

# 13<sup>th</sup> CONFERENCE

## European Wildlife Disease Association

27-31 August, 2018  
Larissa, Thessaly, Greece



University of Thessaly  
School of Health Sciences  
Faculty of Veterinary Medicine



**“Wildlife Health and Conservation:  
Expectations in a challenging era”**



# Abstracts Book



**EWDA 2018**  
**13<sup>th</sup> International Conference**

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**Wildlife Health and Conservation:  
Expectations in a challenging era**

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## ***Keynotes***

### **How do we get action for a healthier planet? The lead ammunition case study**

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***Keywords:*** *Global health, lead poisoning, ammunition*

Global health over the last century has shifted from one characterised by the burden of infectious diseases and under-nutrition to one of the vast array of health challenges posed by globalisation, food production systems, climate change, loss of biodiversity and extreme disruption of ecosystems. Despite this we are arguably better prepared than ever to tackle these challenges with technologies and advances in medical and veterinary medicine together with broader improvements in standards and preventative approaches, excellent communications and knowledge transfer. Typically as a health community we begin with evidence and develop appropriate responses from there. However, recent times show us that facts and evidence are not necessarily as influential as they once were. Furthermore if the solution to your health problem challenges big industry then science alone is irrelevant. Lead poisoning from ammunition sources has no doubt been going on since the first shot were fired from 18th century guns. What has changed is our appreciation of its multiple impacts on wildlife, soils and importantly human health for those consuming game meat which we now know is (not surprisingly) contaminated with this toxic non-threshold substance. The development of non-toxic ammunition provides a tantalisingly straightforward solution – no-one needs to stop what they are doing, and the benefits are manifold, not just for health but also for the image of shooting in a modern society. Great advances have been made in realising a non-toxic future but biology and evidence alone are not convincing and do not bring about behaviour change. This issue typifies many environmental problems relating to human behaviour and also business. The problem and solutions for lead poisoning from ammunition lie squarely in the arena of socio-politics and our training does not necessarily equip us for this. ‘Carrots’ of persuasion and ‘sticks’ of regulation are both needed. The solutions lie in understanding the human dimensions of the problem, appreciating the social perspectives and motivations of the multiple stakeholders, mapping where power lies and overcoming barriers to change. We have to truly work across the disciplines, in partnerships, understanding our audiences and creating engaging advocacy tools and approaches. Wider than lead ammunition, we have the concept of ‘health’ on our side which when applied in a ‘systems thinking’ approach to managing practices throughout society should be a very attractive policy proposition. How we ‘sell’ this is our challenge. Whilst we need to trust that faith in evidence will endure, we need to ensure our collective voices and perspectives are heard and in a persuasive manner which will only be achieved by imaginative transdisciplinary working.

## Wildlife and Vector-borne diseases in Europe

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**Keywords:** *Vector-borne diseases, Europe*

Vector borne diseases are transmitted by arthropods vectors such as ticks, mosquitoes, sandflies or biting midges (culicoides). Many of these diseases which affect humans and/or domestic animals involve wildlife as a reservoir or amplifying host or carrier of infected vectors (e.g. in Europe: West Nile, Tick-borne encephalitis, Crimean-Congo Haemorrhagic fever, borreliosis, anaplasmosis...). However, the role of wildlife in the transmission cycle of these diseases is sometimes poorly recognized or only suspected (e.g. Sandfly fevers). Sometimes wildlife appears to participate to the cycle of transmission of diseases at the same level than other animals (e.g. Bluetongue, Schmallenberg, ...). For some diseases like tularaemia, wildlife is the reservoir and source of infection of sensitive hosts; in another hand arthropod vectors (mosquitoes, ticks...) are involved in the transmission cycle in northern Europe. African Swine fever, an emerging disease which spread in Europe affect wild boars and pigs. Wild animals participate to the dissemination of the disease by contamination of the environment. Soft ticks *Ornithodoros* sp are considered as the reservoir of disease in southern Africa; the role of these long living ticks in the maintenance of the disease is only suspected in Europe. Many vector-borne diseases are considered as emerging threats in Europe; they require an integrated surveillance in humans and animals including wildlife, as well as an entomological (distribution, abundance and seasonality of vectors and biting habits...) surveillance and an close environmental monitoring. Long-term investigations using One Health Observatories in various ecosystems are global approaches to better understand the involvement of each actor in the transmission cycles of these infectious diseases in a changing environment, to model the risks of emergence and spread or disappear, strengthen effective and preventive control measures according to different local contexts.

