* admissions to WRC have increased, while *T. alba* and *A. otus* decreased. This information leads to the suspicion that *S. aluco* is finding new niches in urban environments, compared to other species such as *T. alba* that were common in human infrastructure. This can related to many factors, such the decrease in agriculture areas and the increase in florest. The main causes of admission to the centre were 348 nestlings, 239 unknowns, 47 run over by a vehicle, 40 captures, 38 transferences from other centres, 18 collisions with infrastructures, 10 captivity, 8 electrocutions, 6 gunshots, and 1 predation. Analysis of outcomes data showed that 53% of the animals that arrived at the centre were rehabilitated and released back into the wild. Thirty-eight per cent of the animals died during treatment. Although the data is limited, it can provide valuable information. The rehabilitation and release of admitted birds to the wild can help buffer the negative effects of anthropogenic and non-human activities, especially for those species that have conservation concerns. Further study needs to be conducted to improve and define predictive indicators of survival, the possibility of release and threats.

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A retrospective study on morbidity and outcomes in free-living raptor populations admitted at a wildlife rehabilitation centre in North Portugal between 2005-2023

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Abstract

Birds of prey play a crucial role in maintaining ecological balance and contributing to the ecosystem's health. The causes of morbidity and mortality of free-ranging raptors range from anthropogenic (e.g., collision, gunshot, electrocution) to dynamic environmental conditions that may affect habitat suitability, prey availability or disease outbreak. Portugal is home to several species of birds of prey and hosts migratory species. some of them migratory, endangered or with other priority conservation statuses of concern. Wildlife rehabilitation centres (WRC) offer important insights regarding the threats associated with raptor casualties. A retrospective study was conducted to identify the causes of morbidity and outcomes of free-living diurnal raptors in WRC in northern Portugal, from 2005 to 2023 (vultures were excluded from this study). The study included 878 diurnal raptors, representing 17 different species. The most common species admitted were Common Buzard (*Buteo buteo)* (41%, n=364), Eurasian sparrowhawk *(Accipiter nisus)* (16%, n=145), and Northern goshawk (*Accipiter gentilis)* (8%, n=79). During this period the species considered Endangered according to the Red Book Vertebrates of Portugal were Montagu's harrier (*Circus pygargus)* (n=50), Golden Eagle (*Aquila chrysaetos) (n=2)*, and a Critically Endangered Spanish imperial eagle (*Aquila adalberti*) (n=1). The number of admissions in 2022 (n= 87) is the year with more. All animals were collected in localities from the north of Portugal. The main causes of admission to the centre were 388 unknowns (no information is available regarding the cause), 114 transferences from other centres (animals that came from other centres without admission information), 107 gunshots, 63 electrocutions, 47 nestlings, 25 captures (animals capture in urban areas), 32 captivities (animals that were in illegal captivity during a period), 33 born WRC, 31 collisions with infrastructures, 25 run over by a vehicle, and 6 apprehensions. Analysis of outcomes data showed that 38% of the animals were rehabilitated and released back into the wild and that 54% of animals died during treatment. The long-term epidemiological research conducted at the WRC determined the main environmental and anthropogenic causes of morbidity in wild raptor populations of Northern Portugal. Identification of potential anthropogenic threats and their impact on the morbidity and mortality of raptors can provide important information regarding high-risk areas associated with raptor casualties helping to implement in the future conservation efforts for the raptors. Therefore in the future it will be possible to work with authories and the public to decrease the risk factors to free-living raptor population.

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Congenital anomalies found in American crocodile (*Crocodylus acutus*, Cuvier, 1807) from Colombia

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Abstract

American crocodile (*Crocodylus acutus,* Cuvier, 1807) (Class Reptilia, Family Crocodylidae) is a species of crocodile that inhabits the Neotropics. According to the International Union for Conservation of Nature (IUCN) Red List is considered a Vulnerable. Congenital defects have been described in almost every vertebrate group. In crocodiles, teratology alterations have been described in captive animals (pets, zoos, farms) such as *Crocodylus niloticus* or *Gavialis gangeticus*. The present study aimed to characterize congenital malformations of an *C. acutus* from a hatchery in Colombia. From the eggs examined a total of 43 different types of anomalies were observed. Limbs and tail malformation (31.3%) were the most common changes observed, followed by body (28.9%) and head (25%). Possibly these malformations are associated with a combination of many factors, such as the age of the females, inbreeding, errors in incubations, nutrition and chemical pollutants. It's important to note that research in teratology not only enhances our understanding of the biology of crocodiles but also plays a role in their conservation and management, helping to ensure the long-term viability of these species in their natural habitats. Identifying the causes of developmental abnormalities can contribute to conservation efforts by addressing potential threats and improving management strategies. Although other studies have reported the occurrence of malformations in crocodiles, there is still a large gap concerning the classification of these malformations and the frequency with which they occur. Examining abnormal development can also offer insights into the genetic diversity of crocodile populations, potential genetic stressors, and alterations in the incubation parameters.

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How effective and efficient is your surveillance scheme?

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Abstract

Background: Across Europe, during the last 30 years, a significant number of national wildlife disease surveillance schemes have been set up and developed. These play an important role in the previously neglected assessments of the involvement of wild animals in the epidemiology of diseases important to humans, domestic and wild animals, assessment of the impact of diseases on wildlife populations and in the examination of the potential role of wildlife disease as a portent of environmental change. Evaluation of disease surveillance schemes is required to determine if they are meeting their objectives effectively and efficiently. The schemes differ from country to country in their surveillance approaches and their objectives but share many commonalities. The EWDA and WDA, often in conjunction with WOAH, have worked hard to support these national wildlife disease (or health) surveillance schemes in Europe, however regular evaluations are required if improvements are to be identified and implemented. Evaluation should benefit the funder and policy holder (often government but not invariably). The question therefore arises as to how best to evaluate wildlife disease surveillance?

Objectives: The aim is to discuss how national wildlife disease / health surveillance schemes in Europe may be improved in their efficiency and ability to meet their objectives, by the use of regular evaluations. We summarise what has been achieved by these schemes and suggest how they may be developed in line with the increasingly diverse wildlife health surveillance questions and One Health priorities.

Methods and Results: We provide a short review of several evaluation approaches that may be appropriate to wildlife disease surveillance. The presenter, drawing from his PhD research (title *'National wildlife health surveillance: evaluation of current practise and development of surveillance fit for the future'*) will use examples to illustrate approaches and results from wildlife disease surveillance evaluations.

Conclusions: Epidemiologists across the health surveillance disciplines consistently encourage the use of structured evaluation processes following published formats. Published examples of wildlife disease surveillance evaluation are uncommon and it is tempting to conclude therefore that they may not be essential. However, as the schemes mature with time, the requirement for formal evaluation, particularly by government funders keen to see accountability, will certainly increase. This presentation will illustrate the benefits of engaging in wildlife disease surveillance evaluation to encourage a greater uptake among the wildlife health community.

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The effects of West Nile Virus and their economic factors

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Abstract

The West-Nil-Virus (WNV) is a mosquito-transmitted virus and can lead to severe infections and illness in humans, horses and birds. Most of infections are asymptomatic, but about 20% of both infected humans and horses develop symptoms like fever. Around 1% fall ill neurologically as a result of encephalitis or meningitis. Treatment of a WNV-infection is to date only symptomatic.

To prevent the spreading of WNV, control measures can be implemented, for example the reduction of the mosquito population or the vaccination of horses. For humans, no vaccine is approved yet. To prevent human to human transmission, blood donors can be tested.

For planning any disease control program, it is essential to assess the impact of the disease as well as the costs for control measures. Because of the lack of information in Germany, the aim of this study is to get an overview on research assessing the impact of WNV. This will be used to develop an economic model for Germany in order to assess the costs and benefits of disease control strategies.

A literature research was carried out for WNV and economic in three databases, namely "Web of Science", Scopus" and "Pubmed". After removing duplicates and non-relevant publications, 78 paper remained for analysis.

These publications were analysed in terms of financial impact with regard to treatment costs, control measures and prevention. In addition, we examined the connections between the authors, topics and local relations.

Most of the studies address costs of illness and eventual benefits of different control measures in the US. Those specify mostly on medical costs and the created productivity loss in humans.

Publications from Italy and Belgium focused on the financial burden in the horse sector and the surveillance in animals and humans.

Costs of treating infected people vary greatly, from 21-74€/case (diagnostics only) up to 418.000€/case for inpatient treatment of patients with neuroinvasive West Nile infection.

The studies mostly analysed the costs and control measures retrospectively. A large proportion of the studies are country-specific because of the unique health and surveillance systems. They aim primarily for economic costs and productivity loss, but demonstrate rarely the impact of deaths in connection with WNV or the influence on the environment.

In conclusion, data of the impact of WNV is scarce and dependent on the origin and extend of the study. For future work with the data, assumption for some cost fields have to be made.

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Initial findings on the occurrence and distribution of *Baylisascaris procyonis* in raccoons in central France

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Abstract

Baylisascaris procyonis is the etiological agent of larva migrans in humans (neural, ocular, visceral or asymptomatic), which can lead to severe neurological sequelae and rarely fatal cases. The raccoon (*Procyon lotor*) is the primary definitive host of the nematode, harbouring adult worms in its small intestine. Female worms shed millions of eggs into the environment in the raccoon’s faeces. Transmission to paratenic hosts (such as rodents, birds and rabbits) or to humans occurs through

accidental ingestion of embryonated eggs. Raccoons acquire the infection by the same route or by predation on paratenic hosts. Raccoon is an invasive species in Europe. The presence of *B. procyonis* in wild raccoons has been reported in several countries in Western Europe. In France, raccoons are currently established in three separate and expanding populations, in the north-east, central (Auvergne) and south-west of the country. They are the result of at three independent voluntary or accidental releases of a few individuals since the 1960s, the 1990s and 2007, respectively. In recent surveys, we have found no evidence of *B. procyonis* in 92 and 206 raccoons in the south-west and north-east populations respectively, with the exception of a single infected animal on the north-east border of the north-east population, which is under further investigation. In parallel, thanks to local trappers, we are investigating the presence of *B. procyonis* in Auvergne, where the status of raccoons was previously unknown. The aim of this study is to gain knowledge about the presence of the parasite and, if necessary, propose preventive measures to limit human exposure. The study began in 2023, and 24 raccoons have already been caught by trappers (4 females, 20 mâles ; 5 juveniles and 19 adults), frozen and sent to the laboratory. Necropsy and opening of their intestines revealed the presence of *B. procyonis* (morphological and molecular identification) in 16 individuals (66.7%) from 11 different municipalities in the region. The study will be continue in 2024 and all the parasitological data obtained up to mid-2024 will be presented, as well as a first map of the parasite's distribution in Auvergne. These preliminary results already indicate local environmental contamination by *B. procyonis* eggs in areas frequented by raccoons in Auvergne, and a local risk for humans from ingestion of parasite eggs present in these areas, justifying preventive information for people in contact with raccoons (particularly trappers and hunters).

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Pathology and viral tissue distribution of high pathogenicity avian influenza H5N1 in wild black-headed gulls (*Chroicocephalus ridibundus*) in France.

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Abstract

The emergence of new viruses from the high pathogenicity avian influenza (HPAI) H5Nx clade 2.3.4.4b lineage over the last three years has deeply changed the spectrum of wild birds species affected. Colonial marine birds have been especially impacted by recent outbreaks and became new hosts contributing to epidemiology of HPAI. During the winter 2022-2023, massive mortality events involving black-headed gulls (*Chroicocephalus ridibundus*) were recorded across the entire French territory thanks to the mortality wildlife surveillance network. AMany French wildlife rehabilitation centers have also faced an increased number of submissions of sick birds. Both mortality and morbidity were ultimately attributed to HPAI.

In order to better understand the potential epidemiological role played by black-headed gulls, as well as the impact of HPAI virus infection in this species, a study of pathological modifications and viral tissue distribution associated with HPAI H5N1 clade 2.3.4.4b virus was conducted.

Thirteen birds naturally infected with HPAI virus, collected in the North-West of France between January and February 2023, were selected. Affected birds were found dead or euthanized after exhibiting neurological signs. A complete necropsy was performed and sections of feathered skin, nasal cavity, eyelid, eye, brain, trachea, lung, heart, skeletal muscle, liver, spleen, kidney, digestive tract, gonad, nasal, adrenal, Harderian and thyroid glands were sampled and routinely processed for histopathology. Slides were stained with hematoxylin and eosin and an anti-influenza A virus nucleoprotein immunohistochemistry.

Gross lesions included pancreatic necrosis (4/8) and multiple hemorrhages in mediastinum (2/8), meninges (1/8), periocular tissues (1/8) and breast muscles (1/8). Mild to severe necrotizing and/or lymphoplasmacytic encephalitis (13/13), endophthalmitis (10/13) and pancreatitis (9/13) were the most consistent findings identified at histopathology. Viral antigens were most commonly detected in brain (13/13), eye (10/13) and pancreas (8/13) followed by uropygial gland (4/7), lung (3/13), feathered skin (2/13), visceral ganglia (2/13) and adrenal gland (1/9).

This is the first report of ocular lesions associated with HPAI H5N1 natural infection in black-headed gulls. Furthermore, results suggest the role of the integumentary system and specifically the uropygial gland and feathers as potential shedding routes of HPAI viruses in this species, which could have an impact on the spread of virus within this colonial species. Overall, these results will allow to adapt and ameliorate screening protocols in the field, supporting epidemiological surveillance and diagnostic investigation.

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Using Next-Generation Sequencing to Evaluate Cross-Transmission of Feline Leukemia Virus (FeLV) Between Chilean Domestic and Non-Domestic Felids

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Abstract

Feline Leukaemia Virus (FeLV) is a gammaretrovirus of cats. FeLV is widespread among domestic cat populations and can produce a serious disease threat and significantly reduce the domestic cat lifespan. FeLV can be transmitted from domestic cats to non-domestic felids, and severe outbreaks have been described in non-domestic felid populations. The main consequences of FeLV infection are hematopoietic disorders and neoplasia. Domestic cats have both exogenous (exFeLV) and endogenous (enFeLV), while non-domestic felids lack enFeLV. EnFeLV are copies of the virus in the cat genome that may have protective factors against exFeLV. There are six FeLV groups (FeLV-A, B, C, etc), which arise from exFeLV mutation (FeLV-A) or recombination with enFeLV (FeLV-B). Traditionally, FeLV-A is solely transmitted in non-domestic species, however, a recent study in Pumas from North America demonstrated that FeLV-B variants can also be transmitted horizontally.

Although the FeLV circulation has been successfully controlled in developed countries, it is in contrast to the higher prevalence in South American countries. This study will apply amplicon-based sequencing through next-generation methods (Illumina and Nanopore) to compare and determine the envelope gene diversity and transmission dynamics of FeLV variants in Chilean domestic cats and free-range guiñas (*Leopardus guigna*) positive to FeLV.

We detected multiple variants and viral quasispecies in the domestic cat samples, forming a Chilean cluster. These also demonstrated artificial clustering according to the sequencing method. Nanopore methods were successful in detecting recombinants whereas Illumina methods were more reliable for evaluation of SNP variations. Guiña sequences are still being processed for comparison with domestic cat sequences. In conclusion, NGS has been successful in analysing longer and hypervariable genes, helping to understand FeLV diversity and how this circulates between domestic and non-domestic species.

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***Besnoitia tarandi* infection of the male reproductive system and mammary gland in barren-ground caribou (Rangifer tarandus)**

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Abstract

The objective of this study was to determine the prevalence and cyst density of *Besnoitia tarandi* in the male reproductive system and mammary glands of barren-ground caribou and to document the pathological changes associated with this infection. Additionally, possible risk factors for infection and inflammation were investigated. Samples from a total of 149 male and 229 female caribou were collected from 7 different herds across Canada, Alaska (USA) and Greenland between 2007 and 2009. Biopsies were taken from the skin covering the mammary glands for female caribou, and from the epididymis, pampiniform plexus, scrotal skin, seminiferous tubules and tunica albuginea for the male caribou. The cyst density (cysts/mm^2) of *B. tarandi* in each tissue, as well as the distribution, severity and type of associated inflammation were determined by histopathological examination. Individual (sex and age) and environmental (season, year and herd) risk factors were investigated for infection, cyst density and inflammation. The association between the cyst density and inflammation level was also investigated. *Besnoitia tarandi* cysts were detected in 22.2%, 31.3% 20.4%, 11.2%, 10.4% and 8.0% of the sections of epididymis, pampiniform plexus, scrotal skin, seminiferous tubules, tunica albugina and mammary gland skin respectively. The median cyst densities varied from 0.07 cysts/mm^2 in the seminiferous tubules to 1.02 cysts/mm^2 in the mammary gland skin. Males, adults and post summer months were seen as risk factors for infection. Subadults demonstrated a higher cyst density, and a higher risk of inflammation was present during the winter months and for females. A very good agreement was observed between infection of the different reproductive structures within each male (percentage of agreement: 89.2; coefficient type 1 of Gwet's agreement: 0.94). Overall, the intensity of inflammation was light to mild and a neglegible correlation was found between the cyst density and the severity of inflammation (r_s=0.24, CI=0.02-0.44, *P*<0.05). When a concomitant inflammation was present, the odds of having a *B. tarandi* infection was 2.94 times higher compared to the absence of a concomitant inflammation (95% CI=1.66-5.22, *P*<0.001). Additionally, for the pampiniform plexus, a 68.5% higher chance of infection was

present when a concomitant inflammation was present (*P*<0.001, 95% CI: 0.56-0.81). This study not only highlights the importance, but also provides the basics for monitoring of *B. tarandi* infection and the associated inflammation in the testicular tissues and mammary gland in barren-ground caribou.

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A new wild rodent model of retroviral germline colonization and the potential origin of the woolly monkey virus

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Abstract

Retroviruses cause disease by infection. However, upon infecting the germline they can be transmitted as Mendelian traits. Currently the most accessible model for studying the process and consequences of retroviral germline colonization is the koala retrovirus (KoRV) in its koala (*Phascolarctos cinereus*) host. KoRV is genetically similar to the Gibbon Ape Leukemia Viruses (GALVs), relatives of which have been found in bats and rodents in Indonesia, New Guinea and Australia. In an attempt to identify the ancestors of GALV/KoRV and their natural hosts, we screened hundreds of rodent and bat samples from New Guinea.

We hypothesized that the GALV/KoRV group originated in New Guinean mammals based on identification of similar viruses to GALV as dysfunctional germline integrations in Australian and Indonesian Melomid rodents and in some bat species in the region.

We screened 278 samples representing 1 rodent and 7 bat families from Papua New Guinea by gammaretrovirus specific PCR. Five of 10 *Melomys leucogaster*

samples were positive which were then subjected to target enrichment hybridization capture and high throughput sequencing to obtain full length viral genomes. Based on the viral genomes, phylogenetic analysis was performed, structural protein modelling and theoretical functional analysis was done and the consensus sequence of the positive samples was used to generate viral constructs to determine viral replication dynamics in cell culture followed by EM to determine viral structure and subcellular location.

Melomys leucogaster was the only positive species and contained fully intact single copy of WMV in one location in the genome but only in some individuals of the species. This non-fixed endogenous retrovirus called complete Melomys WMV (cMWMV) is genomically intact and replication competent in both human and mouse cell lines, is anti-retroviral sensitive and can be characterized by EM with particles budding at the cell surface membrane.

cMWMV represents a new rodent model of retroviral genomic colonization with similarities to KoRV but at a much earlier stage of colonization represented by a single endogenized copy in a population of *M. leucogaster*. The results also suggest that WMV, if not all GALVs, originated in rodents from New Guinea.

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Relevance and feasibility of involving wildlife rehabilitation centres for disease surveillance in France

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Abstract

Biodiversity loss is a major societal preoccupation of our time and is suspected to be a key factor in the emergence of various diseases. Wildlife has been shown to play an important role in the emergence and maintenance of various diseases impacting animal and public health, but is also a victim of theses emergences, which can affect species of conservation concern. Consequently, wildlife disease surveillance appears as an essential tool for the protection of human and animal health, and for species conservation.

In France, wildlife disease surveillance systems are mostly based on the continuous, opportunistic surveillance of wildlife mortality. This event-based surveillance system proves efficient for the detection of emergent diseases in wildlife, but is insufficient in terms of geographic, species, and type of affection coverage. Therefore, complementary surveillance devices need to be found in order to enhance wildlife disease surveillance as a whole. These complementary devices could be based on data collected from moribund animals, thus requiring the involvement of new stake-holders. We suggest that wildlife rehabilitation centres (WRC) could be relevant candidates. About 100 wildlife rehabilitation centres are currently operating in France, treating each year 100.000 distressed animals.

Our aim is to investigate the relevance of French WRC contributing to wildlife disease surveillance and to what extent, as a complementary device to wildlife surveillance networks already in place. Indeed WRC collect mostly moribund animals, thus potentially allowing the detection of sub-lethal pathologies, in individuals belonging to more anthropophilic or small size species.

Firstly, we will consider the use of admission data collected by WRC across France to implement syndromic surveillance, using time-series analysis, to rapidly detect and respond to abnormal morbidity or mortality events. Secondly, we will assess to what extent WRC can contribute to either the better understanding of the circulation and evolution of three viruses (HPAIV, CDV and WNV), or their potential clinical impact, or their surveillance. Finally, we will explore the acceptability of this possible new mission for WRC, and identify the barriers and benefits of implementing the above-mentioned measures on the long term.

The integration of the three components will allow us to determine whether including WRC in disease surveillance is relevant, and to provide recommendations on the most adapted implementation modalities, considering the aim of this surveillance and the capacity and willingness of WRC to assume this new mission.

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Characterization of the cloacal and oropharyngeal microbiota of Atlantic gannets (*Morus bassanus*) naturally infected with highly pathogenic avian influenza virus (H5N1)

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Abstract

The Atlantic gannet (*Morus bassanus*) was among the seabird species most significantly impacted by highly pathogenic (HP) H5N1 avian influenza virus (AIV) infection in 2022. The microbiota appears to play a crucial role in the health of wild birds, but can be altered by several factors, including AIV infection. The objective of this study was to characterize the cloacal and oropharyngeal microbiota of HPH5N1 positive and negative Atlantic gannets. Therefore, 20 gannets arriving to the Northern Spain coast (9 positive; 11 negative) were sampled between September and October 2022. The amplification of the 16S rRNA gene, the construction of libraries and their sequencing with Illumina technology were carried out, and the bioinformatics analysis performed. A total of 1,240,851 bacterial sequences were obtained from cloacal swabs and 204,173 from oropharyngeal swabs, representing 16 or 14 phyla, respectively. The five most abundant phyla account for 88.2% (cloacal) or 92.7% (oropharyngeal) of the relative abundance. The identified bacteria were grouped into 208 genera for cloacal samples and 114 for oropharyngeal samples, of which 89 and 54, respectively, were identified to the species level. No significant differences were observed in the relative abundance and alpha diversity of the detected bacteria concerning HPH5N1 infection. The most frequently detected phyla in cloacal swabs from H5N1-infected versus non-infected specimens were Planctomycetes (32.3% in positives; 16.3% in negatives), Epsilonbacteraeota (19,3% in positives; 37.8% in negatives), and Synergistetes (15.4% in positives, 0.4% in negatives). While in the oropharyngeal swabs they were Planctomycetes (25.0% in positives; 17.7% in negatives), Actinobacteria (21.2% in positives; 31.1% in negatives) and Acidobacteria (20.0% in positives; 13,2% in negatives). Also noteworthy is the identification of certain species of bacteria of sanitary interest: *Coenonia anatine*, *Ornithobacterium rhinotracheale*, *Chlamydia abortus*, *Suttonella ornithocola* or *Erysipelothrix rhusiopathiae*. In conclusion, high variability and significant heterogeneity within the different analyzed groups was observed in the gannets microbiota. But some trends in the relative abundance of certain bacteria can be appreciated. Further studies are needed to determine the role of the identified microbiota in the health status of the Atlantic gannet.

Grant PID2020-114060RR-C31 funded by MCIN/AEI/10.13039/501100011033; grant VIGIA-2200003 founded by Basque Government.

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Causes of death, exposure to toxicants and hemopathogens of European hedgehogs (Erinaceus europaeus) from a french wildlife rehabilitation centre

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Abstract

The European hedgehog (*Erinaceus Europaeus*), common in rural and suburban environments, is perceived as declining within its native range, prompting conservation concerns. This study, conducted at a French rehabilitation center, aimed to 1) identify causes of death in European hedgehogs, to detect potential emerging diseases, and 2) systematically screen dead hedgehogs for toxicants and hemopathogens.

Using clinical information, necropsy examination and ancillary testing in 159 dead hedgehogs, we identified the primary cause of death of 92% of them. The leading causes of death were an impaired general condition (30%), bacterial infections (26%), frequently caused by *Salmonella* Enteritidis, and traumatisms (20%). We also identified factors contributing to death in 78% of individuals, including a high proportion of cases with significant parasite infestations. The cause of death corresponded with the initial admission diagnosis in only 40% of the individuals.

Toxicological screening revealed that 42% of individuals had been exposed to anticoagulant rodenticides, including 6.4% animals with levels compatible with acute intoxication. Animals were also contaminated by trace metals, especially lead, but no pesticides residues were detected. The hemopathogen screening revealed a low diversity and prevalence of blood pathogens with 3.6%, 2.9%, and

2.9% individuals infected by *Anaplasma phagocytophilum*, *Mycoplasma turicensis* and *Mycoplasma wenyonii*, respectively.

In conclusion, a multidisciplinary diagnostic approach involving a rehabilitation center unveiled the multi-factorial causes of mortality of a sample of free-ranging hedgehogs in France. The variety of diagnoses suggests that there is not a unique prominent or emerging disease responsible for the mortality in hedgehogs. This study also highlighted the high exposure of hedgehogs to various toxicants. To further investigate the extent and causes of population declines, health information issued from several centers should be combined with demographic data, as well as ecological assessments on the availability and quality of resources.

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Coypus (*Myocastor coypus*): a potential role in the epidemiological cycle of botulism in wetlands?

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Abstract

Botulism in wild birds is a widespread bacterial disease mainly affecting *Anatidae* and raising important conservation issues. The epidemiological cycle of *Clostridium botulinum* in ponds relies on complex transmission pathways between different biotic and abiotic compartments. Decaying carcasses of wild birds are known to amplify and perpetuate the disease through the "carcass-maggot" cycle. However, the role that other vertebrates, particularly sympatric mammals, may play in this cycle is poorly understood. Among these, the role of coypus (*Myocastor coypus*), an abundant and widespread alien invasive species known to be susceptible to botulism raises questions. The aim of our study was to investigate the occurrence of *C. botulinum* type C/D (responsible for avian botulism outbreaks in France) carriage in coypus outside a clinical botulism outbreak in a pond with a known history of recurrent avian botulism outbreaks. A total of 106 coypus (juveniles and adults) were killed for regulation in the pond (118 ha), followed by opportunistic swabbing in situ. Swabbing was carried out from December 2021 to January 2022, just after a major botulism outbreak that occurred from July to November 2021. For each swab, the gene encoding the botulinum neurotoxin C/D was detected using real-time PCR after an enrichment step allowing the bacteria to grow. Samples positive for *C. botulinum* type C/D in coypus and in wild birds during previous outbreaks were compared using a typing method (MLVA). Seven of the 106 rectal swabs were positive for *C. botulinum* type C/D (four young females and three males - two adults and one juvenile). MLVA comparison of a selection of positive samples showed that the same MLVA profile was detected in swabs collected from coypus and in liver samples collected from dead birds during previous outbreaks of avian botulism. Our results show that coypus can be asymptomatic carriers of *C. botulinum* type C/D strains with an MLVA profile identical to that of the strains responsible for clinical signs in birds. This suggests that coypus may play a role in the epidemiological cycle of botulism, including the shedding of *C. botulinum* type C/D outside outbreaks resulting in its dispersal in the environment or may even participate in the carcass-maggot cycle when coypus mortality occurs. This study sheds light on a possible contribution of coypus to the epidemiology of avian botulism that should now be further investigated.

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First report of fatal infection of zoonotic liver fluke (*Pseudamphistomum truncatum*) in a Baltic ringed seal (*Pusa hispida botnica*)

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Abstract

The Baltic ringed seal (*Pusa hispida botnica*) populations were severely reduced in the 20th century due to hunt and contaminants. Although populations are recovering, the rate is slower than expected. This subspecies is currently listed as vulnerable in the HELCOM (Baltic Marine Environment Protection Commission) assessment. Identified threats include global warming, bycatch, and persistent organic pollutants. Additionally, environmental stressors can negatively impact immune status and lower the threshold of susceptibility to disease.

Pseudamphistomum truncatum is a newly emerging zoonotic liver fluke in Baltic grey seals (*Halichoerus grypus*) causing hepatobiliary disease. *P. truncatum* has a wide mammalian host range with the roach (*Rutilus rutilus*) as a paratenic host.

Fluke infections in grey seals are predominantly mild but severe cases with development of liver failure have been reported.

The distribution of Baltic ringed seals overlaps with the Baltic grey seals, however, reports of *P. truncatum* infection in ringed seals have not been as numerous. The first confirmed case of *P. truncatum* in a hunted or bycaught Swedish Baltic ringed seal was registered in 2009 by the Swedish Museum of Natural History. In the last decade, confirmed cases of *P. truncatum* in Baltic ringed seals have been increasing, but occurrence remains low.

In April 2023 a juvenile ringed seal was found dead on Gräsö, an island off the Swedish Baltic coast north of Stockholm. Necropsy was performed at the Swedish Veterinary Agency, Uppsala, Sweden. The seal was in normal body condition and icteric. The liver showed marked parasitic infestation of liver flukes in bile ducts and gall bladder. Microscopic evaluation of liver revealed severe, chronic parasitic cholangiohepatitis at pre-cirrhotic stage with biliary obstruction and intralesional bacteria and liver flukes morphologically consistent with *P. truncatum*. An extension of the inflammatory process was seen in the pancreas. Additional findings included chronic inflammation in lungs and persistent frenulum.

This case confirms that, in addition to other species, even ringed seals can suffer from severe liver fluke infections that lead to impaired liver function and liver failure. Increasing number of cases of this zoonotic liver fluke, with potentially fatal outcome, in Baltic ringed seals highlights that this parasite may pose a threat to an already vulnerable subspecies of ringed seals. Additionally, it may signal increasing parasite prevalence in the ecosystem, posing a threat to other mammals, including humans.

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Toxic Trails: Exploring Environmental Hazards and the Rise of Hedgehog Diphtheric Disease in Flanders (Belgium)

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Abstract

The hedgehog population in Flanders, Belgium, has drastically declined by nearly half from 2008 to 2018 (Van der Veken et al., 2019), reflecting broader trends across Europe (Van De Poel et al.,2015; Krange, 2015; Williams et al., 2018; Taucher et al., 2020). Concurrently, an emerging ulcerative skin disease known as Hedgehog Diphtheric Disease (HDD), linked to *Corynebacterium ulcerans* infections, has become a notable concern to Belgian hedgehogs (Martel et al., 2021; Terriere at al., 2022). In the context of studying HDD, we conducted a preliminary toxicological screening to investigate the exposure of deceased hedgehogs to environmental pollutants.