## **Current epidemiological data for Rabies in Greece. Steps to eradicate the disease**

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**Keywords:** *epidemiological, Rabies, Greece*

Following Rabies reoccurrence in animals in Greece in 2012, this zoonoses still remains a priority for the Greek Veterinary and Public Health Authorities. Until May 09th, 2014, 48 animals, mostly wildlife, were laboratory tested and confirmed positive in Lyssavirus infection, at north-western and central part of the country. Passive surveillance rates were not considered satisfactory during the years 2014-2015, however these rates were significantly improved the period 2016-2018, attributed mainly to the remuneration provided to the hunters when delivering dead foxes for the needs of the programme. Since October 2013, eight Oral Rabies Vaccination (ORV) campaigns of red foxes (*Vulpes vulpes*) were conducted, by aerial distribution of baits-vaccines for wildlife immunization, in order to halt the spread of the disease. The laboratory results obtained by animal samples collected following especially autumn vaccination campaigns, revealed high seroprevalence and tetracycline detection rates (vaccine-baits intake), while these rates were significantly reduced following spring campaigns. The importance of surveillance of Rabies has been recognized in Greece and constitutes the main tool for the epidemiological investigation of the disease. Along with effective implementation of other control measures, it may allow the country to regain Rabies-free status in the near future.

## *Oral presentations*

### **British Veterinary Zoological Society (BVZS) Good Practice Guidelines for Wildlife Rehabilitation Centres**

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**Keywords:** *Rehabilitation, Regulation, Standards*

There are approximately 80 wildlife rescue centres in the United Kingdom (UK) dealing with an estimated 30-40,000 animals per annum, although true numbers may be double this figure. Unlike other countries, including Australia, New Zealand, North America and elsewhere in Europe, where some statutory framework exists, there is currently no licensing of wildlife rehabilitation facilities or practices in the UK. Instead, the legal management of indigenous wildlife in captivity relies upon the application of legislation controlling animal welfare (Animal Welfare Act, 2006), medicines (Veterinary Medicines Regulations, 2013) and veterinary procedures (Veterinary Surgeons Act, 1966). This lack of formal regulation of wildlife rehabilitation and wildlife centres can result in poor animal welfare. The availability of facilities, suitably-trained personnel, and funding have all been shown to be limiting factors in the success of wildlife rehabilitation. A good working relationship between lay rehabilitators and veterinary surgeons has also been found to be an essential part of any successful rehabilitation program. Unfortunately, these relationships can sometimes be poor, with veterinary professionals often criticised for a lack of knowledge and interest in wildlife, and disagreements arising when emotive subjects such as euthanasia are discussed. Within the wildlife veterinary community there are concerns regarding the variable standards of veterinary care within rehabilitation centres, which vary from full time on-site veterinary provision to no veterinary involvement beyond the supply of medicines. Areas such as patient records and post-release monitoring are of particular concern. Such concerns are sadly supported by an increase in the number complaints by members of the public to the Royal College of Veterinary Surgeons (RCVS) in recent years relating to the poor treatment of wild casualty animals. In an attempt to promote better standards for both wildlife centres and veterinary surgeons, the British Veterinary Zoological Society (BVZS) has published Good Practice Guidelines for Wildlife Centres. In writing the Guidelines, a range relevant reports and standards from other organisations were reviewed and incorporated. The Guidelines also followed a similar layout to the UK Zoo Licencing Act, 1981, in order to make them comparable to legislative controls. The Guidelines followed and incorporated the 2014 BVZS Guidelines for the prescription, supply and control of prescription only veterinary medicines (POMs) in zoological collections and wildlife rescue centres. Other subjects covered by the Guidelines include; animal accommodation, feeding, record keeping, safety and security, veterinary care, release and post release monitoring, staff education, ethical review. The Guidelines were reviewed by the British Wildlife Rehabilitation Council (BWRC) and have been promoted via their website, newsletter and conferences. The Guidelines have also been approved and promoted by the RCVS, British Veterinary Association (BVA) and Veterinary Medicines Directorate (VMD). It is hoped that these Guidelines will improve standards of indigenous wildlife care, in both veterinary practices and wildlife rescue centres in the UK and may be useful to wildlife veterinary surgeons elsewhere.