Thirty liver samples underwent examination for trace elements using inductively coupled plasma mass spectrometry (ICP-MS). Additionally, both first-generation and second-generation anticoagulant rodenticides were quantified using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). A multi-screening technique (LC-MS/MS) was used to detect the presence and concentration of agricultural herbicides and insecticides in twenty liver samples.

The results revealed widespread exposure to a range of pollutants. Insecticides showed limited levels of banned Persistent Organic Pollutants (POPs) such as DDT (P=5%; $\bar{x}$=0.001 ± 0.005 mg/kg ww), heptachlor (P=10%; $\bar{x}$=0.002 ± 0.0083 mg/kg ww), and aldrin/dieldrin (P=5%; $\bar{x}$= 0.001 ± 0.0025 mg/kg ww). Herbicide exposure was notable, with tembotrione detected in 90% of samples ($\bar{x}$=0.17 ± 0.27 mg/kg ww) and mesotrione in 50% ($\bar{x}$=0.05 ± 0.11 mg/kg ww). Heavy metals presented an alarming exposure, particularly cadmium (P=100%; $\bar{x}$=4.11 ± 2.46 mg/kg dw) and lead (P=100%; $\bar{x}$=2.19 ± 1.28 mg/kg dw). Anticoagulant rodenticides were also prevalent, with significant levels of brodifacoum (P=47%; $\bar{x}$=12.62 ± 44.72 µg/kg ww), bromadiolone (P=43%; $\bar{x}$=22.05 ± 107.45 µg/kg ww), and difenacoum (P=57%; $\bar{x}$=21.87 ± 63.40 µg/kg ww). Other rodenticides detected included coumatetralyl (P=13%; $\bar{x}$=0.25 ± 1.02 µg/kg ww), flocoumafen (P=27%; $\bar{x}$=0.12 ± 0.30 µg/kg ww), and difethialone (P=6%; $\bar{x}$=0.61 ± 3.28 µg/kg ww).

These findings highlight the extensive contamination of hedgehogs by various environmental toxicants and underscore the urgent need for comprehensive environmental monitoring and conservation strategies to mitigate further declines in hedgehog populations. Future research will focus on expanding the sample size

to further explore health implications, specifically investigating potential correlations between rodenticide exposure and the prevalence of HDD.

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Faecal Metagenomics as a surveillance tool for the health of red deer

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Abstract

Parasites and bacteria carrying antimicrobial resistances (AMRs) not only endanger the health of wild animals, but also that of livestock and humans. Since the habitats of wildlife are continually breached by anthropogenic influences, possibilities for spillover infections in either direction emerge. In Germany, red deer (*Cervus elaphus*) populations face severe threats like fragmentation of and displacement from natural habitats. Under these aspects a need to monitor the health of red deer becomes apparent.

By using faecal shotgun metagenomics, the resistome, parasitome and microbiome of hunt-harvested red deer will be analyzed. This method allows for the assessment of the gastrointestinal health, while only requiring small amounts of sample material. In addition, we will compare the results with those obtained from fresh droppings to assess whether a non-invasive sampling strategy could be used. Since metagenomic analysis is not common for the investigation of gastrointestinal parasites, a histopathological examination of five H&E-stained tissue samples along the lower gastrointestinal tract (abomasum, jejunum, ileum, caecum and colon) will be performed to validate the metagenomic results. Additionally, swabs of the sampling material will be cultivated on selective media to isolate and characterise ESBL*E. coli*, if present. These investigations are simultaneously performed on sympatric free range cattle to gather insight into the wildife-livestock interface.

So far, five of the 70 red deer and two of the catttle investigated were positive for ESBL*E. coli* and isolates of the strains were obtained. The use of metagenomics in wildlife monitoring is still quite new but allows for a wide range of investigations with little sampling and preparation effort. This offers a number of possibilities to monitor and investigate wildlife diseases.

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Transmission dynamics of HPAI in wild birds since 2020: a scoping review

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Abstract

1. Background

Highly pathogenic avian influenza (HPAI) H5Nx clade 2.3.4.4b has emerged as a significant threat and has led to widespread outbreaks across the world in recent years. Since 2020, the epidemiology of HPAI has undergone a notable shift, characterized by a rise in both the global number of cases and the diversity of infected wild bird species. This has resulted in year-round spillover to poultry, even extending to infect mammals. Wild bird populations are strongly impacted and are acknowledged as primary vectors of HPAI viruses, playing a key role in the introduction and transmission to poultry. Therefore, gaining insights into the new transmission dynamics of HPAI in wild birds becomes crucial.

2. Objectives

The objective of our study is to systematically investigate and identify the factors contributing to the new transmission dynamics of HPAI in wild birds and at the interface with poultry. Understanding the evolving nature of these epidemics is paramount for developing more effective strategies to counter the virus and implement efficient measures aimed at protecting both poultry and wildlife.

3. Materials and methods

We undertook a systematic literature review focusing on peer-reviewed papers addressing HPAI H5Nx clade 2.3.4.4b in wild birds, specifically examining epizootics occurring since 2020, on a global scale. After a screening process, which included the evaluation of titles and abstracts followed by a full-text assessment, we extracted pertinent information from 140 studies that met our predefined criteria.

4. Results

We identified key elements in the transmission dynamics of HPAI categorizing them into four main segments that mirror the epidemiological chain of infected populations:

-Exposure: this category explores the primary routes through which naive bird populations are exposed to HPAI viruses, along with the birds' behavioral ecology influencing this exposure.

-Infection: this segment focuses on the impact of infections on various groups of wild birds, assessing their resilience levels.

-Geographical spread: focusing on migration routes and key species involved, this category delves into the geographical aspects of HPAI spread.

-Transmission: this segment establishes the pathways of transmission in wild bird populations and at the interface between wild birds and poultry as well as environments facilitating transmission.

4. Conclusions

This review offers valuable insight into our current understanding of the transmission dynamics of HPAI in wild birds. It enables to identify critical knowledge gaps and provides directions for future research.

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Bat-borne Issyk-Kul virus in Italy: European emergence of a neglected zoonotic agent

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Abstract

Background: The study of bat-borne viruses has grown considerably in recent decades, primarily driven by the increasing concern for their potential to trigger epidemics. *Issyk-Kul virus* (ISKV), *Nairoviridae* family, is a zoonotic virus first detected in '70-'80 in Central Asia from *Nyctalus noctula* bats and bat ticks. Recently, ISKV was detected in bats in Germany, Sweden and Italy, suggesting that its geographical range expanded to Europe.

Objectives: This study describes the isolation and genome characterization of ISKV detected in bats in Italy and aims to acquire epidemiological data on ISKV viral ecology and diffusion. ***Materials and methods:*** Dead bats were collected from wildlife rehabilitation centres from 2017 to 2023. DNA barcoding was used for the bat species identification. The carcasses were subjected to necropsy, and tissue samples were taken to be subjected to a broad-spectrum of analyses that included, in addition to viral genera already long investigated (e.g. coronavirus, lyssavirus, orthoreovirus), more specifically, ISVK. Laboratory methods included virus isolation on cell culture, electron microscopy and PCRs.

Results: Overall, 488 bats belonging to 13 species were collected. The first detection of ISKV was from an adult female of Hypsugo S*avii* in 2022. The full-genome sequence of IT-ISKV, obtained through a standardised NGS protocol, revealed the three typical nairovirus genome segments: L (11,978nt), M (4,907nt) and S (1,457nt), and their highest nucleotide identity was to ISKV detected in *Myotis brandtii* and bat-associated tick in Sweden (strain Sun-2020, k99_1658, k99_589) and in *Eptesicus nilssonii* in Germany (strain PbGER). Therefore, a specific ISKV end-point RT-PCR targeting L gene (844pb) was developed and used to conduct a specific and retrospective ISKV-surveillance. Eight more ISKV PCR-positive bats were detected, seven from *Hipsugo savii* and one from *Myotis mystacinus,* recovered in 2017 and 2020-23. Phylogeny, performed with all ISKV partial sequences detected in this study, showed high genome identity among IT-ISKVs. A recombinant ISKV-N protein and anti-ISKV monoclonal antibodies were produced to enhance laboratory diagnostic capacity, and their characterization is currently in progress.

Conclusion: The ISKV circulation among synanthropic and sedentary bat species, such as Savi's bat in Northern Italy, indicates the likely emergence of a neglected zoonosis agent in the Mediterranean area and suggests potential public health implications. Ongoing studies will contribute to filling the current gaps in knowledge of the ISKV ecology, its diffusion in bat populations, viral tissue distribution, and pathogenesis.

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Bird flu, the new mortal enemy of peregrine falcons

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Abstract

Background. The latest highly pathogenic avian influenza (HPAI) epidemics have caused repeated mass mortality events among wild birds. Since 2020, HPAI H5 virus has been regularly detected and is now endemic in many European countries, including the Netherlands. The effect of the infection is less obvious for more solitary and less abundant bird species, such as the peregrine falcon. The peregrine falcon is an iconic, ubiquitous raptor species. Only recently its populations were able to recover from the threat posed by the unregulated use of pesticides. As its diet is primarily based on consumption of other bird species, peregrine falcons are particularly vulnerable to HPAI virus-infection, by consuming infected preys.

Objectives. The objective of our investigation was to quantify the consequences of the persistent presence of HPAI H5 virus on peregrine falcon populations in the Netherlands.

Materials and methods. A target surveillance for peregrine falcons was set in place to estimate the HPAI virus infection and mortality rates for this species. The numbers of reported dead and infected peregrine falcons during the past four years (2020-2023) were collected and analysed by the Dutch Wildlife Health Centre.

Results. Since 2020, the yearly numbers of reported dead and infected peregrine falcons have progressively increased. The peak of mortalities and infections occurred during the winter months. This season also corresponds to the time of highest presence of this species in the country. The highest rates of mortality and infection were reported during 2023. In this year over 70% of the tested peregrine falcons were positive for HPAI H5 virus. Relative to their wintering populations, over 50% of peregrine falcons may have died.

Conclusions. We concluded that HPAI represents a serious threat for the peregrine falcon and, in combination with other anthropogenic factors, may contribute to the decline of this species. As the dynamics of HPAI are still difficult to predict, targeted surveillance and disease mitigation measures should be set in

place to assure the conservation of the peregrine falcon and other vulnerable species.

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Pesticides and Veterinary Pharmaceuticals: A scoping review of their effects on Neotropical avifauna

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Abstract

Twenty years have passed since landmark publications identified diclofenac to be responsible for the dramatic population declines of *Gyps* vulture species on the Indian subcontinent. Concerns remain across the Old World over agriculturally-derived pesticides and veterinary pharmaceuticals, including the continued approval of diclofenac for livestock use and the introduction of untested compounds into the environment. However, despite an expanding agricultural sector, coupled with high biodiversity, data from the Neotropics on the impact of avian wildlife poisoning is lacking. A scoping review was conducted to address the following question: What is currently understood about the effects of veterinary pharmaceutical drugs and pesticides on avian carnivores native to the Neotropics? A scoping review protocol was written *a priori* and published online. A systematic search of electronic databases (Web of Science, CAB Abstracts, Scopus and MedLine) was conducted on December 5th 2023. All identified articles were exported to Rayyan software and deduplicated. Titles and abstracts were screened by two independent reviewers, excluding studies that a) weren't written in English, Spanish or Portuguese, b) didn't mention avian species native to the Neotropics (as defined by BirdLife International), c) didn't mention a veterinary pharmaceutical drug or pesticide compound and d) weren't primary research. Included articles were downloaded for full-text screening. Articles were excluded if a) the species studied were not carnivorous or omnivorous (as defined by the AVONET database), and b) the article did not pertain to incidents of wild or experimentally-induced toxicity to veterinary pharmaceutical drugs or pesticides in specified avian species. A total of 207 articles were identified for data extraction and analysis. Extracted data included species and compounds studied, date of publication, study design and biological sample type tested for contaminant presence. Detailed analysis was largely conducted on a subsection of extracted data (n=85), discounting sampling studies from outside of the Neotropics. We found an uneven distribution of sampling studies across Neotropical countries, with central South American and Caribbean countries largely unstudied. Recent trends suggest an increase in non-invasive sampling techniques being employed, across a more diverse range of potential agriculturally-derived contaminants. Studies on NSAIDs were underrepresented, notably in avian scavenging species such as vultures, accipitrids and caracaras, raising concerns of unseen impacts on population and ecosystem health. These results help inform directions for future research on a geographical and species level, highlighting potential gaps in ecopharmacovigilance across the Neotropics that may help prevent future consequences for avian, human, and ecosystem health.

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First Trial of Feces-PCR Testing For Malaria In Birds

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Abstract

Avian malaria is a vector-borne disease caused by a widespread bird hemoparasite: *Plasmodium* sp.. Naïve species to this pathogen may be vulnerable and present symptoms ranging from lethargy to sudden death. Given the rapid onset of symptoms, treatment of affected birds is often illusory. Early detection and prophylactic measures on susceptible birds and their environment are so far the best way to protect them. Given this observation, the authors wanted to investigate the possibility to screen birds for the parasite both early and without contention stress. Fecal PCR test, already used in primates was thus tried in birds. 102 carrion crows (*Corvus corone)* were sampled for blood and feces in 2 capture sessions. In the first campaign (April 2019; n=47) 38% were positive for *Plasmodium* sp. on blood and 27% were positive on feces (intestine massage) with only 15% of cross-positivity. In the second campaign (June 2020, n=55) 44% were positive on blood and 5.4% were positive on feces (spontaneous emission) with only 4% of cross-positivity. The PCR-inhibition capacity of the urate deposit has been tested and proven on new samples and certainly explains this difference

(feces emitted with massage lack urate deposit). Further studies are already undergoing with an eviction of urate deposit on spontaneously emitted feces. This technique however already proved useful to detect new reservoir species (*Ardea cinerea, Ciconia ciconia)* in Alsace without any capture (feces are harvested under the nests) and arises great hopes in term of malaria early detection in birds.

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Emergence of Canine distemper in wildlife in France

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Abstract

Since the early 2000's, southwestern Europe has known numerous outbreaks of Canine distemper (CD) in wild canids and mustelids (Italy from 2006, Germany from 2008, Switzerland from 2009, Belgium from 2012, Spain from 2020).

In France, prior to 2019, only sporadic cases of CD were detected through the French wildlife mortality surveillance network (SAGIR OFB FNC) and epidemiological surveillance implemented in national parks. However, starting in 2019, clusters of clinical cases were detected in European badgers (*Meles meles*) and Red foxes (*Vulpes Vulpes*) in the bordering departments of Switzerland (Doubs and Haut-Rhin), as well as in other alpine departments (Savoie and Isère). It raised the question of the emergence of a virulent strain of Canine Distemper Virus (CDV) as well as of its impact on the health of wild populations.

To investigate this emergence, we jointly analyzed the data collected from the event-based surveillance of the participatory SAGIR network at the national scale and the data collected through reinforced event-based surveillance of the Parc National des Pyrenees (PNP) carried since 2010.

In the French Pyrenees, systematic virological screening confirmed a strong increase of viral circulation since 2018, with the proportion of virus infection increasing from 0% before 2018 to 17% in both 2019 and 2020. The proportion varies between species, with Red foxes (17%), Pine martens (*Martes martes*, 8%) and European badgers (5%) being the top three of infected species.

The national screening of deceased wildlife through the SAGIR network confirmed a gradual increase of clinical cases, with the rate of clinically infected animals increasing from 0% in 2018 to 8% in 2020. Cases cluster in Doubs and Savoy departments and the most affected species is the Red fox (7% of confirmed clinical cases among deceased animals).

Viral strains identified in wild carnivores were compared to those collected in other European countries. Phylogenic analysis showed that France experienced four different introduction events, none of which were epidemiologically linked to strains isolated in domestic carnivores. In the Alps, viral strains showed a limited genetic diversity and geographic proximity with sequences isolated from Swiss wildlife in 2010. In the Pyrenees, isolated strains showed geographic proximity with sequences isolated from Spanish wildlife in 2020.

These findings confirmed the circulation of the CDV in French wildlife. In doing so, they urge to assess the impact of the disease on the conservation of susceptible and endangered species.

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Are red foxes (Vulpes vulpes) a sentinel species for Borna Diseases Virus occurrence in Austria?

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Abstract

Borna disease virus 1 (BoDV-1) is the causative agent of Borna disease, a non-purulent meningoencephalitis in horses, sheep, South American camelids and other domestic mammals in certain endemic regions of central Europe. Recently, the zoonotic potential of the virus has been demonstrated. The bicolored white-toothed shrew (*Crocidura leucodon*) is the known reservoir host of the virus, endemic in Austria and an element of the red fox (*Vulpes vulpes*) diet, thus an exposure of red fox to BoDV-1 is supposable. Cases of Borna disease in few horses, one dog and shrews were diagnosed in three Austrian federal states (Styria, Upper Austria and Vorarlberg). The aim of the present study was to

investigate red foxes for possible BoDV infection in Austrian regions where BoDV has occurred in the past and to investigate if the red fox can serve as potential sentinel species for virus presence in wildlife.

A total of 365 brain samples were tested for the presence of bornavirus RNA by two different RT-qPCR assays, one specific for BoDV-1 and the other detecting a broad spectrum of orthobornaviruses. In addition, 271 serum samples were tested for BoDV-1-reactive antibodies using an indirect immunofluorescence assay (IFAT). Samples with positive reactions were re-analysed using a modified version of the IFAT.

All brain samples resulted both BoDV-1 and pan-bornavirus RT-qPCR negative. Three serum samples (1.1%) showed a positive reactivity using the screening test on BoDV-1-infected Vero cells, only one of these samples showed comparable IFAT titres in the confirmatory test on SK-6 cells. However, since bornavirus RNA was not detectable in the brain of the respective individual, the seroreactivity is likewise assumed to be unspecific.

In our study we examined a various number of tissue and blood samples from Austrian regions known to be endemic for BoDV-1 with negative or unspecific results. Therefore, we conclude that red foxes are not an appropriate species to indicate the presence of BoDV-1 in a geographic area. Our results agree with findings of other studies regarding the importance of red foxes as a sentinel for BoDV-1. Considering the zoonotic potential of BoDV-1, the identification of risk areas for domestic animals and humans may nevertheless be valuable. Therefore, the implementation of a notification requirement could be a practicable way to gather information on the current epidemiological situation in Austria.

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Avian malaria in *Chlamydotis macqueenii*: study of hemosporidia circulating in different regions and countries.

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Abstract

The Asian Houbara Bustard, *Chlamydotis macqueenii* is considered endangered according to the IUCN. This specie is bred in captive breeding centres in UAE, Kazakhstan and Uzbekistan for conservation purposes. Parasitic infections, such as avian malaria, can display high prevalence rates, especially in breeding centres, which can be problematic as they might affect growth, health, and long-term survival. Although hemoparasites have been previously described in houbara bustards, less is known about their clinical importance and their potential impact on production, post-release survival or local populations.

Here we studied the diversity and prevalence of hemoparasites by host and geographic region using multiplex PCR with subsequent sequencing (for the identification of different species of *Plasmodium*, *Haemoproteus* and *Leucocytozoon*) and thin blood smears. Between April 2022 and October 2023, samples from 958 individuals (divided into 5 age groups) were collected in captive breed birds. We compared the prevalence between birds with and without clinical signs and studied possible variations in prevalence by age groups, countries, and forms of management (indoor vs outdoor).

We observed various strains of avian malaria and different prevalences in the study sites. The UAE had the lowest prevalence of avian malaria at 18.13% (N = 97/535), followed by Kazakhstan with 30.73% (N = 55/179) and Uzbekistan with the highest values at 68.44% (N = 167/244). *Plasmodium* species were more prevalent in UAE captive breeding centre; while *Haemoproteus* species were the most frequent in the other study sites. Sixty-six cases of co-infections were also identified, with the most common occurrence being *Plasmodium* + *Haemoproteus* (N = 56), followed by *Plasmodium* + *Leucocytozoon* (N = 8) and then *Plasmodium* + *Haemoproteus* + *Leucocytozoon* (N = 2). Four undescribed species were identified, two of *Plasmodium* and two of *Haemoproteus*.

The results suggest that there is no significant impact on mortality caused by avian malaria in Asian houbaras, since no differences were observed between houbaras with clinical cases and healthy ones. An increase in the prevalence of hemoparasites was observed up to the age of 6 months, followed by a decrease and stabilisation of the rate. Kazakhstan and Uzbekistan had higher prevalence rates which can be explained by a climate that is more favourable to the presence of hemoparasite-transmitting mosquitoes.

Knowledge of the different species of hemoparasites and the genotypes that can be identified in these species, both in the wild and in captivity, is essential to assess clinical or subclinical effects on health, reproduction, and population reinforcement.

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Heterogeneity of pathological prion protein accumulation in the brain of moose (Alces alces) from Norway, Sweden and Finland with Chronic Wasting Disease

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Abstract

Chronic Wasting Disease (CWD), a highly contagious, neurodegenerative, transmissible and fatal prion disease affects captive and free-ranging cervid species in North America and is expanding.

CWD was first detected in Europe in wild reindeer (*Rangifer tarandus*) in Norway in 2016, leading to extensive surveillance in all cervids species in Norway and detection of CWD in moose (*Alces alces*). This led to mandatory surveillance in EU and EEA countries. Further cases were detected in old moose in Norway, Sweden and Finland. The cases in moose differ in their epidemiology from CWD in North America and Norwegian reindeer and it is hypothesised that CWD in European moose is sporadic and less/no contagious under field conditions.

This study characterised the deposition of PrP^{Sc} in the brain of moose affected by CWD in Nordic countries to achieve a better understanding of the disease presentation and of the similarities and variations among the cases.

Thirteen CWD-positive moose from Norway (n=7), Sweden (n=4) and Finland (n=2) were investigated. Following the positive CWD test results the whole brain and additional lymphoid tissues were sampled. Sixteen different brain areas were analysed with ELISA and immunohistochemistry (IHC). For IHC monoclonal antibodies 12B2, 9A2, L42, SAF 84 and P4 were applied. Seven patterns of PrP^{Sc} deposition were identified (fine punctate, coarse granular, intraneuronal, intraglial, linear, perineuronal and stellate) and their intensity and extent were scored.

Core antibodies L42 and SAF84 detected PrP^{Sc} in the brain of all animals, as intracellular and extracellular PrP^{Sc} deposits. PrP^{Sc} was detected with the N-terminal antibodies 12B2, 9A2 and P4 in only 2 moose. Levels of PrP^{Sc} deposition in the different brain areas varied among cases and with different antibodies. Large variation in total PrP^{Sc} accumulation were observed amongst the moose. Lymphoid tissues were negative for PrP^{Sc}, which differs from CWD in wild reindeer in Norway and CWD in North American. ELISA showed positivity in each of the brain areas and each moose, with strong variations between individuals, the results were consistent with IHC. High PrP^{Sc} levels were found in the frontal cortex, thalamus, midbrain, and obex.

The heterogeneity among animals suggests occurrence of prion strain diversity or presence of multiple prion strains. It is important to characterize how different CWD-strains behave biologically to understand the epidemiology and inform surveillance and management strategies.

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Clinical, Epidemiological and One Health Insights from an Mpox Outbreak in Captive Chimpanzees in Cameroon

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Abstract

Background: Monkeypox virus (MPXV) is a re-emerging virus of global concern. A Clade I MPXV outbreak occurred in wild-born, captive chimpanzees (*Pan troglodytes*) at a primate sanctuary with semi-natural habitat in an endemic region of Cameroon in 2016.

Objective: Study the epidemiology, clinical progression and One Health risks to improve understanding and inform future outbreak management.

Methods: Epidemiological and clinical data were recorded. Human and animal contacts were identified and monitored. Diagnostic samples were tested by PCR and genome sequencing. Environmental samples from in and around the affected enclosure, including surfaces, cage bars, food debris and bedding, were tested by PCR to assess transmission risks. To identify potential reservoir hosts, small mammals in the orders Rodentia and Eulipotyphla were captured and tested by antibody ELISA and PCR both during the outbreak and a year later.

Results: There was an 87% attack rate and a 10% case fatality rate in the affected group. Clinical signs included exanthema, facial oedema, peri-laryngeal oedema, and eschar. Peri-laryngeal oedema was associated with more severe respiratory signs. The whole genome sequence shared 99.7% identity with a sequence from a human case in Cameroon in 1989. Of 13 environmental samples tested, 6 were PCR positive for MPXV, including cage bars, food debris and bedding. Of these 6 PCR positive samples, 2 taken from sleeping platforms contained viable virus. Of 162 small mammals sampled, 2 were antibody positive, but none were PCR positive. There were no human cases.

Discussion: MPXV poses a risk to wild primates, captive primates and human contacts in endemic regions. The source of this outbreak was likely direct or indirect exposure to an infected reservoir host, by one or more chimpanzees. Additional animals were likely infected by direct or indirect chimp-to-chimp transmission, potentially including respiratory transmission. Different clinical syndromes observed in this outbreak may reflect distinct routes of infection, as has been hypothesised for outbreaks in wild chimpanzees. Peri-laryngeal oedema likely contributed to airway obstruction and death and may contribute to severe morbidity or mortality in other species, including humans. In under-resourced sanctuaries in mpox-endemic countries, rapid risk assessment could aid resource prioritisation to reduce the risk of zoonotic spillover.

Conclusion: Mpox is a differential diagnosis for facial swelling, peri-laryngeal swelling, and eschar in chimpanzees in endemic areas. Rapid identification of outbreaks reduces the risks to animals and humans. Control measures should follow a One Health approach.

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Expanded health and disease surveillance of marine mammals in Sweden: Cause of death, diseases and other findings in seals and porpoises 2020-2023

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Abstract

Although the Swedish Veterinary Agency (SVA) has performed wildlife disease surveillance for 75 years, marine ecosystems have been underrepresented. In 2020, an expanded health and disease surveillance program for marine mammals was launched. Jointly delivered by SVA and the Swedish Museum of Natural History (NRM), the program is funded by the Swedish Agency for Marine and water Management (SwAM) as a part of Sweden's environmental monitoring.

This national program provides knowledge on health, diseases, mortality and other threats to seals and cetaceans to inform management and protection of these populations. In addition, these marine mammals serve as sentinels for the health of marine environments and threats to other animals including humans. Here we provide a brief overview of this new program and summarize results from animals examined at SVA.

Stranded seals are examined by SVA, hunted and bycaught seals are examined by NRM, all cetaceans are jointly examined at SVA. For animals examined at SVA, carcasses are collected from the field for complete necropsy examination. A comprehensive suite of data and tissues are collected, archived and available for ongoing and future research. Additionally, targeted surveillance for Influenza A virus, morbilliviruses and bacterial culture of lung is performed routinely.

Between 2020-2023, 235 animals have been examined at SVA: Harbour porpoises (*Phocoena phocoena*) (n=157), Grey seals (*Halichoerus grypus*) (n=37), Harbour seals (*Phoca vitulina*), (n=34), and one Ringed seal (*Pusa hispida*). Six other stranded cetaceans were also examined.

The most common cause of death for all stranded porpoises was bycatch in fishing nets. When considering only adults, diseases were the most common. These included infectious diseases but also, for example, dystocia. Skin lesions and

parasitic infections occurred frequently in porpoises. The most common causes of death in seals were diseases and various types of traumas. Young seals often were emaciated with severe parasitic infections. Death from biliary trematode infection was observed in three young seals. Active Influenza A infection was found in one grey seal and one porpoise, *Erysipelothrix rhusiopathiae* was found in nine porpoises.

This expanded surveillance program is continuous, enabling identification of trends indicating changes in both animal health and ecosystems. Examples of health issues we are following include skin lesions of porpoises, trematodes in seals, and influenza A virus spillover to marine mammals in general.

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The second national screening of *Echinococcus multilocularis* in Sweden

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Abstract

The fox dwarf tapeworm *Echinococcus multilocularis* (*Em*) is a zoonotic parasite that was first found in Sweden in 2011 after ten years of surveillance of about 300 hunter harvested red foxes (*Vulpes vulpes*) annually. A first national screening on a larger scale was conducted in 2012-2014. Faeces from almost 3.000 foxes were tested, with four positive samples from four different counties. The estimated prevalence was approximately 0,1% at a national level. A few human cases of alveolar echinococcosis in the last decade that could not be traced back to living abroad or international travel have been found in Sweden, so a continued surveillance of this zoonosis is prioritized by relevant authorities. A second similar national screening during 2021-2024 was conducted to follow up and to update the knowledge about the national situation of *Em*. Using the results from the first screening as a base line, the Public Health Agency could then assess any need to revise the information to the public about risks of acquiring alveolar echinococcosis. Funded by the Swedish Board of Agriculture, the Swedish Veterinary Agency ran the national screening by analysing collected fox scats and faecal samples from hunter harvested red foxes by real-time PCR. Samples were sent in by the public in specific sampling kits, after posting calls for citizen science support on social media. The Swedish Association for Hunting and Wildlife Management tasked selected members of their staff and association members throughout the country to collect samples, with a focus on certain counties of

higher interest and also areas where samples were lacking. Analysis of over 1.600 samples since 2021 has resulted in new findings of single positive scats in three different municipalities. Further sampling around the positive findings has failed to find further positive samples. In addition, the continuous presence of the parasite has been shown in two known hotspots, in the municipalities of Uddevalla in the southwest and Gnesta in eastern mid-Sweden, where *Echinococcus* was already found in 2011. Except in the two known hotspots, *Em* appears to be present very locally or sporadically in Sweden, as cases can be found in single foxes or fox scats if large numbers of samples are collected. So far, all cases have been found in the southern half of Sweden.

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The first outbreak of African swine fever in Sweden, a point introduction in wild boar

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Abstract

After the introduction of African swine fever (ASF) to Georgia in 2007, surveillance efforts were intensified in Sweden. Reporting of found dead wild boar is done online at **rapporteravilt.sva.se**, as part of the national wildlife disease surveillance at the Swedish Veterinary Agency (SVA). Reported dead wild boar are sampled and tested for ASFV. In the last week of August 2023, several separate reports of dead wild boar in Fagersta municipality led to the identification of the first case of ASF in Sweden on September 6, 2023. The finding of ASF in wild boar initiated an immediate response by the relevant authorities, headed by the Swedish Board of Agriculture (SBA) and SVA with the objective to identify the infected area and then eradicate the outbreak.The outbreak was immediately reported to the relevant national and European authorities as well as WOAH. Based on the location of the six first reported cases, knowledge about ASF epidemiology in wild boar, the local wild boar population, and the habitat, SVA suggested a preliminary infected area and the SBA decided on restrictions including ban on public access to off-road land within the area. A local disease control centre and a sample collection centre were set up on-site. All domestic pigs within the area were euthanised within the first week of the outbreak. Carcass search was organised by the SVA and the SBA with the help of volunteering local hunters. A core area where all ASF-positive

cases had been found was fenced in and surviving wild boar were trapped and culled. Surveillance of dead wild boar and domestic pigs in farms was intensified in all municipalities surrounding the infected zone. Taphonomic analysis was done to estimate the time of introduction of ASF. The initial restriction area was 1 000 km^2, reduced to 618 km^2 on November 30. A 114 km^2 core area was fenced in by November 22. Of 87 found carcasses (Feb-24) 62 were positive for ASF, all in the core area. The earliest infected case was estimated to have died between May and July 2023. The last positive material was skeletal remains found on November 18. Multi-agency cooperation and preparation for an outbreak, and participation of local hunters and communities once the outbreak happened, has been pivotal for the early identification, management, and hopefully swift eradication of this outbreak.