## OIEparser: The data management tool for OIE-listed diseases

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**Keywords:** database, epidemiology, notifiable diseases

Wildlife disease management requires access to large amounts of relevant disease information, including date, duration and geographical location of the outbreak, severity of the disease and species affected. The World Organisation for Animal Health (OIE) oversees a list of 117 notifiable terrestrial and aquatic diseases. Outbreaks of these diseases must be reported due to specific hazards or importance in international trade. The reported events are accessible to the public in the form of individual pdf documents at the OIE's World Animal Health Information System (WAHIS). While these reports carry important disease information, up to this point WAHIS could not be searched as a database to give researchers easy access to disease statistics over a set time period or regarding one specific disease.

Our objective was to create an easy-to-use R package which collects data in the interest area of the researcher and facilitates further use for statistical analysis and incidence mapping.

The protocol in case of the OIE-listed diseases is to file a detailed initial report upon the outbreak of the disease, which contains longitudinal and latitudinal coordinates, the number and species of affected animals, the start and end date of the outbreak, and the actions taken. Several follow up reports are also required depending on the type and severity of the disease. These reports per incidence can be linked together via a designated case ID number.

We used the R software to create a package that collects all OIE reports from a time period of interest. The program picks out the relevant information from the individual pdf reports and creates an SQL database with all the acquired information on the user's computer, which facilitates an easier research process when looking for specific but comprehensive information on a single disease, or in a single geographical area.

Furthermore, our R package includes a mapping function that makes surveying all reports of a disease over the examined time period possible via creating a time lapse map of all incidences over the study period. The individual pdf reports are retrievable from the constructed maps as well. The OIEparser package is publicly accessible, and free to download and install from the <https://solymosin.github.io/OIEparser/> website.

Our toolbox allows researchers to navigate the vast WAHIS database with ease, and use all available wildlife and domestic animal disease data in a structured way. Knowledge of incidence rates and appearances have tremendous application possibilities. Data analyzed this way can show trends in disease spread over time and geographical locations, can be incorporated into disease risk analysis, and facilitate preventive measures.

In order to demonstrate the tool, we used real-time data mapping of African swine fever incidences both in wild boar and domestic swine in countries neighbouring Hungary to prepare a risk analysis for national preventive measures.

## Reestablishment of a non-migratory flock of whooping cranes (*Grus americana*) in Louisiana, USA

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**Keywords:** whooping crane, flock management, disease

The whooping crane (*Grus americana*) is named for its “whooping” call and is the tallest bird in North America. The crane is classified as an endangered species, the rarest of the world’s 15 crane species, with a 2017 total population of 775 birds including 163 captive birds. Prior to European settlement an estimated population of over 10,000 cranes resided in North America. By 1870, the number of cranes was reduced to approximately 1,350 with numbers continuing to decline until there were less than 50 birds by 1940. Through extensive conservation efforts and the development of artificial insemination North American whooping crane populations have since begun to increase. Historically the principal wintering range of the whooping crane included the tall grass prairies and the coast of the Gulf of Mexico extending from southwest Louisiana, through southeast Texas, and into northeast Mexico. By 1939, only 13 resident cranes were identified, concentrated within the area that is now the White Lake Wetlands Conservation Area (WLWCA). By 1947, only one bird remained which was captured and relocated to Aransas National Wildlife Refuge, Texas in 1950. In March 2011, 10 juvenile cranes hatched and reared at the USGS Patuxent Wildlife Research Center were released in Louisiana through a collaborative effort involving the United States Fish and Wildlife Service, the United States Geological Survey (Patuxent Wildlife Research Center and the Louisiana Cooperative Fish and Wildlife Research Unit), the Louisiana Department of Wildlife and Fisheries, and the International Crane Foundation. This effort was the beginning of an attempt to reestablish a non-migratory whooping crane population in southwest Louisiana. During the growth phase, juvenile cranes undergo frequent health examinations at captive breeding facilities that include being vaccinated for Eastern equine encephalitis and West Nile virus. The birds have health examinations at weekly intervals until 60 days of age then biweekly thereafter. Two weeks prior to arriving in Louisiana the cranes undergo an examination which includes a complete blood count, serum chemistry panel, *Salmonella* spp. testing, fecal flotation, testing for inclusion body disease of cranes, and infectious bursal disease. The current 2018 maximum population in Louisiana is 66 whooping cranes. Of the 127 cranes released in Louisiana, 125 have been captive raised and two have hatched and fledged in the wild. When cranes arrive in Louisiana they are outfitted with bands and transmitters to help biologists track and monitor the birds. They are then placed in top-netted pens where they are monitored for a few weeks before being released into the wild. There are two release sites in Louisiana, one at the WLWCA and another at Rockefeller Wildlife Refuge, 19 km south. The first eggs were produced by a released pair in 2014, and there have been eight additional pairs nesting since. In 2016, the first chicks were hatched in the wild in Louisiana with one successfully fledging. Unfortunately the cranes have not yet generated the anticipated successful reproductive activity. Additionally, the mortality rate is currently 48%, with gunshot and other anthropogenic influences comprising the greatest number of deaths.