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Unveiling the Global Wildlife Health Landscape: WOAH Stakeholders Inventory 2023 Survey

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Abstract

Wildlife health is a complex matter that requires multidisciplinary teams and cross-sectional work among veterinarians, conservationists, wildlife biologists, wildlife managers, epidemiologists, and many more. The World Organisation for Animal Health (WOAH) aims to unite expertise and sectors through its Wildlife Health Framework (WHF), fostering collaborative efforts to enhance better understanding and responses to wildlife health issues. Understanding the full range of stakeholders involved in surveillance activities, and the barriers they face in establishing effective surveillance systems, is crucial to designing interventions that are fit for purpose.

In pursuit of the objective to produce a global chart, WOAH conducted a Global Wildlife Stakeholders inventory exercise from June to September 2023. An online questionnaire was distributed to WOAH's in-country correspondents in charge of official animal disease notification, on wildlife, and aquatic animal diseases, covering 200 countries and territories. We collected information on the national stakeholders responsible for various steps in the wildlife surveillance chain:

protocol design, field detection and sampling, laboratory, data analysis and management, and communication.

Insights gathered from 91 respondents, representing 78 countries and territories underscore the diversity among wildlife health stakeholders and the complex interactions between the public and private sectors during the investigation of wildlife health events.

This study not only provides valuable insights into the diverse landscape of national-level stakeholders involved in wildlife health but also identifies significant gaps and opportunities for enhancing collaborative efforts. Fostering collaboration between stakeholders highlights an important opportunity to build bridges between sectors. This is an important foundation for protecting the health of wildlife and ecosystems and upholding the principles of One Health for the benefit of all.

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Twelve Years of Research on *Echinococcus Multilocularis* In Alto Adige-Italy

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Abstract

Background

Echinococcus multilocularis is the causative agent of alveolar echinococcosis, a serious disease that in humans can be fatal if not treated. Beside rabies, *E. multilocularis* represents the major zoonotic agent transmitted from foxes to humans, and is ranked as a priority infection among food-borne pathogens in Europe. In this work, we report the results of twelve years of study on this zoonotic tapeworm in Alto Adige (Italy).

Objectives

Since 2012, different aspects of *E. multilocularis* epidemiology have been studied by our research team. In particular: geographical distribution on a regional and local scale, features of the routinary diagnostic tests in foxes, effects of definitive host population density, development and validation of a new molecular diagnostic test on stool samples, reassessment of the prevalence and surveillance strategies.

Materials and Methods

Prevalence in red fox was monitored from 2012 to 2018. In 2019-2020 period, the performances of the routine test (Coproscopy+Multiplex PCR) on faeces were compared to a real-time quantitative PCR (qPCR), by using a scraping technique (SFCT) on fox small intestines as the gold standard. This allowed, in the same period, to reassess the prevalence of *E. multilocularis* in foxes from Alto Adige. According with the studied years, the relation between red fox density and the presence of the cestode was investigated. Fox density was estimated by a non-invasive spatial capture-recapture (SCR) approach on genotyped scats in two study areas: one representing a known hotspot, and another naïve to the parasite.

Results

Our results confirmed an apparent increasing prevalence of *E. multilocularis* in foxes through the years. qPCR showed a much higher sensitivity compared to the previous routine test and allowed to reassess the prevalence in foxes at 14.3%, markedly higher than estimated in the same area in the previous years (<5%). Based on these evidences, Alto Adige can be classified as a highly endemic area. On a local scale, we evidenced how the parasite distribution seems independent from fox density.

Conclusions

We recommend to perform surveillance for *E. multilocularis* in foxes on small geographic areas, applying sensitive diagnostic tools directly on fox faeces, in

order to increase the probability of detection and to provide more accurate information to estimate the exposure risk for humans

The present work is part of the research project IZSVE RC 03/11; IZSVE RC 18/2016; IZSVE RC 05/19 founded by the Italian Ministry of Health.

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Health status monitoring of free-ranging Apennine wolves (Canis lupus italicus) in 2022-2023: a preliminary study

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Abstract

Since the early 1970s, EU directives and national regulations have been issued to protect the wolf, an endangered species. Currently, several public institutions collaborate in a national monitoring plan that aims to define the abundance and health status of wolves in Italy. In particular, IZSLER is involved in performing molecular analyses (PCRs) to detect several pathogens of conservationist and zoonotic interest. Since February 2022, 88 carcasses from different Apennines areas have been subjected to necropsy.

Almost all animals died as a result of collisions with motor vehicles, but in three cases, signs of poisoning were detected and three wolves died from a gunshot wound. Nevertheless, samples from the lungs, liver, spleen, kidneys, and intestine, were collected and analysed to detect Influenza A virus (IAV), Canine distemper virus (CDV), Coronavirus, Mammalian orthoreovirus (MRV), Adenovirus, Carnivore protoparvovirus 1 (CPPV-1), Hepatitis E virus (HEV). Out of 88 wolves, 28 brains, collected in Lombardia and Emilia-Romagna regions, were analysed for Aujeszky disease virus. A total of 3 animals, from the Emilia-Romagna, Marche and Lazio

regions, respectively, resulted positive for CDV (p=3%). Sequencing of the H gene was performed to define the virus lineage. Three other wolves from Emilia-Romagna, Marche, and Umbria tested positive for MRV (p=3%), and the viral strains were then sub-typed. Six wolves from Central Italy and one from the South Italy (Calabria region) tested positive for Canine Adenovirus type 1 (CAdVI) with a prevalence of 8%, all samples resulted negative for CAdVII. A total of 58 (p=66%) animals tested positive for CPPV-1 (56 for Canine Parvovirus type 2 and 2 for Feline Parvovirus, respectively). Indeed, the detection of Canine Adenoviruses and Protoparvoviruses is quite common in wild carnivores, often showing high prevalence. The coinfection with canine distemper, CAV1 and canine Parvovirus was detected in one poisoned wolf, which presented not optimal body condition and was also affected by sarcoptic mange.

All animals resulted negative for Aujeszky disease, IAV, Coronavirus and HEV.

These preliminary results allow us to provide a broader and more in-depth view of the health status of wolves spread along the Apennine area in Italy. This aspect is particularly important in relation to the growth these populations have been experiencing in recent years and the potential overlap with other susceptible domestic and wild species, potentially involved in the spread and circulation of viral agents.

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Exploring Bat-Virus Interactions: Insights from a Study in the Gobi Desert

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Abstract

Background In May 2022, a collaboration effort between the Institute of Biology, Mongolian Academy of Sciences and several Italian scientific institutions resulted in an expedition to the Gobi Desert in Mongolia. The primary objective was to explore the biodiversity of fauna and microorganisms, with a focus on small mammals. Bats, in particular, were of significant interest due to their capability to harbour and transmit emerging and potentially harmful viruses both to other animals and humans. **Objective**. The aim was to collect data on viruses circulating in bats in one of the most remote regions of the planet, the Gobi Desert. **Materials and Methods** Bats were captured using mist nets at six different oases in the Gobi Desert. Each captured bat was identified based on its morphological characteristics, and faecal samples were collected from each animal for virological investigations. These samples were then subjected to PCR protocols targeting viral families and genera known to potentially impact human and animal health, such as Coronavirus, Orthoreovirus, Influenza A virus, Pestivirus, Paramyxovirus, Herpesvirus, Adenovirus, Flavivirus and Phlebovirus. Positive samples were further analysed using Sanger sequencing to better identify the viral genomes detected. **Results** A total of 90 faecal samples were collected from insectivorous bats belonging to the genus *Hypsugo* (n.5), *Plecotus* (n.54), *Vespertilio* (n.7), *Myotis* (n.8) and undefined species (n.16). Eleven samples (12.2%), all collected from *Plecotus* bats, resulted positive for Coronavirus. Blast analysis conducted on a 440 bp of the CoV RdRp gene revealed 95.1% identity with the MERS-related CoV strain BatCoV_B20-180 detected in *Rhinolophus ferrumequinum* in South Korea in 2020. Three samples (3.3%) collected from bats belonging to the genus *Vespertilio* resulted positive for Mammalian Orthoreovirus (MRV) with showing a 100% identity in the L1 region (344 bp) with the MRV strain WIV4 detected in *Hipposideros* sp. in China in 2011. Three samples (3.3%) from *Plecotus* and *Myotis* bats resulted positive for Herpesvirus, and sequencing of the PCR target region (DNApol 250) is currently in progress. Molecular analyses for Influenza A virus, Pestivirus, Paramyxovirus, Flavivirus and Phlebovirus genera yielded negative results. The results of PCR tests for adenovirus, along with the phylogenetic analysis using the obtained sequences of CoVs, MRVs and Herpesviruses, are still

in progress and will be presented at the congress. **Conclusions** This study contributes to filling the current gaps in knowledge regarding the complex ecological and evolutionary relationships between bats and their viruses.

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Top predators as bioindicators for environmental health - living and dying in a polluted environment

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Abstract

Birds of prey at the end of the food chain are known for their potential to accumulate environmental pollutants. Beside being exposed to bioaccumulating substances, raptors are also threatened by ingesting inorganic toxins with their prey. Lead (Pb) poisoning from hunting ammunition remains the biggest threat for white-tailed sea eagles [*Haliaeetus albicilla*] in Germany. Our long-term health monitoring of sea eagles (N=1049) revealed that 80% of deaths were attributed to anthropogenic factors, with lead intoxication being the most relevant. Although the use of lead-based shot gun ammunition for hunting water birds is regulated on a European scale, lead-based rifle ammunition for large game animals is not. Effective Europe-wide restriction is needed to prevent the release of lead into the environment, which poses a threat to top predators, and burdens humans consuming game meat.

While lead exposure in birds of prey is well understood, there is limited knowledge about the lead levels deriving from hunting ammunition in mammalian top predators. A preliminary study on long-term accumulation in wolves (N=32) indicate clearly that clinically relevant lead values are found in their bones, reflecting life-long exposure.

Other environmental pollutants analysed as part of our long-term health monitoring are anticoagulant rodenticides (ARs) but also selected medicinal products (MPs) and frequently used plant protection products (PPPs). We analysed livers of red kites (*Milvus milvus*), northern goshawks (*Accipiter gentilis*) and Eurasian sparrowhawks (*Accipiter nisus*) as well as white-tailed sea eagles and ospreys (*Pandion haliaetus*) from agricultural and urban habitats in Germany. The highest detection of ARs occurred in urban goshawks (81.3%; n = 48), closely followed by red kites (80.5%; n = 41), sea eagles (38.3%; n = 60) and sparrow

hawks (13%; n = 23), whereas no ARs were found in ospreys (n = 13). Among 7 MPs, four were detcetd with Ibuprofen being most frequent. Among 30 investigated PPPs, only two were detected in red kites.

In a wide scope screening, we analysed the livers of 30 white-tailed sea eagles for the identification of over 2,400 contaminants. In total, we detected 85 chemicals of which 27.1% were MPs, followed by persistent organic pollutants (23.5%), PPPs (20%) and industrial chemicals 17.6%. Furthermore, 7.1% were stimulants. Exposures to chemicals in apex predators are complex, depending not only on intrinsic chemical properties but also on other factors such as ecology and land use.

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Veterinarians for non-captive wildlife in France: challenges and motivations

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Abstract

In French veterinary schools, many students aspire to specialize in wildlife. However, once graduated, very few work in this field. This study aims to understand why, once trained, few veterinarians work in non-captive wildlife. It will also establish the profile of wildlife veterinarians and identify the issues related to this specific veterinary exercise.

To answer these questions, several hypotheses have been proposed:
- There are few non-captive wildlife courses in French schools
- There are financial limits to working with wildlife
- Access to additional training is restricted
- The few veterinarians working in wildlife are motivated and passionate

A 10-minute questionnaire, containing between 41 and 57 closed questions, was distributed to wildlife practicing veterinarians through a mailing list, social media platforms, and French Vet Council newsletter. The survey is organized into five parts. The first part corresponds to the description of the current activities of veterinarians in wildlife and those not related to wildlife. The second part focuses on the general training of veterinarians. The third part concentrates on their

experiences in the field of wildlife. The fourth part examines their motivations, and the final part is related to personal information.

Among the 145 responses received, 63% were from women, 36% from men, and 1% identified as "other." Fifty percent of them had taken wildlife courses at school, 63% received additional training after school and 45% found work in wildlife directly after school. The main issues with additional training are the cost and lack of time to attend. Sixty-eight percent of veterinarians working with wildlife do so as an additional activity because a full-time activity does not allow good remuneration. Despite the many difficulties, wildlife veterinarians are motivated and passionate (74%). The sources of motivation are numerous: vocation (95%), the link with their values (77%), the passion for wildlife (74%), the desire to protect wildlife (53%), the attraction of varied activities (50%), constant training (41%) and the love of challenges (27%).

The practice of veterinary medicine in non-captive wildlife has many constraints: employability, lack of remuneration, location of activities, lack of training and free time, significant mental load. Despite the will and passion of practicing veterinarians, students struggle to join this path. To try to remedy this, it will be necessary to: strengthening the economic model, redefining the identity of the "wildlife veterinarian," restructuring training, and creating a directory for students.

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Risk analysis and health surveillance of lynx in France

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Abstract

In France, the Eurasian lynx *(Lynx lynx)* is a protected species, still threatened with extinction. Pathogens may strongly impact the dynamics of small fragmented populations. Early detection of an emerging health problem in lynx populations is therefore essential. Different networks collaborate to ensure event-based

surveillance of wildlife diseases, thanks to detection of morbidity and mortality signs in French lynx populations. However, the event surveillance currently in place is subject to several biases. It must therefore be coordinated with other surveillance methods, in order to be as effective as possible. A multidisciplinary collective expertise was therefore implemented at the request of the species managers to consider integrated surveillance of lynx diseases in live and dead compartments.

We first developed a health risk analysis taking into account 35 hazards, including trauma (collisions, poaching or trapping), anticoagulant poisoning, cardiac abnormalities, tumors, ear malformations, bacteria (5), viruses (11), ectoparasites (5), protozoa (4) and helminths (4). The risk analysis was based on the AFSSA method (2008), which consists of evaluating for each hazard, through a review of the literature, the occurence probability of the health danger (which depends of the release probability and the exposure probability) and the direct and indirect consequences of the danger (sum of individual severity, diffusibility in population and population severity). This method provides for each hazard an overall estimate of risk rated from 0 to 9. Traffic collisions, poaching or trapping, feline immunodeficiency virus (FIV) and feline parvovirus (FPV) were identified as being the most important risks for the lynx populations *in natura*. Furthermore, this analysis highlighted different dangers for which more knowledge is needed for the lynx : ear malformations, *Chlamydophila spp*., *Leptospira spp.,* Canine Distemper Virus, etc.

The risk analysis is a basis to propose actions to complete the surveillance measures already implemented on live and dead lynx (such as systematic radiographic, necropsy and histological examinations on dead lynx, virus detection by PCR, etc.). For example, additional opportunistic samples could be carried out on lynx in distress in wildlife center. Nevertheless, for a lynx in distress, emergency care takes priority over precise diagnosis and epidemiological surveillance. The risk analysis allows managers to focus epidemiological surveillance on hazards that are top-ranked, thus optimizing the compromise between disease surveillance, individual diagnostic and welfare.

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The Wild bird-associated zoonoses network: A bird´s-eye view of flavivirus circulation in Germany

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Abstract

Context: Wild birds act as reservoir and amplification hosts for several zoonotic viruses. The Wild bird-associated zoonoses network (WBA-Zoo) is a German surveillance project focusing on arboviruses such as the flaviviruses West Nile (WNV) and Usutu virus (USUV). In the 2023 transmission season, more than 700 human WNV infections were reported across Europe, six of them in Germany[1]. Although primarily WNV can lead to severe illness in humans, USUV is also considered a zoonotic pathogen. Both viruses can cause lethal infections in birds.

Objectives: Monitoring of flavivirus circulation through active surveillance involves sampling of individuals from diverse avian species and habitats and requires significant resources. Meanwhile, wildlife clinics and rescue centers routinely collect hundreds of blood samples every month from a wide variety of wild bird species. Hence, WBA-Zoo aims to facilitate surveillance of WNV and USUV in Germany by examining these samples originally acquired for veterinary diagnostic purposes.

Materials and methods: Each year, more than 1,000 avian blood samples collected by cooperation partners throughout Germany are analysed. Furthermore, organs from another 1,000 deceased birds are tested annually. The presence of WNV- and USUV-specific RNAs in blood and tissues is identified by RT-qPCR. RNA isolates showing positive RT-qPCR results are submitted to MinION Nanopore whole genome sequencing, eventually. Blood sera are screened for antibodies against flaviviruses by ELISA with subsequent confirmation of reactive results by specific virus neutralization tests.

Results: In 2021 and 2022, RT-qPCR and serological methods confirmed a moderate level of USUV circulation at all major locations, which is consistent with observations from preceding years. Meanwhile, WNV-specific RNA was only detected in birds from regions located in the German east already identified as transmission hotspots. There is, however, serological evidence for silent circulation of WNV at further locations in Germany. The phylogenetic analyses revealed the enzootic maintenance of one dominant WNV lineage 2 subcluster.

Conclusions: Detection of WNV cases in birds often precedes spill-over events to humans. Thus, the "bird´s-eye view" of WNV and USUV circulation provided by WBA-Zoo is a valuable contribution to preparedness in human and animal health as well as to wildlife conservation.

Reference:

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Characterization of Sarcoptic Mange in a Previously Naïve Red Fox (*Vulpes vulpes*) Population

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Abstract

Sarcoptic mange, caused by the parasitic skin mite *Sarcoptes scabiei*, is an emerging disease of canids in Prince Edward Island (PEI). The disease is associated with itching, hair loss, crusting, secondary infections, and in severe cases, emaciation and death. Since it was first confirmed in red foxes through a diagnostic submission to the Canadian Wildlife Health Cooperative (CWHC) in 2016, mange has become the most frequently diagnosed cause of death in PEI foxes. Despite it being described in over 100 mammalian species, the variability in disease presentation, progression and immune response, are poorly understood. This study sought to characterize gross and histopathological lesions in foxes with sarcoptic mange from a naïve, insular, North American population. From 2018-2022, 276 carcasses of foxes were obtained from fur trappers and through opportunistic submission to the CWHC, of which 88 mangy foxes were identified via skin scrape at necropsy. Two significant mange-associated lesions, alopecia and skin crusting, were documented using gross description, standardized diagrams, and digital photography. The body distribution and severity of these lesions were analyzed and described semi-quantitatively across 18 body regions for 51 foxes with sufficient documentation. Overall, the distribution and severity of lesions tended to increase proportionally to each other, however, foxes at presumed earlier stages of disease tended to have more disproportionate distributions. Lesions on the ischium, often reported as one of the first body regions with clinical signs for canids, were rare in this population and only occurred in the most severe end-stage cases. Lesion distribution and severity

seemed to decrease during winter months and during fall 2020-spring 2021. Co-occurrence of body lesions were analyzed by clustering using two matrices of computed similarity coefficients to produce a dendrogram of lesions that commonly presented together and in similar severities, as well as a dendrogram of like-presenting foxes. These diagrams resulted in predominantly ventral and dorsal lesion groupings and created a clear division of three main groups of like individuals. These types of lesion grading schemes could be used for the characterization of other emerging wildlife diseases where experimental studies are not feasible and disease presentations are unknown. Results from this study provided much-needed documentation of a sarcoptic mange outbreak in an insular North American canid population. It also re-evaluated longstanding questions involving disease presentation, progression, and thus transmission, furthering our understanding of the pathology and epidemiology of this disease.

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Application of nanopore sequencing using full-length 16S rRNA gene for identifying tick-borne pathogens in ticks from wild boars

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Abstract

The incidence of tick-borne diseases is rising globally, posing a significant public health concern. Through their role as reservoirs of tick-borne pathogens (TBPs) or tick amplification hosts, wildlife can impact TBPs epidemiology by expanding tick range and abundance. Therefore, there is an increasing need for TBPs surveillance in wildlife, and the emergence of next-generation sequencing (NGS) approaches can provide powerful techniques for detection, discovery, and characterisation of these pathogens.

In Europe, Eurasian wild boar (*Sus scrofa*) populations have severely increased, particularly around urbanized areas, such as the metropolitan area of Barcelona (MAB), Spain. These increasing human-wildlife interactions have given rise to new epidemiological scenarios where TBPs can spread faster. In fact, doctor appointments for tick bites and hospital admissions for tick-borne diseases have increased in the MAB in recent years. Moreover, many of these diseases have an unknown origin, evidencing the need for monitoring and detecting TBPs in ticks from this area. In this context, we conducted a study to evaluate the full-length 16S rRNA gene sequencing, an NGS approach, as a broad screening tool for tick-borne bacteria in ticks infesting wild boars from the MAB.

DNA was extracted from 60 ticks feeding on 12 urban wild boars sampled between 2022 and 2023, their whole 16S rRNA gene was amplified by PCR, and nanopore-based sequencing was conducted on the Oxford Nanopore Technologies (ONT) MinION device. Among all the bacteria genera responsible for most of the reported human TBDs, we identified several *Rickettsia* spp. belonging to the spotted fever group such as *R. slovaca*, the etiological agent of tick-borne lymphadenopathy, aligning with a prior study carried out in the same area.

Data obtained from this study demonstrates that the use of the ONT MinION device provides long read sequence lengths that can identify tick-borne bacteria at species-level and, in some cases, to strain-level, thus offering higher taxonomical resolution compared to other NGS approaches. This constitutes a robust tool for the prevention, monitoring, and control of bacterial tick-borne diseases, particularly in globalized cities and with high wildlife-human interactions.

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Effects of intravenous BIMU-8 and vatinoxan on arterial blood gas composition in etorphine-immobilised sheep

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Abstract

Cardiorespiratory compromise caused by the potent opioid etorphine remains a problem in wildlife immobilisation. Two novel drugs, the 5-HT4 receptor agonist, BIMU-8, and the peripheral alpha-2 adrenoceptor antagonist, vatinoxan, have shown promising properties in counteracting negative cardiorespiratory effects without affecting immobilisation. Our aim was to evaluate whether these two drugs could reduce opioid-induced hypoxaemia and hypoventilation based on arterial blood gas analysis. Healthy adult female sheep (n=6) were used as a model for wild *artiodactyls* in a randomised, prospective, controlled cross-over study. Each animal was immobilised with etorphine (0.05 mg/kg) intramuscularly (t0) three times with a four-week washout interval in-between and received each treatment: sterile water (control), BIMU-8 (1.5mg/kg), vatinoxan (0.15mg/kg) administered intravenously seven minutes (t7) after t0. Blood samples were collected from an auricular arterial cannula at t-5 (before etorphine), t5 (after etorphine), t12 (after treatment at t7), t18 and t24 and blood gases and electrolytes analysed. Body temperature was measured using a pulmonary artery catheter at the same timepoints.

Statistical analysis was done using a linear mixed model with 'time', 'treatment' (control/BIMU-8/vatinoxan) and the interaction of time*treatment as fixed factors, and 'sheep' as random factor.

Etorphine induced hypoxaemia, hypercarbia, and acidaemia in all sheep, which worsened over time. Sheep treated with vatinoxan had lower serum glucose concentrations and body temperatures ($p<0.05$) than animals that received the other two treatments, likely due to alpha-2 antagonistic effects on insulin and the peripheral vasculature. However, neither intravenous BIMU-8 nor vatinoxan improved arterial blood gases (i.e. respiratory compromise) in etorphine-immobilised sheep.

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Necrotising fasciitis by Gram negative bacteria in a wild Western-European hedgehog (*Erinaceus europaeus*)

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Abstract

Necrotizing fasciitis (NF) is a rare, life-threatening acute infection of the subcutaneous tissue that leads to necrosis of the surrounding tissues, including muscle and fat. B-haemolytic *Streptococcus* are the most common agents, but *Staphylococcus pseudintermedius, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Escherichia coli,* and *Serratia marcescens* have also been isolated in canine cases. NF cases in wildlife are rarely reported and these animals are usually in captivity.

A Western-European Hedgehog was admitted at a wildlife rehabilitation centre, after a road traffic accident, non-ambulatory. On X-rays a fracture of the distal ileum was found, and the animal was stabilized to be prepared for surgery. Surgical reduction of the fracture was achieved, and stabilization was acquired with an IM pin and an hemi cerclage wire. During convalescence. a swelling was noted in the shoulder region, extending to the axillary region. The skin was hot and discoloured. X-rays revealed subcutaneous air and a fine needle cytology revealed multiple bacteria and leucocytes.

NF was suspected and surgical excision of the affected tissues was accomplished in the same day. To address the extensive skin defect resulting from excision, two Z-plasty incisions were made to facilitate wound closure and a subcutaneous drain was left in place for 3 days. Surgical excised samples were sent for histopathology and microbiologic culture. Marbofloxacin was elected as a first-choice antibiotic and was administered subcutaneously daily for 21 days, oral meloxicam was added to the therapeutic regime daily for 5 days. Sutures were cleaned and

checked every day initially and every 2 days until suture removal, ten days after surgery. Histopathology revealed panniculitis and necrosis of the fascia and underlying muscle, while Gram staining identified Gram-negative coccobacilli. Microbiological culture yielded a pure Escherichia coli culture, that was sensitive to marbofloxacin. Five weeks after the initial admission, the ileum fracture had healed, warranting the removal of the IM pin. After confirmation of full ambulatory recovery and successful acclimatization to outdoor enclosures, similar to the natural habitat, the hedgehog was successfully released back into the wild.

This case underscores the critical importance of prompt identification and intervention in cases of NF, particularly in wildlife where such occurrences are less frequently reported. By employing a multidisciplinary approach encompassing surgical intervention, targeted antibiotic therapy, and meticulous wound care, veterinarians can effectively manage NF and aim for successful outcomes.

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Integrated wildlife monitoring as a tool to quantify the impact of emerging pathogens in natural populations: the case study of Bagaza in red-legged partridge

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Abstract

Integrated wildlife monitoring (IWM) has been highlighted as a crucial tool for early detection of emerging pathogens in natural populations and for tracking changes in disease dynamics. A mortality event associated with neurological symptoms caused by Bagaza virus (BAGV), an emerging flavivirus in Europe, was detected in a red-legged partridge (*Alectoris rufa*) population in a 3000-ha hunting area in southern Portugal between September and November 2021. Using demographic data, hunting records and climatic variables collected as part of a monitoring program between 2012 and 2024, the objective of our study is to quantify the impact of this outbreak on population size, and thus estimate BAGV-induced mortality. For that we developed a population model using 10 years of demographic data (2012-2021) collected before the outbreak as a baseline to predict the population size for the following three years (2022 to 2024). Specifically, a Generalized Linear Model was designed to model the population size in January, after the hunting season (October-December), using the previous year's July population estimates and the estimated mortality associated mainly with predation between July and December, in addition to hunting records. We also examined the role of climatic variables in improving the model fit, specifically the monthly average temperature and cumulative rainfall between July and December. In addition to the demographic variables, only the average temperature in October improved the model fit. This model had a McFadden's R-squared of 0.994, indicating an almost perfect fit. The predicted population size in January 2022 was 59% ($CI_{95\%}$ 52.0-66.0%) higher than the observed population size, while in 2023 and 2024 the predicted values were 3.0% ($CI_{95\%}$ 0.1-5.8%) and 4.2% ($CI_{95\%}$ 1.1-7.1%) lower than the observed values. Given that management practices remained similar, the discrepancy between the predicted and observed population size in 2022 appears to be due to BAGV, representing a BAGV-induced mortality rate that surpasses previous estimates for natural red-legged partridge populations by more than two-fold. The geographic expansion of BAGV and its detection in other wildlife species underlines the threat posed by this potentially zoonotic disease. Hence, its continuous monitoring in natural populations and at the interface with domestic animals and humans is imperative. Our study, reinforces es the pivotal role of IWM within the One Health framework, offering a proactive approach towards averting future pandemics.

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Dynamics of flavivirus activity in multiple avian species breeding in red-footed falcon colonies of eastern hungary

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Abstract

The emergence and spread of West Nile virus (WNV) in Hungary two decades ago was the first step in the establishment of this previously exotic zoonotic flavivirus in Central Europe. Lineage 2 WNV spread from the Carpathian Basin to the Mediterranean region in 2009 and eventually much further, to Germany and the Netherlands. Identification and characterization of environmental and ecological factors leading to WNV outbreak peaks like the 2018 pan-European epidemic is essential to increase the efficiency of disease control and forecasting.

Our objective was to characterize the seasonal activity of flaviviruses in bird communities of the Red-footed falcon colonies and local mosquito fauna at Vásárhely-puszta, a typical lowland habitat in Eastern Hungary.

During the 2022 breeding season, blood samples were collected from nestlings of colony-breeding and territorially breeding Kestrels (Falco tinnunculus), Red-footed falcons (Falco vespertinus) and Jackdaws (Coloeus monedula). Mosquitoes were trapped weekly from June to September using BG-Sentinel traps at the same sites. Total nucleic acid was extracted from blood samples and mosquito pools and examined for WNV and Usutu virus in a TaqMan RT-PCR. Sera were tested with the Ingezim West Nile Compac ELISA kit.

From June to mid-August, blood samples were collected from 287 nestlings (45 Jackdaws, 181 kestrels and 61 Red-footed falcons). ELISA test yielded positive results in 0% of Jackdaws, 31.5% of Kestrels and 13.1% of Red-footed falcons. qPCR detected Usutu virus infection in one Kestrel, while lineage 2 WNV infection was also identified in a single kestrel nestling. A total of 2746 mosquito specimens were collected and sorted into 393 pools. 91.3% of identified mosquitoes were Culex pipiens. qPCR detected 7 lineage 2 WNV and 7 Usutu virus positive mosquito pools of Culex pipiens, Ochlerotatus dorsalis and Coquillettidia richiardii.

Seasonal flavivirus circulation was detected and characterized in the local bird community and mosquito vectors. Seropositivity of 3-4 weeks old nestlings may be due to either maternal immunity, or a very early infection. ELISA results will be

confirmed by the immunofluorescence test and cross-reactions will be differentiated. Viral circulation detected in mosquitoes at the end of July and mid-August corresponds to the characteristic seasonality of flavivirus activity, but WNV and USUV were already detected in the examined chicks as early as mid-June and July. This study was conducted from research grants SA-99/2021, TKP-2021-EGA-01, RRF-2.3.1-21-2022-00006, OTKA K 120118.

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Case report of na interclavicular air sac fibrosarcoma in a female Carolina Duck (Aix sponsa, Linnaeus 1758)

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Abstract

The Carolina duck (*Aix sponsa,* Linnaeus 1758) is an Anatidae species originating in North America. Several types of neoplasias have been reported in this species. The aetiology is not defined and spontaneous tumors are rare within this species.

The authors present an unusual case of spontaneous fibrosarcoma occurring in an interclavicular airbag in an adult female *A. sponsa*. The animal was found dead in the, without any previous signs of sickness, being the tumour only observed in the postmortem exam. The mass was spherical with 3cm of diameter cranial to the heart, anatomically related to the inter-clavicular air sac. At the cut, the surface presented a colour grey/white and sparkling with a fasciculate pattern. At microscopic examination was possible to observe the proliferation of spindle-shaped cells arranged in an interwoven pattern, with moderate cytonuclear atypia. The neoplastic cells presented scant cytoplasm and elongated to oval nuclei with inconspicuous nucleoli and occasional mitotic figures Cytonuclear atypia was generally moderate, however cellular aberrations were observed. Van Gieson's staining confirmed the diagnosis of fibrosarcoma. Single cases of tumours in Anatidae have been described. However, prevalence surveys, essential for

understanding its aetiology and defining risk factors, are quite rare at the moment. Cancer prevalence seems to be increasing in wild and exotic animals and can have a great impact and significance on the environment-human-animal triad. In the future, a one-health approach to neoplasia is the basis for comparative oncology and enables a different attitude towards cancer as a possible ecosystem disease.

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Assessing avian influenza surveillance intensity in wild birds using a One Health lens

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Abstract

Wildlife disease surveillance systems can support early detection of emerging pathogens, promote preparedness, and provide the contextual basis to inform timely and effective response in the event of an outbreak. Zoonotic pathogen surveillance in particular warrants an integrated approach that maps and evaluates the system within a One Health framework in recognition of the inextricable linkages that exist at the human-animal-environmental interface. This study evaluates the intensity of avian influenza virus (AIV) surveillance in Ontario's wild bird population following the 2021 H5N1 highly pathogenic avian influenza virus (HPAIV) incursion into Canada using a One Health lens across gradients of human, agricultural, and wild bird populations. Specifically, we aimed to 1) identify spatial clusters of high and low wild bird AIV surveillance intensity; 2) describe the spatial and temporal distribution of HPAIV wild bird detections and HPAIV infected domestic premises; and 3) use a One Health approach to identify opportunities to enhance wild bird AIV surveillance. Analyzing 2,562 samples collected between November 1, 2021, and October 31, 2022, in Ontario, Canada, as part of Canada's Interagency Surveillance Program for Avian Influenza Viruses in Wild Birds, we identify spatial variations in surveillance intensity relative to human population density, poultry facility density, and wild mallard abundance. Using the spatial scan statistic, we pinpoint areas where public engagement, collaborations with

Indigenous and non-Indigenous hunter/harvesters, and working with poultry producers, could augment Ontario's AIV wild bird surveillance program. Enhanced surveillance at these human-domestic animal-wildlife interfaces is a crucial element of a One Health approach to AIV surveillance. Our findings underscore the importance of both live, through capture or harvest, and sick and dead wild bird surveillance, and suggest that both programs could benefit from expansion and enhanced support. A targeted and adaptive AIV surveillance strategy acknowledges the capabilities and limitations of each surveillance method, to enable strategic resource allocation and the prioritization of sampling opportunities to maximize coverage. Ongoing assessment of our wild bird surveillance programs is essential for strategic planning and will allow us to refine approaches and generate results that continue to support the program's overarching objective of safeguarding the health of people, animals, and ecosystems.

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Estimating post mortal interval of wild boar carcasses to establish a timeline in the Swedish ASF outbreak 2023

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Abstract

African swine fever (ASF) is a viral hemorrhagic, often fatal disease with high global economic consequences. Sweden was free from ASF until September 6, 2023, when a dead wild boar was sampled in the passive surveillance and confirmed positive for ASF virus. After a thorough search of the outbreak area, a core area was fenced to restrict wild boar movement. Estimating the time of death (TOD) of positive carcasses in an outbreak can give information that helps to understand the outbreak epidemiology. The purpose of this study was to estimate the time elapsed since death for wild boar carcasses from a ASF outbreak to discern the outbreak timeline and epidemiology. Local hunters searching for carcasses were instructed to take photographs of the carcass before it was removed from the forest, sampled, and then incinerated. Information about the

habitat, stage of carcass decomposition, presence of maggots and signs of scavengers was collected when possible, using a checklist. An evaluation was made for all carcasses found up until October 31. In addition, a selected carcass in advanced decomposition was evaluated using a more advanced human taphonomy model that calculated day-degrees needed to reach a certain stage of decomposition. Weather data was received from Swedish Metrological and Hydrological Institute (SMHI). Using these methods, a TOD was estimated for each carcass. Using the photo evaluation, the oldest carcass was estimated to have died between May 17 and July 7 which corresponded well to the more advanced taphonomy model, which for the same carcass indicated an interval from May 8 to June 28. All evaluated carcasses had an estimated TOD from May to late September. No indication of active disease spread has been noted after September 2023. Using photographs of carcasses is an easy yet reliable way to estimate an approximate TOD in wild boar carcasses, if reference material is available. Severely decomposed carcasses render a wider margin of error, whereas fresh cadavers give a narrower margin of error. However, the methods used gave an estimation of time of introduction of ASF in Sweden and when active spread of disease ceased. This epidemiologic information helps managers to adjust disease control efforts, to adapt applied restrictions, and can be used as support for the future declaration of freedom from disease.

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A current snapshot of *Leptospira* presence in wild boar across Germany

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Abstract

Leptospirosis represents a major threat to public health as one of the important re-emerging zoonosis worldwide and is caused by pathogenic species of the genus *Leptospira*. Although wild boar were shown to play a major role in *Leptospira* epidemiology current information on the relating presence in Germany are lacking. Since serological tests are complex or not commercially available for the wildlife sector, molecular diagnosis is essential for large-scale surveillance and

genetic differentiation, but is challenged by the extensive biodiversity of *Leptospira* spp. in the environment.

In order to reveal the current presence and recent development of both pathogenic and non-pathogenic *Leptospira* spp. in the wild boar > 1000 kidney samples collected during hunting seasons from 2017 – 2023 on 21 military training areas from all over Germany were subjected to 7 different *Leptospira*-PCR-assays targeting different genes. For confirmation, 2 commercial PCR-kits specific for pathogenic *Leptospira* spp. were applied. In addition, EDTA-blood-samples from animals tested positive were investigated likewise.

Regarding the pathogenic species, the pathogenic *Leptospira* spp. group II were mostly detected. Highest percentages were found in the south-west (Baden-Württemberg, Bavaria) with 38,6 % of the whole population tested positive in kidneys, followed by northern (Schleswig-Holstein) and middle-west (Hesse) Germany, while lowest percentages were detected in the north-east (Mecklenburg-Western Pomerania) with only 2,28 % positive population in total. Molecular detection of non-pathogenic *Leptospira* spp. revealed highest proportions in middle-west (Hesse) with 35,82 % and middle north-eastern Germany (Saxony-Anhalt, Brandenburg) with 30,56 % respectively. A general increase was observed in all areas during the last 4-5 years, particularly since 2019 until 2021 depending on the region. *Leptospira*-DNA in EDTA-blood samples was detected in 78,9 % of animals with positive kidneys.

The clear geographical differences, particularly concerning pathogenic *Leptospira*, are mostly in line with prevalence data of rodents and current human incidence rates suggesting an important link between human Leptospirosis and wildlife. The comprehensive molecular approach for the differentiation of the pathogenic, intermediate and the saprophyte cluster revealed a clear domination of the recently described *Leptospira* spp. group II that is suspected to play a role in human and animal Leptospirosis. The German wild boar population was shown as substantial carrier for both pathogenic and saprophytic *Leptospira* species. In the one-health context, the steady increase of wild boar population and mixing of habitats from wildlife, domestic animals and humans substantially contributes to the risk of interspecies transmission.

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Identification and Characterization of Circulating Borrelia Strains in an Endemic Area of North-East Italy

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Abstract

Background

Lyme disease is a widespread severe tick-borne zoonosis, mainly transmitted by the *Ixodes* genus, whose etiological agent, *Borrelia burgdoferi* (s.l.), includes several pathogenic genospecies that cause different clinical symptoms in humans. As information regarding the distribution of different *Borrelia* strains is poor, it is therefore important to identify the genotypes circulating in field, in a view to gain a more detailed knowledge of the epidemiology of this disease.

Objectives

This study aims to identify and genetically characterize *Borrelia* genospecies circulating in ticks in a Northeast Italy endemic area. This data will help to improve surveillance of pathologies caused by bacteria of the genus *Borrelia*.

Material and Methods

Ticks were collected in 2021-2023 through active surveillance surveys using the dragging/flagging technique, and through passive surveillance from both wildlife and people accessing the Emergency Room of the Belluno Hospital. Following morphological and developmental stage identification, ticks were ethanol-washed and subjected to DNA extraction. The presence of *Borrelia* and the involved genospecies were investigated using specific qPCR assays. For deeper genomic characterization of positive *Borrelia* samples, Multilocus Sequence Typing (MLST) was directly applied to tick extracts.

Results

Borrelia was detected in 15% of environmental and human ticks, and in 4% of ticks from wild animals. Among infected ticks, *Borrelia afzelii* was the most prevalent genospecies (31%), followed by *Borrelia garinii* (17%). Notably, 7% of

environmental ticks were positive for *Borrelia miyamotoi*, and 8% harbored co-infections with multiple *Borrelia* genospecies. MLST was performed on 30 samples: 27 different Sequence Types (ST) were obtained of which 23 were not previously identified in the MLST database.

Conclusions

Our findings revealed a remarkably high degree of genetic diversity among *Borrelia* genotypes circulating in the investigated areas. Notably, strains infecting environmental ticks displayed no clonal relationship to those found in humans. Although *Borrelia afzelii* was the dominant genospecies, a significant proportion of ticks showed to harbor *Borrelia miyamotoi*, known to cause atypical tick-borne relapsing fever. This finding warrants further investigation, as this pathogen remains poorly understood and likely underdiagnosed in humans.

The present work is part of the research project IZSVE RC 08/2020 founded by the Italian Ministry of Health.

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Serological assessment of the circulation of Crimean-Congo Hemorrhagic Fever in wild ungulates from Southern France.

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a human hemorrhagic fever characterized by severe symptoms and important fatality rates, on the rise in the Balkans and Mediterranean countries. Its main vectors are ticks from genus *Hyalomma* spp. and the virus has been recently identified for the first time in France in *H. marginatum* ticks collected in proximity of the border with Spain (Pyrenees region). While wild vertebrate species, particularly ungulates (wild boar, deer), could play a role in the maintenance and spread of vector ticks as well as in the transmission of CCHF virus, limited information is currently available regarding the presence and spread prevalence of the virus in European wild ungulates. A total of 2182 sera including 869 wild boar (*Sus scrofa*), 80 mouflons (*Ovis orientalis*), 252 red deer (*Cervus elaphus*), 977 roe deer (*Capreolus capreolus*) and

4 foxes (*Vulpes Vulpes)* were collected by different associations of French hunters between 2008 and 2022 from 6 Southern French departments and they were analyzed for the presence IgG and IgM antibodies against CCHFV, using a commercial ELISA kit. Positive samples were confirmed by pseudo-plaque reduction neutralization by the National Reference Laboratory of Hemorrhagic Fever in BSL-4. A multivariate logistic regression model was run to identify potential risk factors of exposure including age, sex, season and forest type. A total of 49 positive samples were detected in wild boar (29% of seropositive sera identified), red deer (18%), fallow deer (26%) and mouflon (2%), distributed in three different departments. An important cluster (more than 90%) of seropositive cases was located in the Hautes Pyrenees Department, close to the Spanish border. Male animals were significantly more exposed than females. Despite investigated wildlife populations have been exposed consistently since 2008, this is the first time the serological activity to CCHF is detected in French wildlife registered. Despite wildlife exposure with CCHF has been previously reported in Spain, this is the first time that results are confirmed by seroneutralization. The levels of seropositivity detected in roe deer are the highest ever reported to date. This study highlights the importance of wildlife population as sentinel species of emerging infectious diseases particularly in transboundary regions.

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Survey of selected pathogens in free-living European wildcats (*Felis silvestris silvestris*) from north-eastern Italy

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Abstract

European wildcat (*Felis silvestris silvestris*) is a carnivore species protected by Italian law, naturally expanding its distribution in the north-eastern Italy (Friuli Venezia Giulia Region). During the period 2020-2023 a total of 54 wildcats were submitted for post-mortem examination in the framework of regional health wildlife surveillance plan. Putative hybrids were evaluated according to ornamentation and gut index towards domestic cat (*Felis silvestris catus*).

Prioritised wild carnivores diseases thoroughly examined were rabies, canine distemper virus (CDV), trichinellosis and *Echinococcus multilocularis* (EM), whereas leptospirosis and sarcoptic mange were investigated only if suspected. Further felid selected pathogens were steadily investigated: feline panleukemia virus (FPV), feline infectious peritonitis virus (FIPV), feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV). We investigated rabies virus by direct fluorescent antibody (DFA) test and the other viruses by means of molecular methods from spleen, gut, brain and lung samples. According to the EC Regulation 1375/15 the magnetic stirrer method was performed to detect *Trichinella* larvae by microscopy after the enzymatic digestion in muscle samples. Eggs of the cestodes were isolated from faeces with a sedimentation/filtration technique; the cestode species was determined by molecular methods. No suspect of leptospirosis neither sarcoptic mange were evidenced during necropsies. All brains tested negative for rabies. Spleen samples were negative for FIV; muscle tissues were negative for the search of *Trichinella* as well as faecal/gut samples for EM. We detected FeLV from the spleens of 3 wildcats (6.82%; CI95% 0.75-13.84%), FCoV and FPV from the guts of 2 different individuals (2,33%; CI95% 0.25-6.58%) and CDV from the lungs of one wildcat (2.17%; CI95% 0.25-6.14%). Aspecific and mild pathological changes were observed in affected animals: spleen and mesenteric lymphnodes enlargement. Rabies and parasites results were in accordance with the epidemiological status of the study area. The prevalence of felid viruses inferred from our study is in accordance or slightly lower than their spread in other European countries. Finally, the detection of CDV may suggest a direct or indirect contact with other carnivore species where the virus show periodical epizootics, such as red foxes (*Vulpes vulpes*) and mustelids. The constant surveillance of wildcats, focusing on pathogens at the interface between sympatric wild carnivore species, domestic cats and their preys, may provide further insights on their epidemiological role and on the impact of these viruses on wildcats health

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Genetic diversity of Pasteurellaceae strains in wildlife with different disease syndromes in Spain

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Abstract

Pasteurellacae are a significant cause of mortality and sporadic disease in wildlife. In this communication, we present preliminary results on the molecular characterization of 105 strains of *Pasteurellacae* isolated from wild animals received from passive wildlife health surveillance in Spain. Isolates originated from a variety of hosts, including wild ungulates (*Cervus elaphus*, *Capreolus capreolus*, *Capra pyrenaica, Rupicapra rupicapra*, and *Sus scrofa*), carnivores (*Lynx pardinus*), lagomorphs (*Lepus europaeus*, and *Oryctolagus cuniculus*), and rodents (*Myocastor coypus*) that had hemorrhagic septicemia, bronchopneumonia, or abscesses. Samples were cultured on chocolate agar plates and bacterial species were identified by the partial sequencing of the 16S rRNA gene. *Pasteurella multocida* was the predominantly identified species (56,2%), followed by *Bibersteinia trehalosi* (21,9%), *Mannheimia haemolytica* (13,3%), *Mannheimia granulomatis* (3,8%), *Mannheimia bovis* (2,9%), and *Pasteurella aerogenes* (1,9%). *Pasteurella multocida* isolates that were associated with bronchopneumonia belonged to capsular type and biovar (lipopolysaccharide) A3, A5 or B8. They showed a strong positive association to the virulence genes *hgb-B*, *tbp-A*, and *nanB586* in ruminants, and isolates from *Sus scrofa* additionally showed positive results for *pfh-A*. Capsular type B and biovar 2 were detected in all *P. multocida* isolates from hemorrhagic septicemia cases in *Sus scrofa* and *Cervus elaphus* and showed a strong association with *pfh-A*, *hgb-B*, and *nanB586* virulence genes. *Mannheimia haemolytica* was isolated from *Rupicapra rupicapra* with bronchopneumonia or abscesses. Most of the isolates belonged to capsular type A and biovar 2, but 21% belonged to other biovars (other than A1, A2, A6). The virulence genes *lkt-A*, *lkt-D*, *lkt-D2*, *irp*, *ton-B*, *sod-A*, *adh*, *lps-A*, and *tbp-A* tested positive in almost all of the *M. haemolytica* isolates. Multilocus sequence typing (MLST) identified several *P. multocida* sequence types (ST), including 12, 31, 50, 64, 132, 370, 393, 445, 446, 473, 564, 565 and novel STs that have yet to be assigned. The MLST in *M.*

haemolytica isolates were grouped into ST 8 and 56 and four novel STs that are pendent to be assigned. We found specific biovars and virulence profile patterns associated with host species, disease syndromes and geographic locations, which enhances our knowledge of these important wildlife pathogens.

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Unravelling the threads: sero-epidemiological dynamics of Bagaza, West Nile and Usutu viruses in red-legged partridge before, during and after the Bagaza outbreak in southern Portugal

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Abstract

Bagaza virus (BAGV), a single-stranded RNA virus in the mosquito-borne cluster of the genus Orthoflavivirus, emerged in Spain in 2010 in red-legged partridges (RLP, *Alectoris rufa*), reappearing in 2019, 2021, and in Portugal in 2021.

Integrated in a monitoring program carried out in an RLP population in southern Portugal since 2018, birds were captured, tagged and sampled before and after the reproductive season. After detecting the outbreak (September-November 2021), sampling was performed every two months. We obtained 468 samples, until November 2022, distributed as: 178 before, 91 during and 199 after the outbreak.

To understand the sero-epidemiology of BAGV, it is crucial to include other flaviviruses co-circulating in southern Iberia, West Nile virus (WNV) and Usutu virus (USUV). Screening was performed using ELISA (n=468) designed for WNV, able to detect other flaviviruses by cross-reaction (i.e. USUV and BAGV). To differentiate specific antibodies virus neutralization test (VNT, n =430) was performed. ELISA showed a flavivirus seroprevalence of 58.1% (2018-2022). By VNT, 66.3% were positive and classified as WNV (28.8%), USUV (2.1%), BAGV (8.1%) or undetermined flavivirus (27.2%).

The first detection of BAGV specific antibodies occurred in October 2021 with a seroprevalence of 8.7%, reaching maximum levels in November 2021 (26.3%) and persisting until October 2022 (2.3%).

WNV antibodies were detected in all sampling periods, with an overall seroprevalence of 28.8% (10% August 2022- 44.8% October 2020), suggesting WNV is endemic in RLP in southern Portugal. Curiously, the highest seroprevalence, observed in October 2020 (44.8%), coincides with the human WNV outbreak in southern Spain in the summer of 2020.

USUV antibodies were detected until the BAGV outbreak in 2021, with low seroprevalence, reaching 6.9% in October 2020. A proportion of VNT-positive individuals (n=117) were classified as undetermined due to similar titres of neutralizing antibodies to more than one flavivirus (< 4-fold differences).

This study shows that long-term monitoring of wildlife populations is essential to detect emerging pathogens such as BAGV and unravel their epidemiological dynamics, including the risk factors associated with their emergence (BAGV) or persistence (WNV).

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Is antimicrobial resistant *Salmonella* spp. in gulls related to that in humans? A case study in Northeastern Spain

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Abstract

Salmonellosis ranks as the second most reported foodborne disease in humans in the EU. While *Salmonella* spp. commonly occur in food animals, they are also prevalent in wildlife, particularly in gull species that scavenge on refuse and frequently interact with urban environments. This suggest that gulls may serve as *Salmonella* reservoirs, including antimicrobial resistant (AMR) strains. Here, using a MIC-based broth microdilution method we investigated the prevalence of AMR *Salmonella* in a yellow-legged gull (*Larus michahellis*) colony with a high anthropogenic pressure on the Medes Islands (Northeastern Spain), from 2013 to 2022. We also assessed by Pulsed Field Gel Electrophoresis (PFGE) the epidemiological link with *Salmonella* spp. responsible for human salmonellosis in a nearby hospital from 2016 to 2018. We focused on serovars Enteritidis, Typhimurium and its monophasic variant (n= 122, 82 from gulls and 40 from humans). Overall, *Salmonella* AMR prevalence was higher in yellow-legged gulls (93.9%, 2013-2022; 84.4%, 2016-2018) than in humans (67.5%, 2016-2018). Multidrug resistance (MDR) between gulls and humans did not differ significantly (64.9% vs. 70.1%) and most prevalent MDR profile in both hosts was Ampicillin-Tetracycline-Sulfamethoxazole. Our analysis identified a total of 23 PFGE genetic profiles, eight of which were shared between gulls and humans, encompassing pan-susceptible, AMR, and MDR strains. These results point to an anthropic origin of isolates from this gull colony, highlighting the important role of seabirds scavenging on human waste in maintaining and spreading zoonotic bacteria and AMR in the environment. Our research confirm gulls can serve as exceptional sentinels for detecting levels of zoonotic AMR bacteria in the environment and underscore the need for a One Health approach to address the challenge of AMR.

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Generalized avian tuberculosis in a free-living Eurasian griffon vulture (*Gyps fulvus*), a case report

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Abstract

Background: Avian tuberculosis (*Mycobacterium avium*) is a contagious chronic disease affecting poultry, birds in zoologic gardens and wild bird species. The cases described in vultures are few and none describe forms that extend to the heart and subcutaneous tissue

Objective: We describe here a case of an adult male, free-living Eurasian griffon vulture (*Gyps fulvus*) that was found in difficulty in the alpine area of Lombardy, North Italy.

Materials and methods: On 09/18/2023 a griffon vulture was found in the province of Sondrio, in the municipality of Campodolcino (46.4012696,9.3411499,15). Sheltered to wild animal rescue center of Sondrio Province. The vulture was in poor general condition (body weight 4.90 kg), severely dehydrated and unable to fly. At clinical examination the lower limbs appeared swollen and ulcerated, in particular the right limb (exudative cellulitis). The vulture underwent X-ray examination, biochemical-clinical investigations and bacteriological, mycological and virological examinations. Despite good initial response to supportive and antibiotic therapy, the bird died on 10/24. A necropsy examination was then performed with specific bacteriological and histological investigations.

Results: Swelling of the lower limbs with elephantiasis of the right and presence of fibrotic eschars covering necrotic-ulcerative lesions. Oral mycoses on the lingual margins and in the buccal fornix. Granulomatous hepatitis with numerous lardaceous whitish granulomas on the surface and in the hepatic parenchyma that appears friable. Presence of large granulomatous lesion in the spleen. Granulomatous lesions are found in the wall of the small intestine, in correspondence of cecal tonsils and mesentery. The heart appears limp and with hydropic alteration of the apex.

Histological examination revealed: Spleen - severe chronic granulomatous splenitis and

widespread with granulomas with a necrotic core and numerous foamy macrophages. Liver- severe and multifocal chronic granulomatous hepatitis with

granulomas with a necrotic center and associated with severe and multifocal acute hepatocyte necrosis. Kidney - multifocal endotubular mineralizations. Heart - severe and multifocal chronic granulomatous myocarditis with granulomas with necrotic center surrounded by a wall of giant multinucleated macrophages. Limb - the subcutis and muscle tissues expanded due to the presence of a fibrous connective tissue in which central granulomas can also be observed

necrotic; fragments of fungal hyphae disseminated in the connective tissue can also be observed

Conclusion: Microbiological and molecular biology tests confirmed generalized *Mycobacterium avium* infection. The case described demonstrates how *Mycobacterium* spp. infections can represent a cause of mortality in Griffon Vultures, causing lesions not limited only to internal organs.

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Prevalence of Escherichia coli producing extended-spectrum β-lactamases isolated from wild European hedgehogs in urbanized areas of Northern Italy

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Abstract

Escherichia coli producing extended-spectrum β-lactamases (ESBLs) have become a major issue for both human and veterinary medicine. In order to monitor the increasing emergence of antimicrobial resistance (AMR), wildlife has been proposed as a sentinel for the spread of AMR in the environment, since it can be a reservoir for AMR bacteria. Thanks to their eating behaviour, hedgehogs represent a promising indicator for evaluating the environmental health. In fact, they are permanent resident animals who live in anthropized areas in contact with both humans and other animal species.

Here we investigated the prevalence of ESBL-producing *E. coli* in wild hedgehogs (*Erinaceus europeus*) from urbanized areas of Northern Italy. Samples of the

intestinal content were collected from hedgehogs deceased in a Wildlife Rescue Center located in Lombardy region. Only the animals without previous antibiotic treatment were enrolled. Bacteriological analysis was performed as follows: each sample was enriched 1:10 in buffered peptone water and, after an overnight incubation at 37°C, it was plated onto CHROMagar^{TM} ESBL plates. AMR profiles of ESBL-producing *E. coli* strains were evaluated by broth dilution (MIC). The presence of the *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CMY-2}$ genes, as well as the genes coding for the resistance to colistin (*mcr-1* to *mcr-10*), was then assessed by PCR.

Overall, the intestinal content of 49 hedgehog carcasses was collected. The prevalence of ESBL-producing *E. coli* was 51% (n=25). All the strains were multidrug resistant (resistant to ≥3 antimicrobial classes); the main resistances were recorded against all the tested β-lactams (cefotaxime, cefazoline, and ampicillin) (100%, n=25), followed by enrofloxacin (92%, n=23), tetracycline (64%, n=16), flumequine (60%, n=15). There were significant correlations ($p<0.01$) within the same antimicrobial class as for quinolones/fluoroquinolones (Spearman's rank correlation coefficient $\rho = 0.85$), or sulfonamides ($\rho = 0.98$). All the strains were sensitive to colistin. Most of them was sensitive to kanamycin (96%, n=24), aminosidine (96%, n=24), and florfenicol (92%, n=23).

The *bla*$_{TEM}$ was the most frequent gene (52%, n=13), followed by *bla*$_{SHV}$ (36%, n=9), and *bla*$_{CTX-M}$ (28%, n=7). Seven strains carried two ESBL genes, five being *bla*$_{TEM}$/*bla*$_{SHV}$, and two *bla*$_{TEM}$/*bla*$_{CTX-M}$. The strains harbored neither the *bla*$_{CMY-2}$ gene nor the *mcr* genes.

Our study provides the first data on the prevalence of ESBL-producing *E. coli* in wild hedgehogs in Northern Italy. The AMR profiles and the genomic characterization of these strains confirms the potential role of hedgehog as an indicator of the AMR spread in urbanized areas.

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Impact of broadleaved hedgerows on the distribution of rodent-associated pathogens and ticks in pine monoculture: insights into the role of a generalist rodent

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Abstract

Understanding the complex relationships between landscape and pathogens distribution in wildlife is critical for evaluating and mitigating zoonotic risks. Human-induced landscape fragmentation and disturbances are key drivers shaping host communities, frequently favouring "generalist" species that may often harbour multiple zoonotic pathogens. Ecological restoration has been proposed as a strategy to enhance biodiversity and, consequently, mitigate zoonotic risks. In Western France, the Landes Forest is a 19th century human-planted forest dedicated to the monoculture of maritime pine (>70%). The plantation of broadleaved hedgerows has been planned to restore biodiversity and enhance the forest resilience against pests, wildfires and wind damages. However, the impact of such hedgerows on the mitigation of zoonotic danger has not been evaluated yet. In this context, we propose investigating whether the quality and connectivity of hedgerows have an impact (i) on the diversity of rodent communities and (ii) on the diversity and prevalence of rodent-associated pathogens and ticks.

Rodent and tick sampling was conducted during the spring and fall of 2023 within the Landes Forest, using the standardised design shaped within the European Superb project. It comprises 24 broadleaved hedgerows characterised by four categories of varying degrees of connectivity and density of broadleaved trees, and 12 control trap lines at the edge of pine forest plots. We detected pathogenic bacteria from rodent spleens, using a metabarcoding approach based on primers targeting the 16S-rRNA and rpoB genes. Tick specimens were collected individually from rodents and through tick-flagging, followed by identification using a microfluidic high-throughput real-time PCR system.

Among the 79 rodents captured, the generalist species Apodemus sylvaticus (wood mouse) dominated with 76 individuals trapped. Interestingly, hedgerows categories did not influence trapping rates. We identified 12 putative pathogenic bacteria in the rodents and three tick species. Mice in lines with a lower density of close broadleaved trees had a higher likelihood of infection with the tick-borne pathogen Neoehrlichia mikurensis. Additionally, a greater number of ticks were collected from the ground in hedgerows compared to pine edges.

Our findings highlight the limited effectiveness of the sampled hedgerows in enhancing rodent diversity. However, they underscore the critical influence of landscape characteristics on rodent-associated zoonotic hazards, emphasizing the

importance of integrating zoonotic risk management into ecological restoration programs.

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Diversity of pathogens in the blood of synanthropic rodents within Poland.

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Abstract

Rodents serve as reservoirs or hosts of a wide range of zoonotic pathogens and therefore play an epidemiological role in the transmission of infectious diseases to humans and animals. Synanthropic rodents are termed vermin given that they impact negatively on human health and economy. To acquire a preliminary unbiased measure of the microbial diversity and abundance in the blood to detect the pathogens carried by synanthropic rodents from various habitats.

Three hundred and twelve synanthropic rodents were trapped live at different habitats (rural, urban and suburb) and blood samples were collected for metabarcoding analysis. In a total of 312 samples, 281(90.06%), 101(32.37%) and 179(57.37%) of the isolated blood samples tested positive to the preliminary polymerase chain reaction (PCR) to test for Bacteria, Apicomplexa and Kinetoplastida pathogens in which Listeria, Babesia and Trypanosoma spp were used as controls of each of the group respectively. Metabarcoding specific adapters for each group will be attached to positive isolates and sequenced on illumina platform to identify the taxonomy of pathogens present. This study provides better understanding on the occurrence, prevalence and risks associated with zoonotic pathogens (bacterial diversity) present in the blood of synanthropic rodents which poses risks to humans. This finding will help provide possible approaches to limit interaction between humans and rodents residing close to human habitation.