## The Great Tit *Parus major* pox disease in France: evidence of emergence and spatio-temporal distributions from citizen science-based data.

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**Keywords:** avian pox, Great Tit *Parus major*, citizen science

Avian pox has been described in more than 200 bird species worldwide and in at least 60 native wild species in Europe. Its nodular proliferative cutaneous form is the most widespread form. It can have little to severe demographic impacts depending on the bird population size, their geographical expansion and their immune status. The Great Tit (*Parus major*, Passeriformes) is especially receptive and susceptible to a viral strain which induces a severe cutaneous form, mainly located around the beak and the eyes. The Great Tit pox disease emerged in Norway in the 1970s, and then spread southward and westward (central Europe in 2005-2009, UK in 2006). In continental France, suggestive cases were sporadically reported in 2012 by the public but the specific situation of the disease remains unknown to date. The goals of the present study were to confirm the presence of the disease in great tits in mainland France, to describe its spatial and temporal distribution, and to investigate the role of migrant great tits as vectors of the disease spread to France. As the Great Tit is a very popular and common garden bird, intensively captured by ringers, and avian pox easy to suspect visually, we used a citizen science approach to collect epidemiological data. We launched surveys during winters 2012-2013 and 2014-2015, asking birdwatchers and ringers to report all cases following standard protocols. Analyses of occurrence data were adjusted for spatial and temporal variations in observer and great tit relative abundances across France. First, our study confirmed the occurrence of the disease in France by histological and/or molecular evidence. Second, cases of the disease were widely distributed across France in winter from the north-eastern borders towards the south-west part of the Massif Central but with very few cases in Northwest France. The report rate of diseased birds significantly varied between French departments, with an apparent higher reporting rates in regions receiving an important influx of wintering birds originating from Northeast Europe. Third, the number of cases was 6 times lower during the 2014-2015 winter than during the 2012-2013 winter, which corresponded to an 8 times lower influx of wintering great tits originating from Northeast Europe (documented by the number of recaptures or recoveries of foreign individuals). During this last winter, at sites where at least one case was reported, the proportion of cases among captured birds ranged from 1.4% to 5.7%. This study demonstrates for the first time that the pox disease is present in great tits in France, probably with a higher prevalence among wintering birds originating from Northeast Europe, in agreement with the recent expansion of the disease. Citizen science surveys were used here for the first time to assess the garden bird health in France, and showed that they are valuable tools for rapidly detecting the emergence and spread at large-scale of a highly visually recognisable disease in a common passerine species. It opens interesting perspectives for the development of garden wildlife surveillance programs in France and in collaboration with other schemes in Europe.

## Spatio-temporal dynamics and aetiology of proliferative leg skin lesions in wild British finches

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**Keywords:** avian papillomavirus, cnemidocoptes, chaffinch *Fringilla coelebs*

The epidemiology and impact of diseases of wild birds that cause morbidity, but are not typically associated with mortality, are often poorly understood. Citizen science schemes offer a means for surveillance of such conditions with externally visible clinical signs when they affect species that commonly utilise peri-domestic habitats. Whilst proliferative leg skin lesions (hereafter 'leg lesions'), most commonly affecting chaffinches (*Fringilla coelebs*), caused by cnemidocoptosis, papillomatosis, or co-infection with both diseases, have been reported for decades from multiple countries in Europe, research to date is limited. The objective of this study was to investigate the spatio-temporal dynamics and aetiologies of leg lesions in British finches. Syndromic surveillance, utilising opportunistic and systematic data collection through a citizen science scheme, the Garden Wildlife Health project ([www.gardenwildlifehealth.org](http://www.gardenwildlifehealth.org)), was conducted in 2014 and 2015 in Great Britain (GB). Participants provided ad hoc reports, or weekly reports throughout the calendar year, of their observations of sick or dead garden wildlife online, including sightings of 'birds with growths on legs', providing digital photographs of affected animals when available. All data were reviewed by veterinarians to identify syndromic reports of leg lesions. Independent exploration of the spatio-temporal trends in both datasets confirmed leg lesions in chaffinches to be widespread throughout GB with other finch species infrequently affected. Seasonal variation was observed, with a winter peak that was more pronounced in eastern than in western GB. The winter population of chaffinches in the British Isles is markedly increased by the immigration of birds from continental Europe, with the birds typically arriving in southern and eastern England. We hypothesise that this winter migration, along with a greater reliance on provisioned food in gardens during the colder winter months, explain the observed seasonal peak in leg lesion reports. Pathological investigations were conducted on 39 chaffinches, four bullfinches (*Pyrrhula pyrrhula*), one greenfinch (*Chloris chloris*) and one goldfinch (*Carduelis carduelis*) with leg lesions, 2005-2015. Whilst the leg lesions were unrelated to the cause of death in the majority of birds examined, this provided opportunity to investigate lesion aetiology. Microscopic examination of lesions and PCR targeting *Fringilla coelebs* papillomavirus (FcPV1) and avian poxvirus were conducted. Cnemidocoptes sp. mites were detected in 91% (41/45) of affected finches and from all species examined. FcPV1 PCR was positive in 74% (23/31) of birds tested: a 394 base pair sequence was derived from 20 of these birds, from all examined species, with 100% identity to reference genomes. Both Cnemidocoptes sp. mites and FcPV1 DNA were detected in 71% (20/28) of birds tested for both pathogens. Avian poxvirus PCR was negative from all 31 birds tested. Hyperkeratosis, hyperplasia and epidermal papillary projections were common histopathological features of leg lesions, however they did not discriminate the relative importance of mite or FcPV1 infection as their cause. Development of techniques to localise FcPV1 is required to elucidate the pathological significance of FcPV1 DNA detection. An integrated approach of syndromic surveillance combined with pathological investigations, achieved through citizen science, has advanced our understanding of this disease presentation of wild finches.

## **Investigation of mass mortality in Arctic terns (*Sterna paradisaea*) at a breeding colony in Wales**

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*Animal and Plant Health Agency and Royal Society for the Protection of Birds*

**Keywords:** *Arctic tern, mass mortality, botulism*

In July and August 2016 over 800 birds, mostly Arctic terns, out of an approximate population of 8,200 terns (4,100 pairs), died at a breeding colony on The Skerries, a group of small islands (17 hectares) in the Irish Sea, three kilometres off the north Wales coast. The colony is managed and monitored by the Royal Society for the Protection of Birds (RSPB) and holds at least 7% of the UK Arctic tern population and 2% of common terns (*Sterna hirundo*). The Animal and Plant Health Agency (APHA) and RSPB's investigation of the mortality covered 3 main areas: 1 A visit to the colony to gather epidemiological information, record clinical signs, view colony structure and habitat; 2 Ongoing review of RSPB monitoring data regarding food provisioning, daily mortality, foraging areas and mortality reports from other colonies; 3 Post mortem examinations and laboratory testing throughout the outbreak. Birds were found dead spread over a wide area, often in sternal recumbency with their wings outstretched. Affected birds that were seen alive had drooping wings and were easy to approach but flew off a short distance before landing, or were seen on the ground with flaccid paralysis and unresponsive before death. 131 carcasses were collected at the visit for examination and disposal, many of which were decaying. The colony is divided into zones for recording. Daily numbers of birds found dead were approximately in proportion to the breeding population in each zone. Chick feeding data indicated provisioning was comparable or better than recent years. Data from birds GPS tagged and also boat tracking data illustrated their foraging area to the west of the islands. Suitable carcasses were examined in detail and over 70 were examined externally and internally. The birds were in poor to moderate body condition with no food in gizzards and minimal gross lesions. *Pasteurella* like organisms isolated from lungs were not associated with any microscopic lesions. *Chlamydia*, pathogenic *Mycoplasma* species and *Cryptosporidia* were not detected. No avian viruses were detected including Avian Influenza, West Nile Virus or Avian paramyxovirus. There was no histological evidence of degeneration, necrosis or inflammation to suggest infectious disease. Toxic levels of arsenic, lead, chromium and cadmium were not detected. Paralytic Shellfish Poisoning or Amnesic Shellfish Poisoning toxins were not detected in livers. ELISA tests were positive for *Clostridium botulinum* toxin type III (C/D) from two of three intestinal samples. A diagnosis of avian botulism was supported by the clinical signs, monitoring data on adequate food supplies, minimal pathological lesions and minimal food material in intestinal tracts, laboratory findings with lack of evidence of an infectious disease, and the bio toxin results. This was an unusual finding in a fish eating species and the source of the toxin was not confirmed. Recommendations for future management were made and in the 2017 breeding season there was a 27% reduction in the number of Arctic tern nests but no repeat of high mortality.