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Mannheimia Granulomatis-Infected Pyogranulomatous Oral Lesions with Secondary AA-Amyloidosis In Roe Deer (*Capreolus Capreolus*)

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Abstract

Mannheimia granulomatis-associated lesions are poorly described compared to those associated with other members of the *Pasteurellaceae* family; they include pneumonia, panniculitis, glossitis, stomatitis, and mastitis in domestic and wild ruminants and hares. This report describes 15 cases of roe deer (*Capreolus capreolus*) showing *M. granulomatis*-infected oral lesions that were detected through SAGIR, a participatory network performing event-based wildlife diseases surveillance in France through the collaboration between the French Biodiversity Agency (OFB), hunters, and local veterinary laboratories. The animals were collected between 2016 and 2023, including nine collected within a localized geographic region over a 14-month period. A full necropsy and additional analyses were performed, including bacteriological, coproscopic, and histopathological examination. Animals submitted were older than 15 months of age based on tooth eruption (15/15), and were thin (1/15), cachectic (13/15) or of unknown body condition (1/15). Gross changes consisted of multiple millimetric to centimetric caseous granulomas within the lips, tongue and/or cheeks (13/15), suppurative lymphadenitis of a mandibular lymph node (1/15), or necrosuppurative osteomyelitis with bone proliferation (1/15). Histopathological examination performed on oral lesions of 10/15 animals consisted of multifocal, severe, chronic pyogranulomatous cheilitis, glossitis and/or stomatitis with intralesional Gram-negative bacterial colonies lined by Splendore-Hoeppli material. Amyloidosis was detected in 4/11 animals for which tissues were submitted for microscopic examination, affecting the lamina propria of the abomasum, the spleen, and a minor salivary gland in respectively 4/6, 3/5, and 1/8 animals for which the organ was assessed histologically. Amyloid deposits showed strong immunoreactivity with a murine monoclonal antibody directed against amyloid AA (mc4; amYmed). AA amyloidosis, rarely described in roe deer, was

considered secondary to chronic inflammation. *M. granulomatis* was cultured and identified biochemically or by MALDI-TOF MS in oral lesions of all individuals. Co-infection with other bacterial species such as *Bibersteinia trehalosi* (5/15) and *Streptococcus* spp. (2/15) was sporadically identified. The combined effect of oral lesions, secondary amyloidosis when present, parasitic infestation of variable severity, and possibly environmental factors, was considered to have significantly contributed to the chronic emaciation and the death of these animals. Careful examination of the oral cavity proved to be useful to detect cryptic pyogranulomatous lesions. Further investigation is needed to understand the epidemiology and pathogenesis of *M. granulomatis* infection in roe deer, including a possible propensity to emerge as temporal and geographical clusters and to induce AA amyloidosis.

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Serological survey of wild wood pigeons (*Columba palumbus*), free-ranging feral pigeons and domestic pigeons (both *Columba livia*) for Rotavirus A (RVA) infections in Germany

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Abstract

Pigeon-type *Rotavirus A* (RVA) of genotype G18P[17] was recently identified as the causative agent of 'young pigeon disease' (YPD) in domestic pigeons (*Columba livia* forma domestica) worldwide. The etiology of YPD had remained unclear for almost three decades. Pigeon-type RVA is usually spread among juvenile racing and ornamental pigeons during the racing or exhibition season, respectively, resulting in seasonal YPD outbreaks with mortality rates of up to 50%. Severe disease outbreaks caused by pigeon-type RVA have also been detected in free-ranging feral pigeons, but little is known about the distribution in their populations. Likewise, the occurrence of pigeon-type RVA infections in wild pigeon species, such as wood pigeons (*Columba palumbus*), is unknown.

In this study we investigated the occurrence of RVA infections by detection of RVA-reactive antibodies in sera collected from wild wood pigeons (n=316), free-

ranging feral pigeons (n=61) and domestic pigeons of different age groups (n=245) in Germany using an immunofluorescence antibody test (IFAT). While almost all of the adult domestic pigeons were seropositive (98.7%; n=75), only 62.7% of the sera from juvenile birds were reactive (n=161). Sera of feral pigeons possessed 82% seroreactivity. In contrast, only 4.7% of the tested wild wood pigeons possessed detectable antibodies and mean IFAT titers were about threefold lower than those of domestic and feral pigeon sera.

Our results demonstrate RVA infection to be widely distributed in feral pigeon populations, making them a possible reservoir for endemic maintenance of the infection and transmission of pigeon-type RVA to domestic pigeons. Wild wood pigeons appear to be only rarely infected. Whether this may be due to a lower susceptibility or due to their more scattered way of life remains to be elucidated.

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Sub-clinical effects of blood parasite co-infections in Namibian cheetahs (*Acinonyx jubatus*)

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Abstract

Co-infection by parasites is ubiquitous in wild animal populations. Pathogen (co-)infections can affect individual health and fitness and have the potential to influence population dynamics. However, these infections often remain sub-clinical, and their effects are therefore unknown or overlooked. To better understand the potential underlying costs and effects associated with long-term co-infections, we characterized inflammatory and biochemical parameters in free-ranging Namibian cheetahs (*Acinonyx jubatus*) in a long-term, cross-sectional study (n = 194). This cheetah population harbours a consistent pool of co-infecting vector-borne pathogens (VBP), including bacteria (*Rickettsia* spp., Anaplasmataceae), protozoans (*Babesia* spp., *Hepatozoon* sp.) and filaria (in particular *Acanthocheilonema* spp.). The inflammatory response was characterized by the levels of proinflammatory cytokines (IL-1β and TNF-α), an acute phase protein (SAA), as well as markers of macrophage activity (neopterin) and oxidative stress (reactive oxidative metabolites and antioxidant capacity) in serum or

plasma. Serum biochemical markers were chosen to cover liver and kidney function and muscle health (AP, ALT, GLDH, GGT, total bilirubin, bile acids, AST, CK, LDH, total protein, urea, creatinine). We investigated the relationships between the co-infecting pathogens, inflammatory and biochemical profiles, life-history parameters and environmental factors. Our results suggest that life-history parameters, such as age and sex are more important determinants of the described immunological and physiological measures than the parasite community in the animals. We discuss the importance of the measured parameters in wildlife health monitoring and what long-term effects the VBP infections can have on the genetically constrained cheetah population.

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Highly pathogenic H5N1 avian influenza virus infections in terrestrial predators, Northern Germany

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Abstract

Background

Infections with highly pathogenic avian influenza virus (HPAIV), currently of subtype H5N1 are detected in Europe year-round in wild birds and poultry. Also, the virus has been found in domestic and wild living mammals. Although high HPAIV infection rates in avian species could be detected in Germany, data on HPAIV infections of terrestrial predators, which feed on waterfowl were not available until recently.

Objectives

To determine whether AIV is present in wild life terrestrial predators, we performed a 1 year-survey. The study focused on detection of HPAIV genomes and characterization of infection. Additionally, an antibody ELISA has been currently validated to detect influenza specific antibodies.

Materials and Methods

Initially, HPAIV infections were studied in 170 wildlife predators of several species (red foxes, racoons, badgers, martens, and racoon dogs) by using PCR and in situ methods. The carcasses originated from different geographic locations in the German federal state of Lower Saxony. Investigations included generation of full-length genomic sequences and virus isolation. Further, the ID Vet Influenza A antibody ELISA was modified to investigate serological reactions in postmortal body fluids and meat juice.

Results

Viral RNA was detected in the brains of 5 red foxes which were submitted with differing preliminary reports partly involving signs of disease specific for the central nervous system. Full-length influenza virus sequences could be recovered in four cases. The H5 sequences belonged to the 2.3.4.4b clade of avian influenza viruses as shown by phylogenetic analyses. They were closely related to sequences previously found in avian and mammalian hosts in Europe and did not cluster separately. A PB2 E627K substitution was found in the consensus sequence in only one of four cases. HPAI H5N1 virus isolates were obtained during the first passage in MDCK II cells. None of the five virus positive foxes showed macroscopic brain lesions. Microscopically, mild-to-moderate multifocal lymphohistiocytic encephalitis with predominant perivascular infiltrations was observed in different brain regions. Influenza A nucleoprotein was detected in morphologically affected and unaffected areas of the brain by using immunohistological technique. Sporadic samples showed positive reactivities in a commercially available antibody ELISA.

Conclusions

Our data of a 1-year survey from the federal state Lower Saxony, Germany, confirmed that wild life predators are susceptible to HPAIV infections and subsequent disease. This underlines the potential of avian influenza viruses to infect terrestrial mammals.

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Survey of the presence of viral agents in wild ruminants in the alpine area of Lombardy, North Italy

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Abstract

Background: Wildlife ruminants are susceptible to multiple infectious agents of public health or veterinary relevance. Therefore, acting as a reservoir, they potentially represent a constant risk of their re-introduction into the human or livestock population.

Objective: To define the presence and distribution of viral agents in wild ruminant populations in the alpine area of Lombardy, North Italy.

Materials and methods: We examined 1224 wild ruminant blood samples collected during three consecutive hunting seasons (2020-2023) within the framework of wildlife monitoring plan. The tested species comprised roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), red deer (*Cervus elaphus*) and mouflon (*Ovis gmelini musimon*), which are all abundant in that area. Serological tests were performed to detect antibodies against viruses affecting domestic ruminants, i.e., Schmallenberg virus (SBV), Blue Tongue virus (BTV), Tick Borne Encephalitis virus (TBEV), Hepatitis E virus (HEV) and pestiviruses. The last were also investigated with virological methods. We employed the following tests: i) for SBV, TBEV and HEV, in-house MAb-based enzyme-linked immunosorbent assays (ELISAs); ii) for BTV a VP7-based competition ELISA; iii) for pestiviruses both an in-house competitive MAbs-based ELISA with recombinant NS3 protein as

antigen and a real-time RT-PCR targeted the 5'UTR region for detecting respectively the antibodies and the antigen.

Results: Antibodies against TBEV were detected in about 1.6% of the samples collected. Only two red deer of unknown age tested positive for anti-pestivirus antibodies, and all samples reacted negative for virological ruminant pestivirus. Thus, even if wildlife ruminants can share the same alpine pastures with domestic livestock, it is very improbable that they represent a significant virus reservoir and could be a source of infection.

Antibodies against the *Culicoides*-transmitted SBV were detected in 58 out of 278 (20.8%) sera samples collected in 2022/2023 in any species sampled. In contrast, all analysed samples tested negative for antibodies against the likewise *Culicoides*-transmitted BTV. The differences in the seroprevalences of antibodies against SBV and BTV in the wild ruminant population and the agreement with the correspondent situation in domestic animals confirm that wild ruminants do not play a significant role in maintaining BTV in a given area. Finally, all tested sera resulted negative for antibodies against HEV.

Conclusion: Serological investigations of wildlife animals represent a cornerstone of diseases surveillance, especially when virus shedding is only transient since antibodies remain detectable for longer periods.

This study was supported by grants from the project PRC 2020012 of the Italian Ministry of Health.

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Towards a common database to improve wildlife disease surveillance in Europe

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Abstract

Over 70% of the zoonotic emerging infectious diseases originate from wildlife. Wild animals are also present in urban areas, that are unique ecosystems that provide ample opportunity for zoonotic pathogen transmission between wildlife, domestic animals and humans. Urban areas can therefore form hotspots of increased zoonotic disease risk. The different disease dynamics in urban areas ask for an improvement of wildlife disease surveillance.

To facilitate wildlife disease surveillance in urban areas, a comprehensive overview of zoonotic pathogens studied in urban wildlife species will aid to determine how to set-up this surveillance and to help prioritize surveillance efforts.

We therefore created a database, based on a systematic literature review in Embase focused on zoonotic pathogens in ten common urban wildlife mammals in Europe, namely brown rats, house mice, wood mice, common voles, red squirrels, European rabbits, European hedgehogs, European moles, stone martens, and red foxes. Search terms included keywords related to (1) the Latin species name and any equivalent English names, AND (2) grammatical variations of the term 'zoonosis', and also (1) AND an elaborate list of important zoonotic pathogens created by the Dutch government. We included all articles published before 01-01-2023. Taken all together, we screened 6,305 unique articles of which 882 articles were included. Examples of data of the articles that was included in the database are: pathogen species, tissue(s) sampled, laboratory methods used, mammal species studied, total number of animals tested, number of positive tested animals, country, and trapping location type.

In total, 186 zoonotic pathogen species were described, including 90 bacteria, 42 helminths, 19 protozoa, 22 viruses, and 15 fungi. Most of these pathogens were only studied in one single animal species. Taking the Netherlands as an example, we show how this database can be used by other countries to identify wildlife disease surveillance gaps on a national level. In the Netherlands, only four percent of all potential host-pathogen combinations have been studied.

In conclusion, this database comprises a comprehensive overview that can guide future research on wildlife-borne zoonotic diseases both on a European and national scale. Even taking into account that some pathogens are relatively species-specific, many European countries have no (accessible) data on zoonotic pathogens in these relevant animal species. Sharing and expanding this database provides a solid starting point for future European-wide collaborations to improve wildlife disease surveillance.

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Over twenty years of health and disease surveillance of large carnivores in Sweden

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Abstract

Background

The regulated handling of found dead or culled large carnivores is the basis for a comprehensive wildlife health and disease surveillance of brown bear, lynx, wolf, and wolverine populations in Sweden. The management of large carnivores must be adaptive and continuously based on new knowledge, which motivates a long-term systematic monitoring to analyse and follow trends. Over the last 20 years, the Swedish large carnivore populations have increased significantly. All large carnivores that are either culled or found dead must be sent to the Swedish Veterinary Agency (SVA) to be examined, measured, sampled, and necropsied. This results in a unique dataset that enables a thorough monitoring of health status, existing diseases, and early detection of new diseases.

Objectives

The objective is to summarise the long-term data to give an overview of the health status of the increasing large carnivore population. This collected data should contribute to an adaptive management and continued conservation of large carnivores based on facts, to ensure long-term population viability.

Materials and methods

All collected data from necropsied carcasses and samples from brown bear, lynx, wolf, and wolverine received during 2001 – 2023 were analysed and summarised. The dataset included results from necropsy and collected biological data such as age, reproduction status, weight, and body size measurements.

Results

SVA has data from over 11 000 large carnivores, either submitted as entire or skinned carcasses and from brown bears culled during licensed or protective hunting, limited to sets of sampled tissues. Results from hunted large carnivores constitute a baseline for the state of health of these species. Major causes of death and other findings at necropsies of found dead large carnivores are listed. Most

large carnivores die due to hunting, either licensed or protective hunting. The second most common cause of death is to be killed by traffic, on road- or railroad. There are few infectious diseases, with sarcoptic mange in lynx and sometimes in wolves being the most frequent.

Conclusions

Our work highlights the importance of a comprehensive wildlife health and disease surveillance of large carnivores. To prevent or mitigate future population declines or issues due to diseases or effects of inbreeding etc, long-term monitoring can identify areas of potential research of importance for the continued management of large carnivores. This will also be of importance when all populations have increased to a level of conservation no longer considered as endangered or vulnerable.

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A rare case of campylorrachis scoliosa in American crocodile (Crocodylus acutus, Cuvier, 1807)

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Abstract

The description of malformations in wild animals is very rare. These congenital malformations can be associated with many factors as hatchlings from very young and very old females, genetic causes, malnutrition of the parents, defective incubation, and carcinogenic agents.

In the case report the authors describe a campylorrachis scoliosa in an captive American crocodile (*Crocodylus acutus,* Cuvier, 1807). The embryo died during development inside the egg and was found by causality during an inspection of non-hatching eggs from a zoocriedero of crocodiles. The embryo presented

curvature of the spine a lack of vertebrae and spinal cord caudal to the thoracic region. Also presented other malformations such as retention of the yolk sac with non-closure of the abdominal wall, maxillary macrognathia acrania and a curved tail.

To the author's knowledge, this malformation has never been reported in this species or other crocodile species. It is difficult to determine the aetiology of this congenital malformation. Probably was the combination of many factors, such as the age of the female, inbreeding, errors in incubation, and chemical pollutants in the water. The description of malformation cases is important to understand crocodile biology and potential threats to the population.

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Gut feelings: Gastrointestinal parasite sharing in co-existing wild and domestic ungulates in Kenya

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Abstract

The co-existence of wildlife and livestock is common, yet there is limited understanding of parasite sharing at the wildlife-livestock interface. Gastrointestinal parasites pose significant concerns due to their association with production losses in livestock and potential negative effects on species abundance in wildlife. These concerns are particularly pronounced in regions experiencing increased interactions between livestock and wildlife, such as the Maasai Mara ecosystem in Kenya. Our study aimed to evaluate the prevalence, infection intensity and community composition of gastrointestinal parasites in co-grazing animal species across different interface areas in this region. We obtained ~1000 fresh faecal samples from 14 sympatric wild and domestic herbivore species (>10 kg) across mixed livestock-wildlife and single-occupancy pastures. From each sample, nematode eggs and coccidia oocysts were quantified using

the Mini-FLOTAC technique and nematode infective larvae were isolated through coproculture. Larval DNA was analysed through metabarcoding of the variable ITS-2 region, and sequences were grouped into Amplicon Sequence Variants (ASVs) and cross-referenced with a database (nemabiome.ca) to identify nematode species. Results show that wildlife-livestock coexistence does not influence the prevalence of nematodes in either group, but animals feeding on mixed pastures have significantly higher intensities of nematodes than those on single-occupancy pastures. For coccidia, we found the opposite: infection intensities are significantly lower for wildlife and livestock when grazing together. We will also present the parasite diversity observed amongst the 14 co-existing host species and highlight the impact of host traits and phylogenetic relationships on the composition of parasites within the sampled animal hosts. Our findings shed a light on the parasite communities inhabiting wild and domestic animals in the Maasai Mara ecosystem and underscore the implications of parasite transmission dynamics in wildlife-livestock interface areas.

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Neurological clinical signs associated with Infectious Keratoconjunctivitis and *Mycoplasma conjunctivae* ocular infections

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Abstract

Infectious Keratoconjunctivitis (IKC) is a highly contagious ocular disease that is caused by *Mycoplasma conjunctivae* in domestic and wild Caprinae. Clinical signs are mainly associated with ocular lesions, yet atypical signs such as circling or

abnormal behaviour have also been reported in severely affected Caprinae. In these cases where circling and abnormal behaviour are evident, lesions in the central nervous system have consistently been dismissed, with assumptions pointing towards disorientation due to blindness as the likely cause.

The aim of this study was to properly describe abnormal clinical signs associated with IKC in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*), assess lesions and possible etiological agents involved. We examined 17 IKC cases in Pyrenean chamois, including animals with abnormal behaviour consistent with neurological signs (IKC-with NS; n=7), animals without abnormal behaviour (IKC-without NS; n=3) and animals with IKC in which no full clinical assessment was possible (IKC-NA; n=7). The presence of ear, ocular and bone lesions were assessed by computed tomography (CT) and histological examination at different anatomical locations, mostly from the auditory and central nervous system: eyes, external ear canals, tympanic bullae, vestibulocochlear nerve, cerebrum, cerebellum and brainstem. *Mycoplasma* conjunctivae presence was investigated by real-time PCR in the same locations and microbiological cultures were performed in selected individuals.

Abnormal clinical signs in the IKC-with NS group included pathological nystagmus (5/7), circling movements (2/7), head excursions (5/7; involuntary lateral movement of the head) and head tilt (4/7). Inflammatory findings in the middle and inner ear were found in chamois from the same group IKC-with NS (6/7), including exudate in ear canals and tympanic bullae, and from the group IKC-NA (3/7). No lesions in the external, middle and inner ear were observed in the group IKC-without NS in which the presence of neurological signs was dismissed (0/3). These findings strongly link the presence of neurological signs with middle and inner otitis. *Mycoplasma conjunctivae* was detected in almost all animals with IKC and it was associated with ocular clinical signs (15/17). It was also detected in the inner and middle ear, although it was not consistently associated with lesions in these anatomical locations. Microbiological cultures yielded diverse opportunistic bacteria from different anatomical locations. Our results indicate that *Mycoplasma conjunctivae* can invade the inner and middle ear and contribute to peripheral vestibular syndrome in IKC-affected animals. However, other infectious agents are likely to play an important secondary role in the progression of the neurological syndrome.

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Monitoring *Batrachochytrium dendrobatidis* (*Bd)* in anuran populations in Schleswig-Holstein, Germany – First insights open up new questions

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Abstract

In accordance to the global picture, amphibian populations in Germany are declining. Among other underlying stressors, the invasive fungus *Batrachochytrium dendrobatidis* (*Bd*) can adversely affect amphibian populations and is responsible for mass mortalities in amphibian populations worldwide. In this study, we examined 373 post-metamorphic anurans of seven species in four study areas in the federal state of Schleswig-Holstein, Germany. Study areas were evaluated beforehand by experienced herpetologists and chosen according to their expected high species diversity. Handling of the animals included, if possible, sex determination and classification into the age groups "adult" and "subadult" as well as adspection for macroscopical signs of disease. Skin swabs were collected from the medial side of the legs and the ventral abdomen and tested for *Bd* via PCR.

Bd was detected in common toads (*Bufo bufo*), frogs of the genus *Pelophylax*, moor frogs (*Rana arvalis*), and common tree frogs (*Hyla arborea*). High prevalences occurred in the study areas "Oeversee" (2022: 48%, 2023: 56%) and "Preetz" (2022: 62%, 2023: 23%), whereas low prevalences were found in the study areas "Quellental" (2022: 5%, 2023: 0%) and "Trappenkamp" (2022: 2%, 2023: 5%). Fisher´s exact test showed significant variation between the four locations ($p < 0.001$) and the years of sampling ($p < 0.001$). Differences between sexes ($p = 0.999$) and age groups ($p = 0.315$) were not significant. Of these cases, 90% belonged to the global panzootic lineage (*Bd*GPL). There was no correlation

between infection and macroscopical signs of disease. Although the study did not include amphibian population mapping efforts, disparities in numbers expected and numbers caught occurred for several sampling locations.

Our study indicates a wide distribution of *Bd*, and especially *Bd*GPL in native amphibian populations in Schleswig-Holstein. Although *Bd*GPL is thought to be the most pathogenic *Bd* lineage, we could not show a relation between *Bd* prevalence and macroscopical signs of disease. Causes for the decline of amphibians are likely multifactorial and include, besides infectious agents, climate change, environmental pollution, and habitat loss. In order to effectively protect amphibians and maintain their populations, future research should assess these external stressors and their interplay. Furthermore, amphibian population monitoring programs can help to gather more data on how *Bd* or other stressors

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The influence of knowledge, attitude, and practice on hunters' infection risk for tularemia and nephropathia epidemica

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Abstract

The zoonotic diseases nephropathia epidemica (NE, vole fever) and tularemia, are caused by Puumala orthohantavirus (PUUV) and *Francisella tularensis*, respectively. Especially tularemia causes an increasing number of reported human cases and shows extended geographical range in Sweden. Despite previous findings linking

hunting to infection risk of NE and tularemia, the hunting-related activities that increase transmission risk are poorly demonstrated.
Most PUUV, and some *F. tularensis* infections, are contracted by inhalation of aerosols from rodent excrements or carcasses. *F. tularensis* transmission routes also include infection by blood-feeding arthropods, through handling of infected animal tissues, and ingestion of contaminated food or water. Risk activities may overlap for NE and tularemia during hunting and affiliated trips, making hunters a potential at-risk group.
Endemic areas cover northern (NE and tularemia) and central (tularemia) Sweden. Hunters who live in areas traditionally associated with NE and tularemia may thus be aware of the risks of these diseases, in contrast to hunters who live in non-endemic areas and only visit endemic areas during hunting trips.
We will use a questionnaire with a KAP (knowledge, attitude and practice) approach, focusing on situations associated with transmission risk, hunting habits, and lifestyle. Combined with a sero-survey of PUUV and *F. tularensis* antibodies in risk and reference groups, we aim to detect peri-domestic, recreational as well as work-related activities associated with infection risk. Results are used to answer how hunting habits relate to seroprevalence, and how differences in KAP between hunters from endemic areas and non-endemic areas (southern Sweden) can potentially drive infection risk of PUUV and *F. tularensis*.

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TB-VAC: Pilot study on vaccinating badgers as a complementary measure to culling to control bovine tuberculosis in Dordogne. Preliminary results.

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Abstract

Mycobacterium bovis has been identified in wildlife species in France (badgers, wild boars, deer, foxes) in the vicinity of some cattle outbreaks, with shared *M. bovis* strains, suggesting transmission. Control measures of bovine tuberculosis (bTB) in wildlife have been implemented, included so far: *M. bovis* detection and monitoring (Sylvatub program); hunting waste management in hunted species (wild boar and deer); wild boar and badgers populations control; improving biosecurity to limit cattle-wildlife interactions.

However, the evolution of bTB in the southwest of France is still worrying. This may be explained by shortcomings in breeding practices and surveillance of TB in cattle and by the persistence of *M. bovis* within the multi-host system involving cattle, the environment and wildlife.

Based on overseas published results, we hypothesised that vaccinating DPP negative badgers with injectable BCG, and culling DPP positive badgers would be associated with a reduced prevalence in badgers and nearby cattle herds, as evaluated through TB specific blood assays. The lateral-flow test known as the Dual Path Platform (DPP) VetTB assay is used to identify antibodies against *M. bovis* antigens (MPB83 and ESAT-6/CFP-10). On this basis, a pilot badger vaccination project was launched in southwest of France for a four-year period (2023-2026) as an initial test phase with deployment and data collection, in a limited area (100 km^2).

Following the assessment of indicators for the feasibility, human and financial costs of the overall strategy over the four-year period (such as trapping capture rate, bTB prevalence in newly captured non-vaccinated badgers, vaccination rate…), a larger deployment is anticipated as a second phase. In the stage 1 deployment, badgers are captured with live-traps or specific restraints and tested for TB with a rapid blood diagnostic test (DPP test) to identify and remove infected animals, vaccinate and release negative ones (Test – Vaccinate – Remove method).

The next capture session is planned in spring 2024, will include a new area (35 km^2) in order to increase the number of active setts in the pilot phase.

Results from the 2 first years are described.

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Importance of surveillance and control of low prevalent diseases in conservation breeding centers.

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Abstract

Some diseases, despite being low prevalent, can pose an important impact in *ex-situ* breeding centers performance and management. This impact is related to loss of individuals with high genetic value, reduction in the breeding performance and increase of resources consumption.

The African Houbara bustard (*Chlamydotis undulata*) is classified as Vulnerable (UICN). With the aim of restoring the natural populations a captive breeding center was established in Morocco. Annual average adult flock is 20000 individuals, including 8000 breeders. The annual average chick production is 20000. Epidemiological analyses conducted on data collected from 2012 to 2022 revealed low prevalences values for three avian common diseases: salpingitis syndrome (average annual prevalence 0.59% [0.07% - 1.64%]), Mycobacteria (0.03 ‰ [0.00-0.14‰]) and Salmonella (0.60‰ [0.00-2.39‰]). Salpingitis syndrome leads to permanent oviduct damage and loss of the breeding capacity. Mycobacteria reduces breeding performance in alive birds and leads to euthanasia when diagnosed. Salmonella reduces breeding performance and increases offspring morbidity and mortality. Mycobacteria and Salmonella are potentially zoonotic.

While such epidemiological analyses yielded limited information due to severe zero inflated datasets, using passive and active surveillance tools we identified control strategies for each disease. General passive surveillance includes postmortem examination of each dead individual. Active surveillance is disease specific.

Salpingitis syndrome's active surveillance protocol is based on clinical history and egg production. Control measures include stopping the use of mix sperm, improved biosecurity, staff training and stamping out of affected females. Mycobacteria active surveillance plan is performed both antemortem and postmortem using medical history, breeding performance and weight. Clinical evaluation and Ziehl Neelsen (ZN) staining are carried out on suspicious individuals. Postmortem surveillance is done in every bird older than 1 year old through ZN staining of tissues and confirmation by qPCR. When confirmed a

quarantine protocol is applied to the epidemiological unit. Salmonella active surveillance is done by periodic nested random microbiology screening of birds and environment. When detected, screenings in the same epidemiological unit are done. Control measures includes biosecurity reinforcement.

This low prevalence implies an increase in detection and control efforts. The epidemiology of these pathogens makes necessary to combine individual and flock medicine approaches to avoid underestimation. The unique nature of the houbara breeding centers, where the flock is greater than the conventional captive breeding center for other species without reaching the flock size of modern poultry industry, provides unusual epidemiological situations that can shed light on disease dynamics in non-conventional species.

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Microbial diversity in choanal swab samples from nestling and adult Dalmatian pelicans (*Pelecanus crispus*) and nestling great white pelicans (*Pelecanus onocrotalus*)

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Abstract

Background: Lake Lesser Prespa (Greece) is home to the world's largest breeding colony of the Dalmatian pelican (DP-*Pelecanus crispus*), which breeds there along with the great white pelican (GWP-*Pelecanus onocrotalus*). Between February and April 2022, an outbreak of avian influenza spread through the DP colonies of southeastern Europe, resulting in the death of 60% of the DP breeding population of Lesser Prespa. The outbreak resulted in a significant impact since it caused the death of almost 10% of the world DP population.

Objectives: to comparatively analyze the oral/choanal microbiota of nestling DP and GWP and nestling/adult DP, as well as to perform the pheno/genotypic study

of isolates obtained to pinpoint differences between species and age animal groups.

Material and methods: 52 oral/choanal samples of pelicans (20 and 11 nestling DP and GWP respectively and 21 juvenile/adult DP) were processed. The samples were seeded on blood agar, MacConckey agar, Slanetz-Bartley agar, mannitol-salt-agar and MRSA-chromogenic plates for isolation of staphylococci (methicillin-susceptible and methicillin-resistant, respectively). Identification was performed by MALDI-TOF. All isolates were studied phenotypically and genotypically.

Results: In the case of adult DPs, all of them presented some *Staphylococcus* spp. isolates. Ninety-four percent of the *Staphylococcus* isolates were pansusceptible, except for one isolate that contained the *blaZ* resistance gene. *S. aureus* was found in 85.7% of the adults. The presence of other staphylococci species was relatively low, as was the presence of *Enterococcus* spp. isolates. The presence of an Extended Spectrum Beta Lactamase producing *Escherichia coli* (ESBL) isolate is noteworthy.

All the nestlings were colonized by *Staphylococcus* isolates. The following staphylococci species were detected (GWP/DP): *S. schleiferi* (100%/85%), *S. aureus* (90.9%/70%), *S. delphini* (72.7%/55%), *S. hyicus* (27.3%/45%), and *S. sciuri* (63.6%/45%). *S. chromogenes* was found in only one DP. The presence of *E. faecium* (72.7%/10%) and *E. faecalis* (45.5%/90%) was remarkable. *E. coli* isolates were found in half of the nestlings (54.5%/50%) and in one DP even *Klebsiella pneumoniae* was found.

In DP nestlings the *Staphylococcus* strains were resistant to aminoglycosides (72.7%/40%) (containing the *ant4* gene) and in GWP were resistant to lincosamides (containing the *lnuA* gene).

Conclusion: Colonization of the choanal and oral cavity of nestling and adult DP and nestling GWP showed differences in terms of composition and prevalence of different and potential pathogenic bacteria.

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Influence of diet and presence of heavy metals in the acquisition of antimicrobial resistance and antibiotic resistant genes in *Escherichia coli* in white stork nestlings

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Abstract

White storks (*Ciconia ciconia*) have adapted their spatial ecology and migratory pattern to use landfills as a food source, increasing probability of acquiring antimicrobial resistant (AMR) bacteria and antibiotic resistance genes (ARGs). Foraging in landfills also implies exposure to pollutants like heavy metals, pharmaceutical residues, and caffeine. The presence of heavy metals in the environment may cause selective pressure on bacteria and induce metal resistance genes (MRGs), that drives ARGs through co-resistance and cross-resistance mechanisms. Thus, feeding nestlings from these food sources could increase acquisition of AMR through the combined effect of urban waste and pollutants.

This study aims to determine the influence of the degree of anthropization of the diet and the presence of heavy metal(oid)s, both dietary and environmental, in the occurrence of AMR bacteria/ARGs in *E. coli* in nestlings. Moreover, this study aims to evaluate the utility of nestling feathers to determine the use of landfills by adult storks through the analysis of the isotopic signature of parental diet, as well as to determine pollution surrounding the nest through metal analysis.