## **Prevalence of avian influenza and Newcastle disease viruses in local populations of wild birds in contact with Houbara bustards conservation breeding programmes in the United Arab Emirates.**

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**Keywords:** *Chlamydotis macqueenii*, wild avifauna, virus transmission

The Asian Houbara bustard (*Chlamydotis macqueenii*) is a vulnerable species, whose populations drastically declined all across its natural range. Conservation breeding projects have been implemented in the United Arab Emirates (UAE) to sustain wild populations by release of captive-bred individuals. Breeding facilities are known to attract wildlife, especially birds, as a source of food and water. This has been observed in the UAE Houbara captive-breeding facilities, which are located in desert environment. To prepare captive-bred Houbara for release into the wild, the final step involves breeding in outdoor aviaries. Recent outbreaks of low pathogenic avian influenza and avian pox in captive bustards have questioned the role of interaction with wild birds. This emphasizes the likelihood for diseases transmission between free-ranging and captive-bred birds and highlights the need to assess sanitary risk in Houbara translocation programmes. Here we aimed to assess whether commensal bird species (Passeriformes, Columbiformes and Galliformes) play a role in the transmission of avian influenza and Newcastle disease viruses to captive bustards. During two consecutive years, more than 4500 individuals were captured on two breeding sites, marked and sampled (serum sample and tracheal/cloacal swabs). Swabs were screened by quantitative PCR for Avian Influenza Virus (AIV, gene M) and Newcastle Disease Virus (NDV, class II, gene M) and serums were analyzed for presence of antibodies against these viruses by competitive ELISA. Preliminary results showed low prevalences for both viruses (0.8% for AIV and 1.0% for PMV, respectively) and seroprevalence of 0.5% and 14.5% for AIV and NDV, respectively. There was noticeable variations among species (from 0.4% to 1.1% and from 1.6% to 61.1% for AIV and NDV, respectively; p-values <0.05). These preliminary results demonstrate that although wild birds are exposed to these viruses, they seldom excrete AIV and NDV. To fully understand the epidemiological risk represented by wild birds for captive Houbara bustards, we will screen for some others pathogens in a close future and we will also investigate the ecological and behavioural components of the transmission process, using Capture-Recapture, counting and tracking data. Key-words : *Chlamydotis macqueenii*, wild avifauna, virus transmission

## **Disease and health implications of free living wildlife on a captive zoo population.**

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**Keywords:** *Mycobacterium, wildlife, Chagas*