We collected cloacal swabs, blood samples, and contour feathers from 86 white stork nestlings from five different breeding colonies. Plasma was analysed for 49 veterinary pharmaceuticals and caffeine. Nestling feathers were used to determine carbon and nitrogen isotope ratios, as well as to measure concentrations of heavy metals and As. AMR *E. coli* and their ARGs were studied in cloacal swabs.

Isotopic signature classified nestlings into natural or urban-waste diet, with the second one associated with caffeine in plasma. Some heavy metals (Al/Ni/Co/Cr/Pb) were related to the urban-waste diet while As, Hg, Cu and Zn were more related to the natural diet. According to the concentration of Hg, As and Pb, some nestlings were influenced by previous mining activity around their breeding colonies. Probability of acquisition of AMR bacteria/ARGs was higher in nestlings fed with the urban-waste diet.

Nest location plays an important role in the occurrence of AMR bacteria/ARGs in nestlings, which receive the influence of the degree of anthropization/contamination of parental diet in combination with the overall pollution around the breeding colony. Both nestling feathers and plasma caffeine can be reliable biomarkers of landfill use by adult storks during the breeding season.

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Quantitative risk assessment of African swine fever at wild boar-outdoor pig farm interface in France

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Abstract

African swine fever (ASF) is a devastating disease of pig industry and can be transmitted at the wild boar - domestic pig interface. Throughout Europe, outdoor pig farming is common and different type of farms are present, thus being exposed to African Swine Fever Virus (ASFV) from wild boars. Previous quantitative risk assessments mainly focused on the introduction of ASFV into a country, however, there is a gap to assess the risk of ASF transmission between wild boar and domestic pigs in outdoor pig farms at the local scale. This study aimed to estimate the risk of infection via three potential pathways and to identify the main determinants of risk of ASFV transmission in outdoor pig farms exposed to a local infected wild boar population during the high-risk period (HRP) prior detection in wildlife. We used stochastic modelling approach to estimate the risk and a global sensitivity analysis to identify the main determinants of risk. Based on existing variation in outdoor pig farming system around EU, a generic layout of outdoor pig farm is also defined. The three pathways considered in this study were: movement of wild boar, contaminated fomites, and swill feeding. The riskiest pathway for ASFV transmission to outdoor pigs was the movement of wild boar (median risk: 0.042, 95% CI: 0.002 - 0.141), followed by contaminated fomites (median risk: 0.009, 95% CI: 0.0002 - 0.0985). Sensitivity analysis showed that the most influential risk determinants from the infected wild boar population are the number of daily visits of wild boar to an outdoor farm and the probability that outdoor activities by farmer or worker were conducted within study area, contributing 56% and 6.3% change in model variance. These results suggest that i)

the most uncertain pathway for ASFV transmission to pigs is the movement of wild boar, leading to direct contact or indirect contact ii) among all possible biosecurity measures, preventing the entry of wild boar on farms should be prioritized to lower the risk of exposure to ASFV virus. It also highlights the importance of continuous surveillance activities to increase our ability for earlier detection of ASF in wild boar population. More generally, our study provides a comprehensive framework which will assist the researchers and risk managers with ASF surveillance and intervention activities at a local scale related to outdoor farming system.

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A syndromic approach to integrated health surveillance in Swiss wild birds

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Abstract

Wildlife health surveillance (WHS) programs are crucial for safeguarding biodiversity, domestic animal and human health. Spikes in wildlife mortality and morbidity events can serve as the initial indicators of disease emergence. Many general Wildlife surveillance systems – including the Swiss WHS – are characterized by legal, geographical, and administrative fragmentation, lack baseline biological data, and exhibit notable gaps in the monitoring of smaller garden wildlife species such as songbirds, hedgehogs, and squirrels. This is a major challenge for early detection of diseases.

Our project aim is to evaluate a syndrome-based, participatory approach towards harmonized collection and availability of health data to improve surveillance. Our current efforts focus on free-ranging birds as a well-defined study group with extensive long-term population data availability and a settled citizen engagement. First, we defined easily recognizable syndromes based on distinct pathological categories and we assigned a diagnosis to each case by analysing necropsy reports of birds submitted to the Institute of Fish and Wildlife Health, Switzerland in the framework of WHS during the last two decades. Second, we analysed how syndromes are linked to diagnoses and to what extent these are related to seasonal, species-specific and spatial trends. Third, together with data scientists,

we will develop a prototype for an online reporting system (ORS) of diseased and dead birds suitable for integration into existing population monitoring tools.

Our goal is to empower wildlife enthusiasts to contribute to health surveillance efforts, provide wildlife professionals with real-life insights into population and spatiotemporal disease trends and provide a transferable use case that can be adopted beyond birds. In the long run, we hope that integrating health data with ongoing population monitoring will forward wildlife health surveillance in Switzerland.

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Exposure to High and Low Pathogenicity Avian Influenza viruses in a multi-species assemblage of UK seabirds

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Abstract

Emerging infectious diseases substantially impact veterinary and human health, often resulting in severe ecological and economic costs. Increasing global trends of disease emergence, largely driven by accelerated anthropogenic activity at the interface of wild and domestic species, are of urgent multi-disciplinary concern. The emergence of H5N1 clade 2.3.4.4b high-pathogenicity avian influenza virus (HPAIV) in Europe has had devastating consequences for both domestic and wild avian species, exemplifying the substantial conservation, veterinary and public health risks of emerging high pathogenicity avian influenza (HPAI).

Since 2021, H5N1 clade 2.3.4.4b HPAIV has caused unprecedented mortality events in previously unaffected colony-nesting seabirds. Unusual in both seasonal timing and species breadth, this epizootic has demonstrated extensive geographical dissemination across continents and spillover events of HPAIV in mammals. The ecology and behaviour of previously unaffected hosts could prove significant in the epidemiology of novel HPAIV infection, perpetuating these unique geographical and species incursions.

Given the unprecedented nature of HPAIV incursion in seabirds, current baseline data of trends in individual, population or species infection is limited, and has to date focused on patterns of mortality. Where outbreaks have decimated populations, understanding whether surviving birds have not been exposed, can act as asymptomatic carriers or have some degree of immunity to infection following exposure and/or recovery from infection, is essential in understanding both the potential impact of infection on different populations and the likely persistence of infection over time.

This study aims to provide baseline evidence of infection, mortality and exposure to H5N1 clade 2.3.4.4b HPAIV within a multi-species assemblage of breeding seabirds. To do so, we collected blood, cloacal and choanal swab samples from outwardly healthy seabirds of five distinct species upon the Isle of May National Nature Reserve, SE Scotland during May to July 2023. Plasma samples were assessed for antibodies indicative of previous exposure to any avian influenza virus (AIV) subtype (H1-H16) via commercially available ELISA kits, and for subtype-specific antibodies via both haemagglutination inhibition assay and competitive ELISA. Swabs samples were tested for AIV RNA via real-time reverse-transcription-PCR.

Results suggest species differences in AIV antibody prevalence. This highlights the ongoing necessity for studies of AIV within multi-species assemblages to understand potential routes of transmission, and the need for improved understanding of species differences in disease tolerance and consequences during novel emergence events.

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Two Decades of Border Disease in Pyrenean Chamois in Catalonia, Ne Spain

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Abstract

In 2001, a Border Disease virus (BDV, Genus Pestivirus) was identified as the cause of a previously unreported disease in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*). Since then, their populations have experienced severe outbreaks of disease and associated mortality, resulting in significant population declines. Following these epizootics, BDV has continued to circulate in some chamois populations while fading out in others, resulting in diverse epidemiological scenarios and population trajectories. However, monitoring of BDV infection in chamois has decreased in recent years due to the absence of overt disease outbreaks.

The aim of this study is to analyse the population dynamics in different protected areas, primarily National Hunting Reserves (NHR), in relation to BDV infection, with a focus on one region of the Central Pyrenees where the chamois population has been declining since the first outbreak of disease. Our hypothesis is that BDV remains a major driver and a population-limiting pathogen for chamois population trends in some areas.

Chamois census data from the last 23 years was analysed in six areas in Catalonia, NE Spain. Serological and virological analyses for pestiviruses were performed in chamois and other ungulate species, and Border Disease (BD) cases from the passive health surveillance system were compiled to assess spatio-temporal distribution. In addition, population dynamics of other ungulate species (mouflon, red deer, fallow deer, roe deer, Iberian ibex) were also explored.

Population declines were observed in two of the six protected areas after the initial outbreaks of BD. High seroprevalence and sporadic cases of BD were detected in the selected study area with a decreasing population. In contrast, chamois populations in which no BD cases have been diagnosed in recent years have experienced significant recovery to its historical highest numbers or have remained stable over time.

BD remains a limiting factor for the recovery of the Pyrenean chamois population in certain areas of the Catalan Pyrenees. Other factors such as increased competition for space and food resources with other ungulate species (red deer

and mouflon), and the rise in tick infestation (potentially causing tick-borne diseases) should be carefully analysed. Priority measures for the species should include strengthening BD and health surveillance throughout the territory and evaluating environmental changes and increasing competition with other ungulates.

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End-stage liver and systemic amyloidosis in a Baltic grey seal (*Halichoerus grypus*) from Gotland, Sweden

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Abstract

For the past five decades Sweden has maintained a comprehensive monitoring system of the seal populations living in its waters. The program is based upon their role as sentinels for the environmental contaminants still present in the Baltic Sea, but it also follows their reproductive status, overall health and impact of infectious diseases.

In July, 2023, a female grey seal (*Halichoerus grypus*) on the Baltic island of Gotland was shot by a hunter due to its extremely poor body condition and lethargy. The animal was sent to the Swedish Museum of Natural History for post-mortem examination to investigate the cause of its condition.

A complete necropsy was performed, followed by virological, bacteriological and histopathological analysis and age was determined.

The main finding was hepatomegaly with severe cirrhosis, loss of architecture, encapsulated cyst-like structures in the liver parenchyma and fibroplasia. Other findings included cachexia, jaundice, several skin ulcers, subcutaneous petechiae, interlobular emphysema of the lungs, peritoneal adhesions, dark tarry intestinal content, mild thickening of the adrenal cortex, a cyst in the right kidney, mild parasitism and mild arteriosclerosis. No thymus was evident the tonsils were small. The gallbladder was unremarkable and no liver flukes were found.

The lung was screened by PCR for morbillivirus and influenza A virus with negative results. Bacterial culture of the lung revealed moderate growth of *Streptococcus canis/equisimilis* (betahemolytic streptococcus) in mixed flora. Bacterial culture of the liver and kidney cystic structures did not detect any specific infection.

The animal was six years old and nulliparous.

Microscopically, the liver and kidney exhibited extensive amorphous, eosinophilic proteinaceous deposition along the sinusoidal spaces and in the renal cortex and medulla, respectively. Liver architecture was also altered, with loss of definition of lobule limits and extensive fibrous trabeculae throughout the parenchyma. Congo red histochemical staining confirmed severe amyloid deposition, consistent with hepatic and renal amyloidosis

The etiology of amyloidosis could not be determined, but likely represents secondary reactive amyloidosis from chronic antigenic stimulation which is the most common form in animals. The lesions would probably have been fatal had the seal not been killed. The most likely differentials include chronic viral or other infection, chronic toxic insult, autoimmune disease, neoplasia or hereditary conditions. Sporadic cases of amyloidosis have been previously described in grey seals and this is to our knowledge the first case of systemic amyloidosis in Swedish seals. As such, continued monitoring is warranted.

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Highly pathogenic avian influenza in wild birds in the united kingdom 2022-2023: impacts, conservation and knowledge gaps

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Abstract

Since the start of the recent 2021 outbreak of H5NI Highly Pathogenic Avian Influenza (HPAI) in the UK, a number of different quantitative data sources on the mortality associated with HPAI have been collated by government nature conservation bodies, along with conservation and volunteer-based organisations. These ranged from detailed post-hoc assessment of mortality in individual sites (e.g. reviews by site managers, or individuals tasked to undertake a detailed analysis of mortality data), through ongoing data entry into three national databases by site managers, to ad-hoc observations submitted by the birdwatchers in the BTO's bird listing app (BirdTrack) and website. The UK hosts internationally important numbers of breeding waterbirds and this species group was most affected with minimum estimates of mortality in some common species exceeding 10% of the UK's breeding population. Most affected were Great Skua *Stercorarius skua*, Northern Gannet *Morus bassanus*, Barnacle Goose *Branta leucopsis* and Black-headed Gulls *Chroicocephalus ridibundus*. Other gull, terns and auk species were also heavily impacted. We review this activity and present updates to quantify the extent of mortality on UK seabird populations and outline management guidelines that have been put in place. We then identify key data gaps and ongoing research priorities.

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Garden Bird Disease Observatory in Catalonia, Ne Spain

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Abstract

Garden birds are species of birds that visit gardens and urban areas for food and shelter. Some of these species have experienced a decline, and serious diseases affecting them have been detected. Following the suspicion of *Suttonella ornithocola* in Catalonia (NE Spain) in 2020, in collaboration with the Catalan Institute of Ornithology (ICO), the Animal Health Research Center IRTA-CReSA, and the Government of Catalonia, we aimed to establish a network for monitoring their diseases through citizen science, investigate and analyse the causes of disease and mortality, and identify the risk factors to favour control measures.

From November 2021 to February 2023, 97 reports of sick or dead birds of 30 different species were collected. These were mainly obtained through the Torreferrussa Wildlife Rescue Center (43%), direct contact (33%), and notifications through “ornitho.cat" (10%), the web portal of ICO. On 25 birds we performed postmortem examination, and when feasible, histological, microbiological and serological/virological analyses of Avian Influenza, Newcastle Disease and West Nile fever.

The quick response, information feedback, and consistent dissemination over time have been crucial in fostering citizen collaboration. The most common cause of mortality was trauma (44%), mainly related to anthropogenic factors such as collisions with glass, road traffic accidents, and predation. Infectious diseases have accounted for 14% of the deaths, with outbreaks of Newcastle Disease and isolated cases of salmonellosis, mycobacteriosis, coligranulomatosis, knemidocoptic mange, fungal pneumonia and septicemia. Additionally, significant findings and pathological conditions such as nephropathy, cystic feather granulomatosis, nutritional hyperparathyroidism, tick infestation, hemosporidian infections, endoparasitosis (trematodes, nematodes, cestodes and coccidia) as well as avian keratin disorder, were identified.

Preventive measures are essential to reduce mortality associated to anthropogenic factors, such as constructing windows with materials that minimize collisions. It is important to avoid situations that concentrate high number of birds when infectious diseases are detected, such as feeding birds in parks or bird feeders in gardens, as these practices can increase disease incidence.

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Patterns of exposure to APMV-1, the causative agent of Newcastle Disease in wild populations of seabirds.

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Abstract

Viral pathogens have the potential to impact the demography and viability of animal populations and often leave lasting signatures of infection in the form of antibodies. Antibody levels can aid the identification of individuals in a population that have been exposed to infection but survived. In addition, antibodies can alter responses to future infection, both directly in the host, and indirectly in offspring via the transfer of maternal antibodies in early life. However, the dynamics of natural infection are difficult to study in wild settings due to the complex interplay of exposure, immune response magnitude and antibody persistence that culminate in differences in antibody profiles between individuals. Therefore, sampling individually marked populations of animals is key to understanding the impact of host traits on exposure and response to key viral infections.

Avian paramyxovirus type 1 (APMV-1) is a globally important virus, and can cause major mortality in poultry under the name Newcastle disease. APMV-1 also circulates in wild birds, with the majority of infections sublethal, but high mortality events periodically recorded, most notably in the cormorant family. Despite this, little is known about the factors that shape infection and susceptibility to APMV-1 in wild populations.

Here, we aimed to use multiple years of antibody data to investigate the patterns of APMV-1, in seabirds with particular focus on a species of cormorant, the European shag (*Gulosus aristotelis*). We collected blood samples from a range of seabird species on the Isle of May, Scotland, and from both adults and their offspring in a population of European shags for which life history data is routinely collected, including differences in migratory behaviour during the non-breeding season. Antibody prevalence was quantified using enzyme-linked immunosorbent assays (ELISA).

We found consistent evidence of antibodies for APMV-1 across multiple years in adult European shags, despite no antibodies in other species breeding at the same location. We report how patterns of antibody prevalence are distributed between the sexes and birds differing in winter migration strategy. Maternal antibodies to APMV-1 were detected in newly hatched chicks, reflecting both maternal antibody levels, and individual chick traits. Identifying the factors that influence viral antibody prevalence both within populations and between species, can contribute to our understanding of historical exposure and the vulnerability of populations to future outbreaks.

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Crayfish plaque - a widespread threat to German crayfish populations

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Abstract

Background

Crayfish plague is one of the most important diseases of crayfish and is widespread worldwide. The causative pathogen *Aphanomyces (A.) astaci* belongs to the oomycetes. These filamentous, fungus-like eukaryotes are classified within the class *Stramenopiles* with about 600 species.

In the mid-19th century *A. astaci* was introduced from North America to Europe with North American crayfish species such as *Pacifastacus leniusculus, Orconectes limosus* or *Procambarus clarkii*. Although these species can be infected, they rarely show clinical symptoms. However, resistant crayfish are carriers and responsible for further pathogen spread. Spores can also be disseminated via inanimate vectors.

A. astaci reproduces asexually in the aquatic environment. Outside the host they survive for up to 14 days. Highly susceptible native crustaceans such as *Astacus astacus, Austropotamobius torrentium* or *Astacus leptodactylus* are encountered through filament penetration of the cuticle and deeper organs. Susceptible crayfish develop clinical symptoms after an incubation period of about 10 days with high mortalitiy rates.

Objectives

In the event of mortality or as part of preventive measures in crayfish populations, samples from various German federal states are regularly sent to the Food and Veterinary Institute Braunschweig/Hannover, Germany, for examination.

In addition, a project analysing the pathogen’s spread in Lower Saxony, Germany, local waters has been initiated.

Material and Methods

Cultural verification procedures are very time-consuming in comparison to PCR techniques. Here, a *Taq*Man PCR with a minor groove binder probe is applied, targeting a 59-bp sequence of ITS1 from the nuclear ribosomal gene cluster.

Results

A. astaci has been recently detected several times in crayfish taken from waters located in Lower Saxony as well as other German federal states. In the last 10 years we have examined about 250 German samples of different crayfish species. Affected federal states repeatedly initiated disease control measures due to mortality events in crayfish caused by the crayfish plague (For instance: Alatsee, Bavaria, Germany).

Conclusions

The crayfish plague is spread through invasive species and poses an enormous ecological threat for indigenous crayfish.

Regular detections of the pathogen *A. astaci* in both susceptible and vector species confirm its widespread distribution in Germany. Numerous European countries are fighting the crayfish plague as well.

To prevent further disease spreading, biosecutity and disease control measures are necessary as well as the collection of epidemiological data to document the status quo. Furthermore, stocking with disease-free animals is recommended. In this regard, national, and Europe-wide cooperation is of great importance.

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Immunological response and safety after vaccination with a COVID-19 mRNA-vaccine in guinea pigs

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Abstract

During the SARS-CoV-2 pandemic, innovative mRNA-vaccines were approved with unprecedented speed. The COVID-19 mRNA-vaccines consist of mRNA coding for SARS-CoV-2 spike-glycoprotein, enclosed by lipid nanoparticles (LNPs). The vaccines reliably protect from severe disease and allowed for the transition from a pandemic to an endemic phase. The risk-benefit assessment was in favor of the vaccines, but rare adverse reactions such as myocarditis have been reported, the pathogenesis of which is still unclear.

The goal of our study is to understand how mRNA vaccines modulate the immune system, which may allow to decouple safety related effects from efficacy-defining mechanisms. We aim to understand the increased risk of myocarditis. Potential explanations could be an aberrant expression of spike-protein in the myocardium leading to apoptosis, a direct negative impact by LNPs on cardiomyocytes or a generalized inflammatory response.

An animal trial was conducted, in which guinea pigs were immunized with mRNA-vaccine or empty LNPs. Two and seven days after each immunization animals were sacrificed to perform necropsies and to collect organ-samples. Samples were analysed by qRT-PCR and histopathology for cytokine and SARS-CoV-2 spike expression and for inflammatory processes in the tissue.

Preliminary data show elevated levels of the inflammatory chemokines, CCR3 and CXCL10, at the injection site and the heart. High amounts of IL-1, CCL3 and CXCL10 were found in spleen and liver. Vaccine spike-mRNA was detected in large quantities at the injection site, spleen and liver but only at low levels in the heart. Immunohistopathology indicates inflammatory processes at injection site, heart, spleen and liver shortly after immunization. We aim to find out, if these inflammations can be correlated with the presence of mRNA vaccine particles.

In view of their versatility and excellent immunogenicity mRNA vaccines have become an indispensable part of our pandemic-preparedness-armamentarium. However, any potential safety issue has to be elucidated and resolved. Our efforts will contribute to this process and thereby enhance general vaccine acceptance.

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Increasing preparedness by networking: 20-year network "Rodent-borne pathogens"

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Abstract

Rodents are important as pests in agriculture and forestry, model organisms for biomedical studies and pathogen reservoirs. These pathogens may have the potential to cause disease in domestic animals and humans, or might be rodent-specific and have no or yet unknown zoonotic potential. The network was established as a platform for an interdisciplinary collaboration of scientists in mammalogy, ecology, genetics, immunology, toxicology, epidemiology, virology, microbiology, parasitology and human and veterinary medicine.

The objectives of the network are: (i) to identify the diversity of viruses and other pathogens in rodents and other small mammals, (ii) to estimate their geographical distribution and host specificity, (iii) to understand processes that may lead to outbreaks and to characterize consequences of rodent population dynamics on pathogens, (iv) to prove the zoonotic potential of novel pathogens, and (v) to develop tools and workflows for pathogen detection.

Over the last 20 years, about 30,000 rodents and other small mammals were collected by network collaborators from forests, grasslands, zoological gardens, urban landscapes and pet rat breedings and other husbandries. Specimens were dissected and species determined following corresponding standard protocols. A variety of pathogen-specific, generic and open view molecular methods were used to detect and identify known, and yet undiscovered, pathogens.

This network was involved in the discovery of numerous rodent- and shrew-specific herpes- and polyomaviruses. Based on clinical cases in humans, domestic, wild and zoo animals, novel zoonotic and animal pathogens were identified, e.g. variegated squirrel bornavirus 1, squirrel adenovirus and rustrela virus. The network enables the detection of several viruses of unknown zoonotic potential, e.g. novel hepeviruses in rats, common voles and shrews, and the identification of the rodent reservoirs of rustrela virus and lymphocytic choriomeningitis virus. In addition to eight hantavirus species in wildlife, the zoonotic Seoul hantavirus was recently identified in pet rats in Germany.

In conclusion, the network provides an important infrastructure for interdisciplinary scientific work in a One Health perspective and allows a holistic view on pathogen interactions including viruses, bacteria and endoparasites.

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Significance of Porcine circovirus 2 in wild boar in Sweden

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Abstract

Porcine circovirus is common in domestic pigs in several parts of the world. Porcine circovirus type 2 (PCV-2) targets lymphoid tissues, leads to immunosuppression and can also cause reproductive disorders. The virus has been detected in wild boar in Europe and has been shown to cause disease in them. In southern Sweden, 99 % of wild boar have antibodies against PCV-2, however the presence of the virus has not been investigated. Due to the immunosuppressive nature of PCV-2, co-infections are of interest, and domestic pigs co-infected with PCV-2 and *Salmonella* Choleraesuis show more severe clinical signs than those only infected with *Salmonella* Cholerasuis.

The aim of this study was to determine the presence of PCV-2 virus in wild boar in Sweden and to investigate the link between PCV-2 and infection with *Salmonella* Cholerasuis.

During 2023, lymph nodes were collected from 103 wild boar and analysed for PCV-2 using real-time PCR. Fifty-eight lymph nodes were collected from seemingly healthy, hunted wild boar. Remaining 45 samples were collected from diseased wild boar through the wildlife disease surveillance program at the Swedish Veterinary Agency (wild boar either found dead or euthanized due to illness). Routine culture for *Salmonella* spp. was performed on 100 of the 103 samples.

PCV-2 was identified in lymph nodes of 50% (n= 52) of the total analysed wild boar. Out of the wild boar shot during hunt, 40 % (n= 23) were positive for PCV-2, whereas 64% (n= 29) of clinically ill or found dead wild boar had detectable virus in the lymph nodes. Histopathological lesions associated with PCV-2 antigen were detected with immunohistochemistry, primarily in the lymph nodes and lungs of young animals found ill or dead. *Salmonella* was detected in 13 wild boar, and 10 of these were positive for both *Salmonella* and PCV-2.

The results indicate that PCV-2 virus is common among wild boar in Sweden, particularly in diseased or animals found dead. Pathological lesions associated with PCV-2 in wild boar were observed in some of the PCR-positive wild boar, however the significance of the disease alone and in relation to other infections remains unclear and needs to be studied further. Most of the animals carrying Salmonella were also positive for PCV-2, indicating that co-infection of the pathogens is common and that this possibly is of importance for the wild boar population.

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Highly pathogenic avian influenza (H5N1) virus with evidence of widespread systemic infection in wild red foxes (V*ulpes vulpes*) in Sweden 2021-2023

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Abstract

Since the influenza season 2020-2021, highly pathogenic avian influenza (HPAI) (H5Nx) clade 2.3.4.4b has resulted in an unprecedented number of infections in wild and domestic birds across the globe, with numerous spillover events to wild and domestic mammals. In Sweden, infection with HPAI H5N1 clade 2.3.4.4b was diagnosed in 2 red foxes (*Vulpes vulpes*) in 2021 and in 14 red foxes in 2023. Ten foxes were euthanized with neurologic signs including ataxia, circling and /or convulsions, 5 were found dead, and one was shot during a normal hunt. Macroscopic lesions were confined to the lung and, in one case, to the liver. Lungs were oedematous and had multifocal to coalescing areas of consolidation. The liver of one fox was discoloured yellow tan and the carcass was icteric. Feathers and bird feet were found in the stomach of one fox and feathers in the faeces of another. Microscopic lesions were most often found in the brain (non-suppurative meningoencephalitis) and in the lung (necrotizing interstitial pneumonia), but in several cases lesions associated with viral antigen (detected by

immunohistochemistry) were also found in the heart, liver, spleen, kidneys, pancreas, and eyes. Of the 14 foxes diagnosed in 2023, 10 were temporally and geographically associated with an outbreak of HPAI H5N1 in black-headed gulls (*Chroicocephalus ridibundus*) in Stockholm. Analysis of genetic sequences shows that the viruses isolated from the foxes were closely related to the viruses found circulating in wild birds. The viruses from cases in 2021 and one from January 2023 belonged to three separate genotypes while the rest of the sequenced viruses (May to August 2023) all belonged to genotype BB, which was the genotype most often found among gulls in 2023. While microscopic lesions in the brain and lung are consistent with those previously described in foxes infected with HPAI H5N1, lesions in other tissues are rarely described and descriptions of the eye are lacking. The presence of viral antigen in the retina of foxes investigated here shows that the virus can infect the eye and may explain why some infected foxes found alive with neurologic signs also appear blind, as reported by several authors. The viral sequences from the infected foxes in Sweden suggest that they were infected through ingestion of, or close contact with, infected wild birds. These findings reinforce the importance of both passive and active surveillance of HPAI in mammals, especially carnivores and scavengers.

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Emmonsia spp. infection in coypu (Myocastor coypus) from Lower Saxony, Germany

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Abstract

Background

Originating from South America, the coypu (*Myocastor coypus*) is listed as an invasive alien species (IAS) of Union concern according to EU regulation 1143/2014. It was introduced to Germany roughly a hundred years ago and is considered established in all German federal states. According to literature, infections with the saprophytic fungus *Emmonsia* spp. occur in a broad range of mammalian hosts and also occasionally in humans. The infection may lead to

adiaspiromycosis, a pulmonary and occasionally systemic disease possibly leading to death.

Objectives

Between 2019 and 2021, 128 coypus were harvested during legal hunting procedures and received a full post-mortem examination in order to get insights on diseases as well as injuries resulting from live trapping of these animals.

Materials and Methods

All animals were necropsied at the Institute for Terrestrial and Aquatic Wildlife Research (ITAW). Samples of selected organs were fixed in formalin and routinely processed for histopathology. For further investigations, additional tissue samples were frozen.

Results

Among 128 harvested coypu, the lungs of five animals histologically showed multiple granulomas with intralesional intact or degenerate adiaspores of up to 500 µm that were characterised by a thick, three-layered, hyaline wall around a basophilic, granular core. Based on morphology and size, an infection with *Emmonsia crescens* is most likely.

Conclusions

Burrowing animals are considered to be affected by *Emmonsia* spp. infections due to inhaling spores from soil or nesting materials. The disease was consistently reported in muskrats, another invasive alien species with semiaquatic, burrow-building lifestyle in Europe. Coypu are susceptible and develop pulmonary adiaspiromycosis and therefore might facilitate the spread of the fungus. Carnivores ingesting infected animals shed adiaspores in their feces, where spores germinate and develop. Depending on weather conditions, both animals and humans may inhale airborne conidia released from the soil. Therefore, *Emmonsia spp.* should be considered as a potential cause of disease, warranting further investigation.

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Vector-Borne RADAR- An enhanced surveillance programme for mosquito-borne viral diseases of wild birds in the UK

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Abstract

The United Kingdom (UK) was free of mosquito-borne viral zoonotic diseases until summer 2020 when Usutu virus (USUV; *Flaviviridae*) was detected in Greater London in both avian hosts and mosquito vectors. USUV Africa 3.2 lineage isolates with a shared most recent common ancestor have been confirmed each subsequent year at the index site, 2021-2022 in wild birds, thus virus overwintering and autochthonous transmission in mosquitoes is the likely mechanism of local persistence, and not repeated incursions. USUV-mediated population declines of Eurasian blackbird (*Turdus merula*), have been documented across continental Europe. A reduction in blackbird reporting rates and breeding populations in Greater London since 2020 indicate that a disease-mediated decline may also be occurring in the UK. Although the related flavivirus, West Nile virus, has not been detected in UK, its occurrence in neighbouring countries requires vigilance for potential incursion. The Vector-Borne RADAR (Real-time Arbovirus Detection And Response) project (www.vb-radar.com) was established in 2023 to enhance mosquito-borne flavivirus surveillance in the UK. The objectives are to 1. develop early warning surveillance of flaviviral disease outbreaks in wild birds and 2. investigate risk pathways for flavivirus incursion and transmission within host and vector populations. Active surveillance of live wild birds will commence in spring 2024 at a network of bird ringing observatories across southern England. Cloacal and pharyngeal swabs, and contour feathers, will be collected for PCR and blood samples taken for serological testing (ELISA and virus neutralisation). Geographically-associated mosquito populations will be trapped for species identification and flavivirus PCR. Meanwhile, VB-RADAR has enhanced existing passive surveillance networks for wild passerine mortality, delivered through citizen science (www.gardenwildlifehealth.org), by increasing engagement with stakeholders, including wildlife rehabilitation centres and zoological collections. Post-mortem examinations are conducted on passerines

and brain and kidney samples are tested for flaviviruses using PCR. In 2023, 4 of 131 dead wild birds tested USUV-PCR positive: three blackbirds and one feral pigeon (*Columba livia*). Sequence analysis confirmed Africa 3.2 lineage as in previous years. Additionally, approximately 20,000 mosquitoes have been collected, the majority of which were *Culex pipiens,* an ornithophagic mosquito and key vector of flavirivuses in continental Europe. Some *Culex pipiens* larvae caught at the index site and reared to adults tested USUV-positive, indicating transoviarial transmission in vector populations as a mechanism for local persistence. The VB-RADAR team comprises governmental animal and public health organisations, in collaboration with conservation NGOs, exemplifying a One Health approach to protect UK biosecurity.

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Echinococcus multilocularis (fox tapeworm) egg contamination of commercially grown berries and infection of foxes in the Dutch province of Limburg

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Abstract

Background: *Echinococcus multilocularis,* the fox tapeworm, has been recognized as the most important food-borne parasite in Europe. Foxes are the main definitive hosts and humans can accidentally get infected by ingestion of eggs. This may cause the life-threatening disease alveolar echinococcosis (AE), where the larval stage of the parasite develops into a tumor-like structure in the liver with the potential to metastasize. After the first detection of *E. multilocularis* in foxes in the provinces of Limburg and Groningen in 1996, an increase in fox prevalence and human cases has occurred in The Netherlands. Since ingestion of eggs might cause AE, it is important to not only monitor the presence of *E. multilocularis* in foxes, but also in food. This is especially important in produce that is eaten raw, since cooking will inactivate the eggs.

Objective: To assess the prevalence and associated risk factors of *E. multilocularis* on fresh produce in the Dutch province of Limburg.

Materials and methods: In 2022, 8 strawberry and 6 blueberry samples were bought from farm stands in Limburg and tested for *E. multilocularis* DNA using a washing and filtration protocol, followed by DNA isolation and an *E. multilocularis*-specific qPCR.

Results: In the 2022 study, *E. multilocularis* DNA was found on 1/8 strawberry and 1/6 blueberry samples from Limburg.

Conclusion: The results of the 2022 study show that commercially grown berries from the endemic area can be contaminated with *E. multilocularis*.

Follow-up study: To better estimate the prevalence of *E. multilocularis* on fresh produce, in the summer of 2024 30 commercial soft fruit growers in Limburg will be visited twice to take samples, identify company characteristics, and collect any present carnivore feces. The prevalence of *E. multilocularis* on fruit samples will be studied using the 2022 method. For the fecal samples, host type will be identified using a PCR with primers targeting the cytochrome b gene. Presence of *E. multilocularis* in the feces will be determined using an *E. multilocularis*-specific qPCR. Additionally, the spread of *E. multilocularis* in the fox population will be estimated by a large field study sampling foxes in Limburg in 2024-2026. These results will contribute to estimating the public health risk of *E. multilocularis* and the development of prevention strategies.

This work was done as part of the MEmE project, supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

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Improved stability and specificity of baits for oral administration of substances to wild boar

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Abstract

Oral vaccination is one of the most attractive wildlife disease control options. As a result of the recent global reemergence of African swine fever and ongoing classical swine fever and animal tuberculosis, oral vaccination of Eurasian wild boar (*Sus scrofa*) has revived renewed interest. Even when several baits for wild boar and feral pigs have been described, developing more stable and specific formulations is important. This communication proposes a new bait formulation primarily composed of corn flour, piglet feed, sugar, and honey as a binder to obtain improved elasticity. The bait consists of a matrix with no protective coats, has a hemispherical shape (ø 3,4x1,6 cm), and displays an anise aroma and blue color. The color and aroma did apparently not affect bait choice by wild boar, while bait coloring contributed to avoid consumption by non-target species (corvids). Baits with the new formulation were significantly more resistant to humidity and high temperatures than previous versions. Simulations suggest that baits with the new formulation are elastic enough to resist impacts from a maximum altitude of 750 meters. Thus, the new bait prototype solves several problems of previous baits while keeping a format that can be consumed by piglets and adult wild boar.

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Genetic determinants of H5N1 avian influenza viruses that led to an unprecedented mass mortality in gulls in 2021/2023

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Abstract

Background

As reservoir hosts for avian influenza viruses (AIVs), wild waterfowl usually show no clinical signs of infection, while there is a constant circulation of low pathogenicity AIVs (LPAIVs) in the population. Since 2021, an increasing number of wild birds, including gulls (Laridae), have died after infection with HPAIV subtype H5N1 clade 2.3.4.4B. The genomic changes that led to the widespread and high virulence of this subtype in gulls remain to be elucidated.

Objectives

We aimed to identify the effects of genetic markers that contribute to the high virulence of contemporary H5N1 clade 2.3.4.4B in gulls.

Materials and methods

More than 40,000 sequences of AIV gene segments were analysed. Cloning and rescue of different H5N1 clade 2.3.4.4B viruses is underway.

Results

Sequence analysis revealed striking differences between the viruses originally circulating in gulls and the new H5N1 viruses. Interestingly, the internal H5N1 gene segments appear to have completely replaced the segments of the original European gull-adapted viruses. Detailed analysis of selected gene segments from both clusters has been undertaken to identify potential key mutations that could lead to the unexpected increase in virulence in gulls. Laboratory experiments are underway to investigate the impact of these mutations on viral fitness.

Conclusion

We describe mutations associated with the high virulence and widespread distribution of clade 2.3.4.4B viruses in gulls in 2021/2023.

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Using integrated wildlife monitoring to understand biodiversity-disease dynamics at the wildlife-livestock interface.

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Abstract

In the One Health context, Integrated Wildlife Monitoring (IWM) merges wildlife health monitoring with host community monitoring to early detect emerging infections, to record changes in disease dynamics and in wildlife population trends, and to allow assessing the impact of eventual interventions. While most wildlife disease research has addressed specific hosts and pathogens, we aim at using IWM to understand complex multi-host and multi-pathogen networks. This holistic approach enables addressing questions such as which are the links

between biodiversity and pathogen circulation, or what is the effect of pasture-based livestock farming on both, biodiversity, and pathogen circulation.

To address this challenge, we choose 18 natural areas in a broad range of natural settings representative of the Iberian Peninsula. In each site we characterized the vertebrate host community (mammals and birds) using audio recordings and camera-trap (CT) networks. Mammal cross-species interactions were analysed in detail based on space/time coincidences per CT and social network analysis. We obtained 434 sera from our indicator species, the wild boar, which were tested for antibodies against 14 pathogens. We also sampled surfaces (15 samples per site) for environmental nucleic acids to detect selected pathogen markers.

We identified 108 bird and 22 mammal species, with a mean vertebrate richness per site of 45 (range 26 to 58). Livestock was present in 13 of 18 sites and was abundant in 9 of 18 sites. The wild boar, which is a central species within all 18 host communities, was exposed to a mean of 8.5 (range 4 to 12) pathogens per site. We found that biodiversity (species richness) had no effect on pathogen richness. However, species richness was negatively correlated with specific pathogens including *Erysipelothrix rhusiopathiae* and mycobacteria. Livestock abundance as quantified by CTs had no effect on biodiversity and pathogen richness. However, we found effects on specific pathogens including *Salmonella* spp. and *Toxoplasma gondii*. Detecting *Escherichia coli* markers in environmental samples was an indicator of livestock abundance.

The dynamics of generalist pathogens in biodiverse host communities remains a major frontier in disease ecology, wildlife management, and animal science. Insights obtained from this IWM scheme suggest that host communities influence ecosystem functioning and pathogen diversity and maintenance.

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Avidity of 11 commercial conjugates to 10 small mammal species

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Abstract

Serological assays are important tools for the detection of (past) infections with micro-organisms. For most domestic animal species species-specific conjugates

are available to be used in these serological assays. However, for many wildlife species no species-specific conjugates are available. For these wildlife species, conjugates for closely related domestic species or immunoglobulin-binding proteins, such as protein A and G, are often used. However, the use of these conjugates is often not validated and may result in false-negative results.

In our laboratory, we perform various serodiagnostic assays on a growing number of small mammal species. Therefore, we wanted to assess the useability of commercial conjugates for small mammal research.

We tested 11 commercial conjugates, including protein G and species- or family-specific secondary conjugated antibodies, on eight rodent species and two insectivore species. Using direct Enzyme-linked Immunoassays (ELISAs), the differences of avidity of conjugates between species and within species was studied.

Large differences in avidity of the conjugates to the various small mammal species were noted. Some conjugates only bound to antibodies of one specific species, whereas others showed a broad binding range across species. The strength of antibody-conjugate interaction varied between species and also depended on the individuals immune response, but overall avidity patterns of conjugates remained the same. In general, the conjugates had stronger antibody-conjugate interactions for rodent species than for shrews, as only two conjugates showed limited response to antibodies of shrews. For shrews, further testing with other conjugates may need to be performed to find more suitable conjugates.

Our study underlines the importance to confirm the avidity of a conjugate for antibodies of a new species, even if the conjugate is known to bind to antibodies of a closely related species.

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Cerebral high-grade astrocytoma with angiocentric features in a canine distemper virus-positive red fox (*Vulpes vulpes*)

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Abstract

Here, we present a case of a high-grade astrocytoma with angiocentric features affecting the brain of a free-ranging, adult, male red fox tested positive for distemper. The animal exhibited ataxia, severe apathy, and dyspnoea. It was shot for animal welfare reasons and submitted for postmortem examination to the Institute of Fish and Wildlife Health, Switzerland, within the general wildlife health surveillance.

Gross examination revealed generalized icterus and traumatic lesions attributed to the culling of the animal. Additionally, the left cerebral hemisphere exhibited focally extensive, grey blurring of the striatum and of the grey-white matter interface of the ventral frontal lobe, olfactory peduncle and nucleus.

Histopathology revealed a poorly demarcated, infiltrative, and densely cellular neoplastic mass extending subpially from the ventral cortex to adjacent structures, predominantly effacing the basal nuclei, ventral capsula interna and olfactory structures. The mass exhibited a prominent and peculiar palisading pattern with numerous pseudorosette-like structures that were partially perivascular and variably intermixed with areas of neoplastic cells arranged in fascicles. Frequently, neoplastic cells entrapped neurons. Neoplastic cells were elongated with frequent bipolar cytoplasmic processes, irregular oval nuclei, and rare mitotic figures. A large proportion of neoplastic cells strongly expressed glial fibrillary acidic protein (GFAP) in their processes, indicative of an astrocytoma.

Other neuropathologic findings included marked perivascular edema and mononuclear perivascular cuffs (composed mainly of CD3+ T cells) throughout the brain, consistent with encephalitis. Simultaneous infection with canine distemper virus was confirmed by PCR. In addition, a focal granuloma containing nematode larvae, compatible with larva migrans, was present in the occipital lobe. Multifocal-confluent, interstitial, mixed-cellular pneumonia with intralesional nematodes and proliferative endarteritis was also observed, suggesting infection with Angiostrongylus vasorum, possibly associated with cerebral larva migrans. Other findings, including necrotising hepatitis, splenic lymphocytolysis and interstitial nephritis with acute tubular injury were likely associated with a disseminated intravasal coagulopathy affecting multiple organs, including the kidneys, due to terminal septicaemia.

This is the first description of an astrocytoma in a free-ranging fox species. Moreover, astrocytomas displaying an angiocentric pattern, reminiscent of the rare angiocentric glioma occurring in humans, are exceedingly rare in the veterinary literature and have only been reported once in a cat. These findings demonstrate the potential co-occurrence of neoplastic processes and infectious

agents, which in a case with a common first finding (e.g. distemper in a neurologic fox) might be overlooked, highlighting the importance of thorough pathological examination.

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Corona of the king of the Polish jungle - prevalence of coronaviruses in European bison (*Bison bonasus*) in Poland

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Abstract

Coronaviruses have been confirmed to infect variety of species, but only one case of associated winter dysentery of European bison has been described. The aim of the study was to analyze the prevalence, define the impact on the species conservation, the source of coronavirus infection and role of the European bison in the transmission of the pathogen in Poland. Molecular and serological screening was performed on the 409 European bison from 6 free-ranging and 14 captive herds over the period of 6 years (2017-2023). Presence of coronavirus was confirmed in 3 nasal swab samples by bovine coronavirus (BCoV) specific real time RT-PCR and one nasal swab by pancoronavirus RT-PCR. Detected virus showed high (>98%) homology in both RdRp and Spike genes to BCoV strains characterised recently in Polish cattle and strains isolated from wild cervids in Italy. Antibodies specific to BCoV were found in 6.4% of tested samples, all originating from free-ranging animals. Seroprevalence was higher in adult animals over 5 years of age ($p = 0.0015$) and in females ($p = 0.09$). Our results suggest that European bison play limited role as reservoirs of bovine-like coronaviruses. Although, the most probable source of infections in the European bison population in Poland is cattle, other wild ruminants could also be involved. In addition, the zoonotic potential of bovine coronaviruses is quite low.

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Multi-host modelling of brucellosis at the wildlife-livestock interface in the French Alps

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Abstract

Emerging or re-emerging diseases involving both livestock and wildlife represent a prominent challenge to disease management. Indeed, knowledge on the hosts or the disease, as well as surveillance data, are often scarce in such cases. This effectively limit our ability to understand transmission dynamics and predict the efficacy of management strategies. However, when enough data or prior knowledge is available, mechanistic modelling can help us to better understand disease transmission within multi-host systems. In particular, it can disentangle the contribution of the different host populations to the maintenance and the transmission functions. The maintenance function is defined as the capacity to sustain the infectious agent within the system. In contrast, the transmission function is defined as the capacity to transmit to the target population, e.g. domestic livestock. A reservoir can then be defined as one or several host populations that fulfil both the maintenance and the transmission functions. The

aim of our study was precisely to better understand which host populations contribute to the maintenance and transmission functions in the case of brucellosis in the French Alps. This multi-host system includes Alpine ibex (*Capra ibex*) and Alpine chamois (*Rupicapra rupicapra*), as well as domestic ruminants (cattle, sheep and goats). Evidence of cross-contamination was found in ibex, chamois and cattle, with very close genetic distance between the isolated *Brucella melitensis* strains. We developed a multi-host, compartmental, deterministic model of *Brucella* transmission within and between these different host populations. Taking advantage of surveillance data in both wild and domestic species, we estimated within- and between-population transmission rates. Contrary to previous studies in animal health, where contact patterns were hypothesized, we also integrated results from observational studies on direct and indirect contacts between species to inform our estimations. From the estimates of the transmission rates, we were then able to calculate the within- and between-population reproduction numbers, defined as the expected number of new infections in a host population generated by a single infectious individual in the same or different host population. By calculating which population or sets of populations had a reproduction number above one (the threshold for infection persistence), our results allowed to bring insights on the populations' contribution to the maintenance function. Similarly, we identified the populations that contributed to transmission to domestic cattle. These results will inform surveillance and management of brucellosis in this multi-host system.

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Insights on the ecology of lyssaviruses associated with the Schreiber long-fingered bat (*Miniopterus schreibersii*)

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Abstract

European bats carry several members the genus Lyssavirus, all considered able to cause Rabies. Among species identified in Europe, West Caucasian bat virus (WCBV) and Lleida bat virus (LLEBV) pose particular concern because of the inefficacy of prophylactic and therapeutic agents. These viruses have been both associated with the long-fingered bat, and WCBV caused rabies in a cat in Italy. However, we are far from understanding their ecology and the factors driving spillover events.

This study provides a preliminary investigation on the ecology of lyssaviruses associated with the long-fingered bat that will help informing targeted surveillance in the future with the final aim of understanding drivers of emergence and design effective mitigation strategies.

We used serology to investigate virus circulation within bat populations of long-fingered bat. We screened bats across in eight European countries across the range of the species exploiting existent surveillance plans and using archive samples whenever available. In Italy, we performed active surveillance in three sites over three years. We assessed the presence of virus-neutralizing antibodies against WCBV and LLEBV. We further tested a set of sera against other lyssaviruses of philogroup I, namely EBLV-1, EBLV-2 and DUVV.

We founded neutralizing antibodies against WCBV and LLEBV in 8/8 and 6/8 countries respectively, with positivity dating back 2012. The sampling effort differed in terms of timing, sample size and test sensitivity, hampering effective comparison between countries. Longitudinal data obtained in Italy suggested seasonal variations, with peaks after aggregation and the weaning of pups that might differ between viruses. In addition to confirming the circulation of LLEBV and WCBV, cross-reaction analyses showed two additional clusters, one reacting against EBLV-1 and the other cross-reacting using EBLV-2 and DUVV.

This study showed a wide distribution of divergent lyssaviruses across the range of the long-fingered bat, with longitudinal data suggesting seasonal dynamics. WCBV and LLEBV co-circulate in most settings, challenging previous hypotheses of one host - one lyssavirus. In addition, preliminary evidence indicates the presence of other lyssaviruses of philogroup I that might either be EBLV-1, DUVV, EBLV-2 or unknown viruses with antigenic similarities.

In order to unravel the complicated ecology of miniopterus lyssaviruses it will be necessary to harmonize sampling strategies across its geographical range and to obtain small-scale longitudinal data to feed mathematical models that might help identifying windows for increased risk.

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Survey of dermatophytes with possible pathogenic action on Alpine Marmots (*Marmota marmota*) captured in the municipality of Livigno, Italy

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Abstract

Background: Wild rodents are affected quite frequently by dermatophytes. There is only a few evidence in the literature of the isolation of dermatophytes on the skin and fur of *Marmota marmota*, but cases of partial alopecia have been reported in many subjects in the study area

Objective: We wanted to investigate the circulation of mycotic species capable of giving clinical forms in the marmot through a systematic collection of samples within a project of capture and translocation. The aim of this work is to investigate the presence of pathogenic dermatophytes, responsible for dermatomycosis in marmots living in the alpine area of Lombardy, North Italy.

Materials and methods: As part of the provincial relocation plan, alpine marmots were captured in different areas of the municipality of Livigno (46°32′10″ N , 10°08′00″ E) in the province of Sondrio. The animals were measured and checked for the absence of clinical signs. In the three-year period 2021-2023, hair samples were collected from 201 marmots. In particular, 135 hair samples and 66 sponges rubbed on the body of the marmots during the capture operations. All samples were subjected to culture examination by direct sowing in selective Dermatophyte Test Medium (DTM) and incubation at +30±2°C for 10 days. Then the suspicious colonies were subjected to morphological identification through microscopic examination (scotch test) to highlight the species-specific conformations of hyphae and fruiting organs with dermatophyte lactophenol blue coloration.

Results: 147 samples tested negative for dermatophytes, while from 54 samples it was possible to isolate fungi responsible for animal and human dermatomycosis. The most isolated species was *Microsporum nanum* with 36 isolations, followed by *Trichophyton terrestre* with 9 isolations and *Trichophyton verrucosum* with 6 positive samples. In three samples it was not possible to identify species, but they appeared to belong to the *Microsporum* genus. *Microsporum nanum* (*Nannizzia nana)* is a geophilic and zoophilic fungus that frequently causes chronic non-inflammatory lesions in pigs a cause of tinea in humans. Also present in the soil of pigsties. Infections in humans are usually contracted directly from pigs or from fomites.

Conclusion: The study demonstrated the circulation of *Microsporum nanum* in a marmot population. Subjects captured in places almost in the vicinity of zootechnical farms (one subject was even captured inside an animal shelter) demonstrated that the close interaction and/or sharing of territory between wild and domestic animals could result in the passage of pathogens between different species.

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Surveillance of antimicrobial resistance in wildlife animals in Mecklenburg-Western Pomerania, Germany

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Abstract

Background: The project "Wildlife disease monitoring in Mecklenburg-Western Pomerania (MVP) through an integrated One Health Surveillance-Response System (WiMoPOH)" aims to establish a systematic framework for the continuous and systematic collection of wildlife samples in Mecklenburg-Western Pomerania

(MVP). WiMoPOH focuses on a better understanding of the potential health risks arising from human-livestock-wildlife cross-transmission by investigating the presence and prevalence of zoonotic infectious agents, e.g. viruses, bacteria, and parasites, in wildlife animals and their potential role as reservoirs.

Within this larger project, our work focuses on the need to study AMR issues using the One Health approach in MVP. The close contact and proximity of wild animals to agricultural-livestock facilities and human-contaminated environments predispose the transmission of antimicrobial-resistant (AMR) bacteria to wildlife. In addition to commensal bacterial strains, this includes opportunistic pathogens such as extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, e.g. high risk-clonal lineages of *Escherichia coli* and *Klebsiella pneumoniae* (even hyper-virulent and converging strains), and methicillin-resistant *Staphylococcus aureus* (MRSA).

Hypothesis: Carnivores, ruminants, and wild boars could act as reservoirs, and contribute to the spread and persistence of AMR between human-livestock-wildlife.

Materials and Methods: Nasal and faecal swabs were collected from 352 hunted animals in Mecklenburg-Wester Pomerania in 2023 (October-December). These included ruminants (fallow deer, roe deer, red deer, and mouflon), carnivores (fox, red fox, racoon, and raccoon dogs), and wild boars. Carnivores were hunted only on the Island of Rügen and frozen at - 80°C before sampling. We screened for ESBL-producing *Enterobacterales* and MRSA on the respective chromogenic agar plates. Species identification and phenotypic antibiotic resistance profiles were assessed using the VITEK II Compact System.

Results: To date, we have isolated nosocomial bacterial pathogens of clinical relevance, (ESBL)-producing *Klebsiella,* (ESBL)-producing *Escherichia coli* and colistin-resistant *Acinetobacter baumannii*, from 98 wildlife samples. Culturomics could be performed on samples obtained from -80°C freeze-dead carnivores. Analysis of the remaining isolates will be continued.

Conclusion: This study has isolated AMR ESKAPE-organisms from wildlife animals, highlighting their potential role as reservoirs, and bioindicators of AMR bacteria in the environment of northeast Germany. Ongoing whole-genome sequencing followed by in-depth bioinformatics analyses will shed light on clonal lineages, carriage of resistance- and virulence-associated genes, and phylogenetic relationships, and will evaluate the role of this environmental area in a One Health context.

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Paired metagenomic and metatranscriptomic sequencing reveals the gastrointestinal resistome and microbiome of yellow perch (*Perca flavescens*) in Minnesota lakes.

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Abstract

Wastewater of anthropogenic origin can influence the wildlife living in natural water bodies, for example, altering the health, behavior and reproduction of wild fish. These fish serve as host for a variety of microorganisms, including commensal and pathogenic bacteria. When exposed to various biotic and abiotic pressures, bacteria can develop antimicrobial resistance; in some cases, commensal bacteria can transfer antimicrobial resistance genes to pathogens within the microbial community. When pathogens acquire antimicrobial resistance, they can pose a health risk if subsequent infections become more difficult to treat in either humans or animals. The aim of this study was to analyze the microbial community (microbiome) and profile of antimicrobial resistance genes (resistome) in the gut of yellow perch collected from two categories of lakes under anthropogenic pressure: wastewater effluent-impacted lakes, and undeveloped lakes (lakes with no human development along shorelines nor wastewater effluent). Yellow perch were sampled from five lakes in Minnesota, United States, three of which were wastewater effluent-impacted, and two that were undeveloped. DNA and RNA were extracted and subjected to shotgun sequencing to characterize and compare the microbiome and resistome of wastewater effluent-impacted and undeveloped lakes. Our results suggest that the resistome and microbiome of the yellow perch gut differs between lakes, and some of this difference may be due to different levels of anthropogenic pressure. The resistome predominantly

consisted of macrolide resistance genes, specifically the group MLS23S, which accounted for 53% of all resistome sequences generated from effluent-impacted lake samples, and 73% of resistome sequences from undeveloped lake samples. The microbiome was dominated by the phyla Proteobacteria, Firmicutes and Actinobacteria. At the species level, the opportunistic pathogens *Plesiomonas shigelloides* and *Aeromonas veronii* were found in higher abundance in samples from effluent-impacted lakes compared to undeveloped lakes. Construction of metagenome-assembled genomes (MAGs) is currently ongoing, which may reveal potential novel components of the yellow perch microbiome and resistome. Metagenomic analysis of wild fish samples is a promising tool to investigate the impact of anthropogenic pressures on the microbial communities within water bodies.

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M. bovis in the territory of the marsican brown bear (Ursus arctos marsicanus, Altobello 1921)

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Abstract

Background or context

Mycobacterium bovis transmission from domestic animals to wildlife and back (known as spillover and spillback), complicates *M.bovis* eradication efforts worldwide. Complete disease eradication is unattainable while wildlife reservoirs persist. Despite the Abruzzo and Molise regions in central Italy being declared MTBC-free since 2022, sporadic MTBC cases have emerged in wildlife. These areas are home to a critically endangered population of Marsican brown bears

(*Ursus arctos marsicanus*). To date, only one fatal *M.bovis* case in a Marsican brown bear has been confirmed.

Hypothesis or objectives

Our goal was to explore M. bovis transmission dynamics in the Abruzzo region, examining correlations between wild animal cases and livestock outbreaks. Analyzing spoligotypes helps understand M. bovis's genetic diversity and informs control and prevention efforts.

Materials and methods

From 2009 till 2023, 72 outbreaks in cattle (most in fattengin beef farms with outdoor aptitude) across the two regions were anlyzed, alongside 5 positive cases in wildlife.Organs with typical lesions from *M. bovis*, founded in cattle at the official inspections at the slaughterhouse and from wild animals found dead, underwent to anatomo-pathological inspections. Samples from lesions were submitted to culture. Identification at species level of Mycobacterium spp. was obtained by an algorithm including a multiplex PCR approach. Spoligotyping and Variable Tandem Repeat approach with a "core" group 12 loci were performed for molecular carachterization. The period considered in this study goes from 2009 to 2023.

Results

Spoligotyping was conducted on 30 farms all the 5 wild positives. A total of 40 spoligotypes and MIRU/VNRT profiles were identified. Cattle spoligotypes included SB0120, SB0134, SB0418, SB0560, SB0829, SB0841, SB0872, SB0919, and SB1324, with SB0120 being most common (20/42). Two wild boars were SB0418, while the other two and the red deer exhibited the SB0120 spoligotype. The deer in 2023 shared the exact spoligotype-VNRT profile with the one Marsican brown bear found positive in the same minucipality in 2014 (Fico et al., 2019). Similar genotypes were also identified in nearby domestic outbreaks around 2014 and earlier.

Conclusions

The variety of cattle spoligotypes reflects beef market dynamics. Wildlife positives from 2019, barring the 2014 Marsican brown bear, hint at shifts in disease transmission, warranting more research. Similar genotypes in wild and domestic cases suggest links with domestic animals, impacting livestock's disease-free status and Marsican brown bear conservation.

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Zoonotic Bacteria in Northern Sweden: Seasonal Trends in Bank Vole Populations

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Abstract

Understanding the interplay between biodiversity, population fluctuations, and pathogen transmission is crucial for effective ecological conservation and public health management. This study focuses on understanding these relationships through an investigation of bank vole (*Myodes glareolus*) populations in northern Sweden, given their role as of zoonotic pathogens causing, leptospirosis, tularemia and pseudotuberculosis.

At the core of this research is the paradigm of the dilution effect, which hypothesizes that higher biodiversity reduces pathogen transmission via different mechanisms including encounter reduction between susceptible hosts and infected individuals, resulting in a lower pathogen prevalence and decreased public health risk. Our main objective is to explore the impact of biodiversity on pathogen prevalence, diversity, and transmission dynamics.

Using a longitudinal approach, we analyze pathogen prevalence in bank vole populations in different forest environments trapped over a complete vole cycle (2020-2023) in northern Sweden. This enables us to shed light on how pathogen load and prevalence vary across seasons, sites and population densities. By capturing these dynamics, we aim to better understand the interplay between host population dynamics, biodiversity, and pathogen prevalence.

This study contributes to understanding the role of community structure among small mammals in mitigating pathogen transmission among host populations.

Furthermore, it enables us to identify spatial pathogen hotspots for zoonotic diseases and facilitates the opportunity for targeted surveillance strategies and public health interventions.

This project is part of the BEPREP project, funded by HORIZON-CL6-2021-BIODIV-01.

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Dynamics of African Swine Fever velocity in wild boar

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Abstract

African swine fever (ASF) virus has been present in the wild boar population of the Baltic countries since 2014. Understanding the dynamics of ASF in wild boar populations is challenging due to limited data on population and movement patterns data, as well as logistical and cost constraints in surveillance efforts. Spatial modelling becomes crucial for anticipating the spread following confirmed ASF events in wild boar, identifying whether the spread is expected to be constant or increasing, or if further cases are expected in other areas and at which rate of occurrence, to implement targeted interventions.

We hypothesize that the spatial dynamics of ASF virus in wild boar in the Baltic countries and Eastern Poland exhibit varying rates of advancement. Such rates can be modelled by estimating the velocity of a front epidemic wave.

We propose a universal kriging model to interpolate the earliest ASF notifications in wild boar from January 2014- January 2022 per 50 sq.km cell in Estonia, Latvia, Lithuania and Eastern Poland. The inverse of the slope surface of the prediction time corresponds to the front-wave velocity of ASF at each location within the study area. The front-wave velocity values (km/month) were explored with descriptive and spatial statistics.

The median front-wave velocity of ASF was based on 2277 predicted points and was of 49.52 km/month. The maximum velocity was obtained for Estonia (740.39 km/month). The median velocity was highest at the beginning of the epidemic, in

the years 2014-2015. Remarkably, it slowed down in 2020. Only Eastern Poland had a sufficient number of predicted points across seasons, and the median velocity seemed to vary very little (from 35.45 in spring to 48.06 km/month in winter). Spring was the season with the fewer predicted velocity points. As for habitat, between 73 and 89% of the velocity points were in high quality wild boar habitat, possibly reflecting wild boar distribution.

The spatio-temporal kriging model has allowed estimating the front-wave velocity of ASF in wild boar. No seasonal variations were detected, but the variation in velocity points predicted could be a reflection in the surveillance efforts across seasons, wild boar habitats, months and years. The velocity was higher at the beginning of the epidemic and took 5 years to slow down.

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A citizen science project shows the occurrence of the fungal pathogen *Batrachochytrium dendrobatidis* in amphibians in the United Kingdom

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Abstract

Batrachochytrium dendrobatidis (Bd) is a fungal pathogen responsible for chytridiomycosis, one of the major causes of amphibian decline globally. Data on its prevalence in the UK are still fragmentary, precluding a full understanding of its potential impact. In this context, a survey was conducted to describe the occurrence of Bd infection in the UK and raise awareness of this disease threat to biodiversity by involving citizens in sampling activities.

In 2008 and 2011, skin swabs were collected from amphibians of England, Wales, and Scotland with active citizenship involvement. Sampling sites were selected

arbitrarily to facilitate the achievement of the target sample size (90 sites, 30 amphibians per site). A Bayesian hierarchical model was used to estimate the percentage of Bd-positive sites and the prevalence of Bd infection within individual sites over a range of potential diagnostic test sensitivities.

Sampling involved 5,766 amphibians in 2008 and 3,106 in 2011, distributed over 123 sites in 2008 and 122 sites in 2011, for a total of 183 individual sites and 346 visits. The sample size of 30 amphibians was reached in 69.94% of visits (n=242/346; sampled amphibians: Q1=28, median=30, Q3=30). Bd was detected at sites in England, Scotland, and Wales. The posterior mean estimates for the percentage of Bd-positive sites in the best-case scenario (diagnostic test sensitivity=1.0) were 21.44% (95% credible interval (CI) 14.56-29.26) in 2008 and 12.79% (95% CI 7.33-19.45) in 2011, while in the worst-case scenario (sensitivity=0.5) were 22.10% (95% CI 15.23-30.07) in 2008 and 13.56% (95% CI 7.90-20.59) in 2011. The posterior mean estimate for the prevalence of infection within Bd-positive sites in the best-case scenario was 15.37% (95% CI 13.50-17.33) in 2008 and 14.73% (95% CI 11.40-18.57) in 2011, while in the worst-case scenario it was 26.49% (95% CI 22.91-30.35) in 2008 and 28.44% (95% CI 21.93-35.65) in 2011.

Given the widespread detection of Bd across the UK, but the relatively low percentage of Bd-positive sites, disseminating good biosecurity practices among citizens who visit ponds could help limit the further spread of the pathogen. These results demonstrate the potential impact of active citizen involvement in resource-intensive studies and highlight the need to investigate the factors affecting the distribution of Bd in the UK. The employed Bayesian hierarchical model represents a versatile framework that can incorporate new data as more studies become available.

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Understanding avian influenza dynamics in wild birds and mammals: A One Health approach

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Abstract

The current epidemic of highly pathogenic avian influenza (HPAI), specifically the A H5N1 clade 2.3.4.4b variant, has profoundly impacted global wildlife, particularly in Europe. While wild birds, especially waterfowl, have traditionally been the primary carriers, this study investigates the involvement of wild mammals in the transmission dynamics of avian influenza. We analyze characteristics and transmission patterns of HPAI in wildlife, pre and post-2020, within the framework of One Health and surveillance strategies.

Drawing upon data from 10,690 HPAI outbreaks reported to the World Organization for Animal Health (WOAH), including 10,392 in wild birds and 298 in wild mammals, we conducted geospatial analyses to discern changes in outbreak distribution. Most of these outbreaks mainly happened in the Americas, mainly impacting wild carnivorous and scavenging mammal groups.

Our findings indicate an increase in outbreaks, particularly in Western Europe and North America, impacting predominantly wild carnivorous and scavenging mammal groups.

Hotspot analysis revealed areas of significant clustering of outbreaks, elucidating distinct geographical patterns in disease occurrence. Kernel density analysis identified regions with high-density outbreak occurrences, notably emphasizing clusters in Western Europe. Proximity analysis uncovered potential transmission routes between bird and mammal populations.

Our results underscore strong connections between bird and mammal outbreaks, particularly in coastal and wetland environments. Vultures emerged as potential carriers between species due to their scavenging habits, while corvids exhibited clear links to mammal outbreaks, suggesting intricate transmission dynamics.

Increased monitoring in mammalian populations is imperative to prevent interspecies spillover events and protect public health. Stringent protocols are essential to curb disease spread among domestic bird populations, mitigating risks to the poultry industry and broader conservation objectives. This study enhances scholarly understanding of avian influenza dynamics, emphasizing the importance of One Health frameworks in wildlife disease surveillance. It provides vital insights for public health initiatives, species conservation endeavours, and the strategic management of avian influenza outbreaks in the poultry industry.(ILINK22023).

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Research cancer in wild fish... leads to an evaluation of parasitism

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Abstract

Two different research studies, one concerning gudgeons and common chubs, the other one concerning European eels, were conducted in the south of France at different times in order to detect tumors/cancers in these wild fish living in different environments (more or less polluted). These research studies were part of larger studies working on these species and environments. No cancer was found macroscopically and microscopically. However, in gudgeons and common chubs, granulomatous coelomitis, pancreatitis and enteritis, associated to parasites (coccidia, metazoans) were noted in 10% to 45% of animals; in many European eels, granulomatous lesions were noted in oropharyngeal and digestive tissues and associated to metazoan parasites, as well as some severe infections by *Anguillicola crassus* noted in swimbladder. In some cases, parasitism was severe enough to affect the life of these animals, particularly in endangered European eels. More studies are necessary to explain the origin, expansion and impact of parasitism in these wild fish.

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Avian Borna Virus Detection in Free-Living Mute Swans (Cygnus olor) in Slovenia

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Abstract

Background. Avian borna virus or ABV is etiological agent of a common psittacine disease, proventricular dilatation disease (PDD) and of clinically and

morphologically similar diseases in some wild bird species (1). **Objective.** The aim of our study was to further investigate the presence of ABV in free living mute swans (*Cygnus olor*) in Slovenia. **Materials and methods.** A total of 146 tracheal and 157 cloacal swabs from 250 wild mute swans found dead were tested. For the detection of ABVs, RT-PCR was used. **Results.** ABV nucleic acid was detected in 5 swans (5/250 (2 %)). In two birds both tracheal and cloacal swabs were positive, in three birds only tracheal swabs were positive, respectively. Through sequencing, the ABBV-1 genotype belonging to the Waterbird 1 orthobornavirus species was identified in ABV-positive mute swans. **Conclusions.** In the present study, we determined the low prevalence of ABVs (5/251 (2%)) in free-living mute swans in Slovenia. However, further studies are needed to investigate if infection can lead to clinical condition.

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Refining iDNA as a tool for biodiversity monitoring and pathogen surveillance

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Abstract

Anthropogenic disturbance is currently causing a dramatic local and global decline of biodiversity. Although the dilution effect remains the subject of controversial debate, there is increasing evidence indicating a link between biodiversity loss and the emergence of infectious diseases. Tropical rainforest ecosystems are biodiversity hotspots and simultaneously harbor important zoonotic agents. The rainforest of Taï National Park in Ivory Coast represents such a dynamic interface, where an anthrax causing agent, *Bacillus cereus* biovar *anthracis* (Bcbva) is causing a significant amount of the wildlife mortality. Increasing human pressure, coupled with climate change, means these dynamic human-wildlife interfaces represent a poorly understood potential hotspot for disease emergence, where new tools are desperately needed to

provide new insights. Here, we leverage the fact that the metabarcoding of invertebrate-derived DNA (iDNA), which can be used to describe vertebrate diversity, can be combined with the detection of pathogens from these same flies. By combining these data streams, fly iDNA can contribute to the understanding of the ecology and distribution of novel pathogens or emerging infectious diseases. We simultaneously surveyed biodiversity and monitored the extent of Bcbva in Taï National Park, at the edges of the forest and in the surrounding villages, by extracting fly iDNA from 500 flies (divided into 100 fly pools). We used metabarcoding for biodiversity assessment and qPCR for Bcbva detection. iDNA generated data about the mammal and fly communities in these habitats, revealing the highest mammal diversity in the forest and considerable changes in fly community composition along the gradient. The generated mammal species accumulation curves reached a plateau in the village-periphery; however, the forest and edges accumulation curves indicated that only a fraction of the mammal community was reconstructed. Bcbva host range estimates from fly iDNA were largely identical to the results gained from long-term carcass monitoring efforts in the region. Bcbva was detected at the forest edge (1/50 pools) and in pristine forest sites (4/25 pools), but all pools from the villages were negative. We are now exploring the number of flies needed to better characterize the mammal communities in these biodiversity hotspots, while also improving cost efficiency by using massive pooling strategies (159 pools, 6392 total flies). Additionally, we want to determine whether Bcbva absence around villages can be confirmed. Results support the use of fly iDNA as a cost effective and efficient tool for measuring biodiversity and studying the disease ecology of some pathogens.

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Spatio-temporal structure of tularemia data on the European hare (Lepus europaeus) species produced in France as part of the SAGIR network from 1993 to 2022

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Abstract

Tularemia is a severe bacterial zoonosis caused by the highly infectious agent Francisella tularensis (Ft). This bacterium, endemic in many countries of the northern hemisphere, can infect a wide range of wild animals. There remain numerous knowledge gaps regarding human exposure.

Among wildlife, the European hare (Lepus europaeus) is highly susceptible to Ft with infection often leading to fatal septicemia. It thus questions its potential role as a natural reservoir. As it is a common game species in Europe, with a limited home range, we used it as a model species to better understand epidemiological cycle of Ft.

In France, a participatory network named SAGIR carries out event-based surveillance of wildlife diseases mainly thanks to hunters and environmental technicians of the National Agency for Biodiversity (OFB). Dead animals collected opportunistically are sent for gross pathological examination to a local laboratory, without standard subsequent testing protocols.

We examined the data collected by this network over 30 years with an exploratory approach, to identify spatial and temporal clusters of positive Ft results and draw hypotheses regarding environmental risk factors for the infection.

Our variable of interest was the result of the lab test carried out on hares collected by the SAGIR network to detect Ft. We focused on regions where the tests have been performed systematically. Analyses were performed both at the regional (ca. 25 000 km²) and departmental (French administrative units, ca. 5 000km²) scales. We highlighted spatial clusters of tularemia cases by mapping the estimated peak areas with a kernel approach. We used generalized linear models to assess the correlation of several landcover variables (density of cereals, wetlands, density of forests, etc.) and seasonality with the probability that a carcass found by the network was positive for Ft.

Our analysis identified distinct areas characterized by clusters of tularemia and revealed inter-departmental heterogeneity of correlation with environmental and/or seasonal factors.

Our findings suggest that the the European hare is part of different, regionally-specific epidemiological cycles, involving or not vector-borne transmission. Future efforts should focus on mitigating data collection biases through more systematic surveillance and incorporating additional relevant variables into modeling approaches.

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Health Surveillance of the Common hamster in the Wild: Using Photo-Trapping for Syndromic Surveillance

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Abstract

In France, the common hamster (*Cricetus cricetus*) is a critically endangered protected species. The primary causes of this decline include intensive monoculture practices, industrial development, climate change, and light pollution, all of which significantly affect the species' reproduction. Although diseases in this species are poorly documented, they may have a substantial impact on population dynamics, especially when populations are small and fragmented. Therefore, early detection of emerging health issues in wild hamster populations is crucial.

Being a small species that lives in burrows, traditional surveillance methods based on mortality detection are challenging to apply to the common hamster. To address this, we have developed an innovative method for early detection of chronic or subacute diseases in wild hamster populations using opportunistic photo-trapping.

By analyzing photographs collected from 2010 to 2023 (sourced from various monitoring projects and studies), and classifying them by year, season, municipality, and burrow, we were able to determine the spatiotemporal evolution of burrows housing individuals with lesions. Our results indicate that skin lesions, ocular lesions, and low body condition scores are the three most frequently observed types of lesions. Over the entire study period, no significant difference was found between areas populated by hamsters from captive breeding and those with wild populations (p = 0,09). However, 2021 showed a significantly higher proportion of positive events compared to other years (p = 0,01), spatially correlated with the release of captive-bred hamsters, though this observation remains unexplained to date.

Photographic analysis conducted by a biologist demonstrated a satisfactory analytical capability (with approximately 80% accuracy in identifying lesions), which supports the expansion of this method to a larger scale.

While further refinement of protocols and integration of this method into biologists' routine practices are needed, these promising results highlight the benefits of syndromic surveillance for the conservation of the common hamster.

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Molecular survey of adenovirus and herpesvirus in wild Iberian wolves (Canis lupus signatus) of Cantabria, northern Spain.

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Abstract

Infectious diseases are significant threats to wild carnivores; thus, their study is essential to carnivore conservation. Adenovirus and herpesvirus are double-stranded DNA viruses able to cause severe disease in carnivores; however, data about their occurrence in Iberian wolves (*Canis lupus signatus*) are limited. Our goal was to molecularly survey adenovirus and herpesvirus in wild Iberian wolves. We tested 119 Iberian wolf spleen samples for adenovirus and herpesvirus, using two broad-spectrum nested PCR protocols to amplify the DNA polymerase gene (215-315 and 224 bp), respectively. All positive amplicons were confirmed by direct sequencing. Samples were obtained during the necropsies of road-killed, dead, or legally hunted wolves in Cantabria, Spain (2017-2021). All procedures were authorized by the Government of Cantabria. No wolves were positive for herpesvirus. Overall, four out of 119 (3.4%) were adenovirus-positive, sampled in 2018 (n=2), 2019 (n=1) and 2020 (n=1). All of the obtained adenovirus sequences were identical to several Canine mastadenovirus 1 sequences identified in a dog (*Canis lupus familiaris*) of Japan, in wolves of France and Italy, and in an Indian jackal (*Canis aureus indicus*) of India. The prevalence observed in Cantabria is apparently lower than the one previously described in Iberian wolves of the region

of Asturias (14%, 21/149), and in wolves of Asturias and Galicia (70%, 38/54), Spain. Canine mastadenovirus A is the causative agent of infectious canine hepatitis, characterized by lesions such as necrotizing hepatitis, vasculitis and disseminated intravascular coagulation, which can lead to encephalitis and glomerulonephritis. This disease is especially important in cubs and can affect endangered carnivores, like the Cantabrian brown bear (*Ursus arctos pyrenaicus*). This study broadens the knowledge of the adenovirus geographic range in Iberian wolves. Further studies are required to assess the presence of herpesvirus and adenovirus in sympatric dogs of Cantabria in order to elucidate a possible co-circulation of these agents in dogs and wolves.

CS, IS and ACE receive a Juan de la Cierva Incorporación (#IJC2020–046019-I) and a Juan de la Cierva formación (#FJC2020-046311-I and #JDC2022-048632-I) fellowships, respectively, granted by AEI/MICIN.

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Reflections from the field on including histopathologic investigation in the diagnostic chain of the event-based wildlife health monitoring program in French National Parks

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Abstract

In 2016, several French terrestrial National Parks committed to developing a unified health surveillance strategy for wildlife in their territories. A seminar in 2017 involving a panel of international wildlife pathology experts and representatives from the French National Parks and the French Biodiversity Office discussed challenges faced by the health monitoring network and called for a change in the diagnostic chain, including exhaustive histological investigation during necropsy, to enhance the sensitivity and specificity of methods for detecting emerging wildlife diseases.

In 2021, finances were obtained and a large-scale histopathologic study was implemented in the event-based monitoring of wild animal mortality in protected natural areas. A triage protocol of carcasses taking into consideration remote locations and access limitation to necropsy facilities was elaborated. A histological sampling protocol was established and shared with each regional veterinary laboratory that routinely performed the necropsies for the respective parks. Training sessions were implemented to sensitize field officers to the use of histology, the importance of transferring carcasses in a timely manner with limited use of freezing and train them in field sampling methods.

A total of 98 requests for histological analysis were received over the course of two years. The state of preservation of the tissues on histology was graded from good to severely altered and a discrepancy was noted with the gross evaluation of the conservation state of the carcass. Seventy-three individuals were frozen before examination and mostly showed marked to severe histologic alteration. Despite recommendation to systematically sample and submit the brain, it was analysed in 15/98 animals only. Histological examination revealed lesions in most cases (n = 90). These made it possible to corroborate the cause of death established during necropsy; diagnose the animal's cause of death when this was not possible during necropsy; refine understanding of the pathological process that led to the animal's death; or to detect lesions involved in pathological processes other than those directly implicated in the animal's death, whether major or minor co-morbidities. Amongst the notable findings, six Alpine ibex showed systemic amyloidosis which was characterized as AA amyloidosis via immunohistochemistry. One also showed pulmonary histologic lesions compatible with caprine arthritis and encephalitis virus infection, confirmed by PCR.

This project demonstrated the difficulties of implementing systematic histopathological analysis in the context of remote mountainous territories. Major setbacks were the state of preservation of the tissues, incomplete data collection and partial sampling, particularly of the brain.

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Ecoepidemiology of Avian Influenza and Flaviviruses in Greek Dalmatian Pelicans and Great White Pelicans after the 2022 H5N1 Avian Influenza Outbreak

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Abstract

The ongoing highly pathogenic H5N1 avian influenza virus HPAIV 2.3.4.4b panzootic has severely impacted many endangered avian species including the Dalmatian pelican (*Pelecanus crispus* DP). In spring 2022, more than 60% of the Dalmatian Pelicans at the breeding colonies in the Lake Lesser/Mikra Prespa died. In an unprecedented study we combined samples from DP and lesser affected great white pelican (GWP *Pelecanus onocrotalus*), ambient and sympatric waterfowl samples to study potential routes of exposure and to understand the difference in impact on the two pelican species. Specifically we collected samples from DP (n=20) and GWP (n=11) nestlings hatched at Lesser Prespa lake in 2022, immature (n=4) and adult DP (n=17) captured for radiotagging in Kerkini National Park in February 2023 and samples from DP (n=72) and GWP (n=39) nestlings hatched in 2023 at four different colonies. Also nesting material from islands affected during the 2022 outbreak (n=34) and freshly deposited faecal samples from pelicans and sympatric waterfowl (geese, cormorants, mute swans, n=98) were collected just prior to the 2023 breeding season. Ambient and faecal samples were tested for AIV by generic matrix gene qRTPCR while samples collected from pelicans were tested for AIV antibodies and RNA as well as for exposure to Flaviviruses.

None of the individuals sampled in both years tested positive for HPAIV H5 by qRTPCR. Avian influenza antibodies directed at subtypes other than H5 were detected in 2/20 (10%) nestling DP hatched after the 2022 outbreak. Among adult and juvenile DP captured for radiotagging in early 2023 23.8% (5/21) individuals had antibodies against H5 AIV, including two individuals with blurry corneas consistent with lesions described In H5N1 infection surviving waterfowl. All nesting material and faecal samples tested negative for AIV. In addition 48% (10/21) adult DP had antibodies against WNV or a cross-reacting Flavivirus, while among

nestlings antibodies were only detected in colonies sampled in and after may 2023, and then significantly more GWP 23% (9/39) than DP 2.2% (1/45) tested positive.

Information obtained to date suggests that exposure is likely key to mortality from HPAIV H5 in DP, and that the virus was not circulating in the DP breeding colonies during 2023. It also provides some circumstantial evidence for potential survival of infected individuals and shows differential exposure to Flaviviruses of GWP and DP.

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Outbreaks of human respiratory syncytial virus in wild, human-habituated gorillas highlight the importance of adopting hygiene measures for risk mitigation

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Abstract

Human respiratory pathogens have been inadvertently transmitted to wild non-human great apes (NHGA) habituated to human presence for research and tourism on multiple occasions and pose a threat to species survival. To mitigate the risk of pathogen transmission at great ape conservation and tourism projects, specific hygiene measures have been developed. These range from wearing surgical masks and maintaining a minimum distance when observing wild animals, to undergoing a 5-day quarantine prior to entering NHGA habitats. These measures have had a concrete effect on reducing human-introduced respiratory outbreaks, but are enforced differently among sites (e.g. depending on infrastructure and site activity) and pathogen introduction may still occur. Implementing longitudinal health monitoring programs of habituated NHGA

represents an essential tool to evaluate the adopted hygiene measures, meet best-practice certification standards and identify areas of improvement.

Here we describe three outbreaks of human respiratory syncytial virus (RSV) type A and B in neighboring groups of wild, human-habituated western lowland gorillas (*Gorilla gorilla gorilla*) in the Dzanga-Sangha Protected Areas (DSPA), Central African Republic, and the Nouabale-Ndoki National Park (NNNP), Republic of Congo, in 2019. At these research sites, gorillas are followed on a daily basis by researchers and are recognized individually. Respiratory signs, such as coughing and sneezing, were observed in September 2019 in 11 individuals of the Mayele gorilla group in the DSPA. In November and December, 14 individuals in the Kingo and Loya gorilla groups within the NNNP showed similar signs. Fecal samples were tested for major respiratory viruses, revealing two positives from DSPA for RSV type A and four positives from NNNP for RSV type B. By implementing target enrichment and high-throughput sequencing, nearly complete viral genomes were obtained from 2 out of 3 outbreaks. Phylogenetic analyses showed that the identified RSV A and B nest within the diversity of human RSV strains.

These outbreaks confirm once again that seasonal human viruses can easily spillover to wild NHGA. Further to the threat this poses to great ape survival, the recent transmission of SARS-CoV-2 from humans to distinct animal species highlights the risk of creating new reservoir hosts where viruses may take distinct evolutionary trajectories. With the ongoing circulation of SARS-CoV-2 alongside seasonal respiratory viruses, great ape research and tourism projects should maximize the efforts toward a sustainable and vigilant implementation of the recommended hygiene measures, which play a key role in prevention.

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Necropsobacter rosorum - A Potential Zoonotic Pathogen Emerging in Nutria (*Myocastor coypus*)

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Abstract

Background: Pasteurellales bacteria encompass diverse species, some known to be zoonotic. However, limited knowledge hinders effective disease surveillance and control efforts.

Objectives: This study investigates a fatal pneumonia case in a nutria (*Myocastor coypus*) caused by missderstood bacterium: *Necropsobacter rosorum,* a gram-negative facultative anaerobe Gammaproteobacteria. We aimed to identify and characterize the isolated bacterium, assess its pathogenic potential and gain insights into the prevalence of *N. rosorum* in humans.

Materials and methods: In 2022, the corpse of one nutria was discovered on the floor of the Borely Park in Marseille (France). The bacterium was isolated from lung tissue and subjected to genomic characterization. This included phylogenetic analysis, virulence gene identification, and comprehensive resistance profiling to evaluate its susceptibility to several antibiotics. Furthermore, we investigated the presence of a CRISPR-Cas system and prophage regions. Finally, a custom script analyzed publicly available genetic data on human Pasteurellales isolates, providing valuable insights into the global prevalence of *N. rosorum* in humans (brain abscess, septicemia and cutaneous lesions).

Results: The nutria's external appearance was unremarkable, with no visible signs of trauma. While the internal examination revealed severe acute pneumonia. The lungs displayed miliary lesions, characteristic of widespread bacterial infection, accompanied by purulent pleurisy. Upon opening the lung abscesses, yellowish pus oozed out. Furthermore, fibro-serous deposits formed flanges around the lungs, and the pleura adhered in some areas to the chest wall. The extent of these lesions suggests the cause of death to be natural. Microscopiccally, the lung tissue revealed widespread necrotizing inflammation within the pulmonary alveoli. This finding supports the diagnosis of severe pneumonia caused by *N. rosorum*. Phylogenetic analysis confirmed the bacterial identity as *N. rosorum*, as well as the pangenome analysis wherein the identity of 99% with the previously strain isolated from human. The virulence profile resembled that of the established zoonotic pathogen, *Pasteurella multocida*. The bacterium exhibited resistance to various antimicrobials and harbored a CRISPR-Cas system and prophage regions. Notably, the data analysis revealed only seven human *N. rosorum*isolates worldwide, highlighting the critical gap in knowledge regarding this zoonotic agent.

Conclusions: This study identifies *Necropsobacter rosorum* in nutria, highlighting its close relatedness to aquatic media, and raising concerns about its potential to spillover to humans and impact public health. The limited data on this bacterium underscores the importance of strengthening ecosystem health and disease surveillance efforts within the One Health framework.

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A longitudinal study of the resistome of wild chimpanzees in Tai National Park, Côte d'Ivoire.

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Abstract

Background:

Infections caused by antimicrobial resistant (AMR) bacteria have become an acute global public health problem: they cause reduced effectiveness of antimicrobial treatments, and increase the severity of infections, associated complications and mortality. The WHO recognizes AMR as one of the greatest threats for global health. The widespread use of antibiotics in human and veterinary medicine has led to a dramatic enrichment and transmission of antibiotic resistance genes (ARGs) between environmental, commensal and pathogenic microbes from diverse habitats.

The increasing fragmentation of natural habitats and the growth of the human population inevitably force wild animals to come into greater contact, directly or indirectly, with humans, increasing the possibility of the transmission of AMR in both directions, and antibiotic-resistant bacteria are increasingly reported in wildlife. However, data on AMR in wild chimpanzees, our closest relatives, is extremely limited. Chimpanzees are susceptible to a similar range of microorganisms, and may serve both a primary and a secondary reservoir for AMR. Objectives
Here, we study ARG in feces of wild chimpanzees collected over a period of 10 years to better understand the diversity and potential spread of ARG in our closest relatives,

Materials and methods

We tested snap frozen feces samples collected from four groups of individually know wild chimpanzees at the Tai National Park in Côte d'Ivoire between 2001 and 2017. We randomly selected 10 samples of 10 individuals per group and year, and pooled DNA extracts. We performed in-solution hybridization capture with RNA baits based on ARG from the CARD database, and sequenced the enriched libraries on an Illumina MiniSeq.

Results

Based on Presence/Absence on the three different sites, we found a certain number of resistance genes, depending on the gene fraction represented and the hits chosen at random. Thus for a gene fraction of 90% coverage and a number of hits greater than 10 we obtained the genes such as: *rmtG, AAC(6'), ANT(3''), A 16S, rrsA, bacA, CblA, cepA, AmpC, QnrB, Sul1, Tet(X), dfrB* and *DHFR etc.* The most abundant genes are the PMRAR, EPTA and PMRF genes from the Pmr gene family (phosphoethanolamine transferase), followed by the HNS genes (efflux pumps_RND_multidrugs), the RMTF and RMTG genes from the 16S_rRNA_methyltransferase family, and finally CBLA and CEPA

Conclusions

The main aim of our work is to compare the dispersal of the resistome between the same species at different sites or between different species through our research at partner sites.

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First detection of Cervidpoxvirus in Europe – disease events among Eurasian tundra reindeer (*Rangifer tarandus tarandus*) in Norway and Sweden

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Abstract

Background: Reindeer herding, based on semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*), is a primary livelihood and cultural cornerstone for indigenous Fennoscandian Sámi people. Norway and Sweden hosts around 455,000 semi-domesticated reindeer. *Cervidpoxvirus* (CPV, family *Poxviridae*), was previously detected in reindeer at Toronto Zoo, Canada, two years after importing reindeer from Finland. CPV has also been found in other Capreolinae species in North America causing lesions on the eyelids, lips, muzzle, vulva, and legs, and corneal ulceration, conjunctivitis, depression, reduced appetite, fever, and mortality. In 2018, Swedish reindeer were observed with lesions around eyes and genitalia and the number of animals showing clinical symptoms has increased, year on year, in some areas. In 2022, an outbreak occurred in a small Norwegian herd.

Objective: This study documents the first identification of CPV in Europe, as a causative agent of disease among reindeer in Norway and Sweden.

Materials and methods: Clinical signs, necropsy findings, and histopathology were documented during disease events. Swab samples from lesions underwent shotgun sequencing using Illumina technology. A draft genome was generated by *de novo* assembly. A qPCR assay was designed and used to select samples for sequencing and to investigate samples from a broader geographical area. Madin-Darby bovine kidney cells were inoculated with lesion material and cytopathogenic effects (CPE) evaluated.

Results: Clinical signs included lesions and crusts around eyes and genitalia, and in the ears, redness and lesions in the nostrils, enlarged lymph nodes, lethargy and weight loss. Some showed clinical signs for 4-6 weeks before recovering. Necropsy and histology findings included fibrinopurulent crusts around the eyes and vulva with intra-epidermal pustular dermatitis, enlarged lymph nodes with oedema, and swollen liver and spleen. *De novo* assembly yielded a draft genome of 163 Kbp in three contigs with 174 genes. This genome is related to CPV from white-tailed deer, but not identical. Cell culture showed CPE after 3 days. CPV was found in two herds in Norway and three in Sweden with qPCR, but clinical observations indicate wider dissemination.

Conclusions: CPV has emerged as a new pathogen among reindeer in Norway and Sweden and this is to the best of our knowledge the first identification of a CPV outside the Americas. Whether CPV has previously circulated and caused disease in these herds is unknown. These events warrant further investigations on the distribution, prevalence and epidemiology of CPV, including host specificity and zoonotic potential.

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Transmission of Mycobacterium bovis from experimentally infected ferrets to in-contacts for 9 months

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Abstract

Bovine tuberculosis is a World Organisation for Animal Health (WOAH) listed disease, subject to mandatory reporting/control throughout Europe. Among the preventive actions required to reduce the transmission of tuberculosis from the environment are the development and implementation of vaccination strategies in infected wildlife, such as badgers (*Meles meles*) in Europe. Oral BCG and killed *M. bovis* have provided some evidence of reducing the development of visible TB lesions in vaccinated badgers compared with controls experimentally challenged with *M. bovis*. However, screening oral vaccine candidates against TB in experimental conditions is challenging in wild caught badgers. Ferrets (*Mustela furo*) are commercially available mustelids that develop macroscopic and microscopic lesions and IFN-g/serological responses similar to those seen in the wild and captive badgers after *M. bovis* tracheal challenge (UK and Irish strains used in badger experimental studies). Such protocols, with large dose of artificially grown bacilli, induce accelerated lesion development in all controls which is not representative of natural TB and may mask the real protective effect of vaccines. A study was therefore conducted to explore the direct transmission of *M. bovis* through direct contact between four experimentally infected ferrets and six naïve animals for 9 months (group composition repeated in four separate cohorts). As in previous studies, the experimentally challenged ferret groups developed TB typical granulomatous lesions and peripheral immune responses. In contrast, most of in-contact animals did not develop detectable lesions and *M. bovis* was rarely isolated in tissues at post-mortem. However, antigenic specific immune responses were measured in most in-contact ferrets, suggesting exposure to *M. bovis*. Further analysis is required to identify blood biomarkers associated with longer-

term disease development in ferrets, and to test if those could be reduced in protected vaccinated ferrets/badgers.

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Safety in the Game Meat Chain – A pan-European Approach

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Abstract

Compared to farmed animals, game is reared in the wild where it feeds mainly on natural food sources, but also on densely cultivated farmland and on feed provided by hunters. This also means that they may be exposed to various environmental contaminants. In addition, wild animals have the potential to act as vectors for the transmission of zoonotic pathogens. It is estimated that 7 million people in the EU engage in hunting, a practice that is widespread in most European countries. However, scientific knowledge on the safety of game meat and the game meat production chain in Europe remains scarce. Despite the relatively modest size of the game meat market compared to that of livestock meat, the European Rapid Alert System for Food and Feed records a notification almost every month.

The COST Action CA22166 Safety in the Game Meat Chain (SafeGameMeat) takes a transnational and multidisciplinary One Health approach to promote and harmonise food safety standards in the context of a growing European game meat market. From September 2023 to September 2027, the network's five working groups focus on various specific issues related to the safety of game meat in the countries and throughout the production chain, from the forest to the consumer. The objective of Working Group (WG) 1 is to gather information on the various practices related to hunting and training, as well as the national legal frameworks for meat inspection. WG 2 focuses on the trade networks and consumption habits associated with the game meat chain. WG 3 is responsible for the identification and assessment of emerging and known chemical risks, with a particular focus on ammunition residues and environmental contaminants such as PFAS. WG 4 will

identify and assess biological risks, including zoonotic pathogens and hygiene risks associated with game meat. The main objective of WG 5 is to disseminate the results of the Action through various forms of networking, including webinars, workshops, and conferences.

The network, which is still growing, currently has over 170 members from 42 different countries, including not only EU Member States but also non-EU countries such as the Western Balkans, Turkey, the United Kingdom, Iceland, the United States of America, Morocco, Switzerland, Nigeria, Tunisia, Zambia and Australia. For more information, please visit www.safegamemeat.eu or contact us at safegame@bfr.bund.de.

European Wildlife Disease Association

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