Several disease outbreaks were noted in an urban zoo in the Southern United States over a span of about two years. Chagas disease, Marek's disease, *Mycobacterium genevense*, and several mouse and rat viruses were introduced into a captive collection via confirmed wildlife vectors. There are some challenges with having a park in a tropical urban area as wildlife have few areas to congregate outside of the park and they have access to food and water via the zoo animals. The zoo has a policy of wildlife removal for some species; however, rats and wild birds were harder to catch and existed in such significant numbers active efforts to remove them were not employed. Also, affected animals living around our zoo were not species we could test or treat but they too also carried disease of concern such as Chagas disease, heartworm disease, canine distemper and rabies. Vaccination and monthly heartworm medications are employed in our zoo animals but many of the wildlife diseases were not preventable. We had wild pheasants introduce Marek's disease a herpesvirus into our zoo chickens with some spread into our valuable collection birds. We had rats that tested positive for antibodies and confirmed by IFA to rat parvovirus, rat theilovirus and mouse/rat adenovirus strain one and two which caused disease and virus transfer to our primates. The American black vulture population introduced *Mycobacterium avium* complex into our closed flamingo collection housed in open air exhibits shortly after a large hurricane had occurred near the zoo. In the southern United States, the Triatominae vector exists; however, *Trypanoma cruzi* had not been identified. The disease has come up in domestic pets coming across from Chagas affected areas. The zoo is also located across from a humane society that has reported *Trypanosoma cruzi* cases. Chagas disease is not a preventable disease and hard to treat as animals can be subclinical until severe clinical signs are noted. The zoo's Sun Bear was identified to be in heart failure with a positive high titer to Chagas and PCR positive for Chagas disease. These outbreaks had substantial economic impacts on the zoo in the form of testing and disease monitoring, loss of valuable species, changes to current infrastructure and exhibit design, chemicals and wildlife control methods, and zoo staff time, training and personal protective equipment (PPE). The outbreaks also required extensive communication between scientific animal and non-animal leadership to accomplish our eradication efforts while recognizing our inability to control disease in the wildlife of concern. Testing wildlife is an important endeavor to fully understand diseases of concern. Staff training, quarantine measures taken between enclosures, exhibit modifications and wildlife deterrents helped with disease control. Disease results, factors that led to outbreaks, control and eradication and communication and monitoring will be discussed in detail.

## **Histopathological study of eye tissues from semi-domesticated reindeer with infectious keratoconjunctivitis caused by cervid herpesvirus 2 in an experimental setting**

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**Keywords:** *Histopathology, infectious keratoconjunctivitis, reindeer*

The aim of this study was to describe the histopathology of eye tissues caused by Cervid Herpesvirus 2 (CvHV2) in experimentally induced infectious keratoconjunctivitis (IKC) in semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*). IKC is a common disease in Scandinavian reindeer and can affect a great number of animals during outbreaks, with calves and young animals being the most susceptible age group. Eighteen 1-year old reindeer calves, seronegative for alphaherpesvirus and culture negative for *Moraxella bovoculi*, were inoculated with CvHV2 (Group 1, n = 5), a combination of CvHV2 and *M. bovoculi* (Group 2, n = 5), *M. bovoculi* (Group 3, n = 5) or sterile saline water as a negative control (Group 4, n = 3). All 10 animals inoculated with CvHV2, alone or in combination with *M. bovoculi*, developed clinical signs of IKC within 2-3 days after inoculation. Animals that were only inoculated with *M. bovoculi* did not develop IKC during the study period. All reindeer eyes, including the tissue from the periorbital region, were fixed with 4 % paraformaldehyde in PBS within minutes after euthanization of the animals. Subsequently, tissues from the upper and lower eyelids, cornea and lacrimal glands were collected from each eye and processed routinely for histological examination. Paraffin-embedded sections stained with hematoxylin and eosin were subjected to a blind histological examination. Major structures in the different tissues were graded in a five-grade severity scale, with 1 being used for normal tissue, 2 for mild pathological lesions, 3 for moderate inflammation, 4 for focal severe inflammation and 5 for general and severe inflammation. A general evaluation of the tissue was also performed, grading the whole section from 1 to 5. Histopathological analysis of the tissues collected from the experimental animals in groups 1 and 2 showed the presence of exudates, edema, hyperemia, hemorrhage, necrosis, vascular thrombosis, vascular necrosis, infiltration of mononuclear cells and neutrophils, pus and lymphoid follicle reaction. In contrast, no apparent pathology of the mucosal or corneal epithelium was visible in the tissues collected from groups 3 or 4. The histopathological examination showed that CvHV2 had the ability to cause severe pathology (i.e. inflammation, necrosis) in all ocular structures examined in our study, e.g. conjunctival tissue, cornea or lacrimal glands, while the *M. bovoculi* isolate used in the challenge experiment did not. These findings are consistent with the macroscopic lesions described in previous studies and support the theory of CvHV2 as a primary causative agent of IKC in semi-domesticated reindeer.



## Spatiotemporal spread of sarcoptic mange in the red fox (*Vulpes vulpes*) and other wild carnivores in Switzerland

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**Keywords:** *Sarcoptes scabiei*, questionnaire survey, camera trapping

Sarcoptic mange is a contagious skin condition caused by the mite *Sarcoptes scabiei*. Its emergence in red foxes (*Vulpes vulpes*) and Eurasian lynx (*Lynx lynx*) in the late 1990s raised the question as whether mange was a new disease in Swiss wildlife, whether it would further spread, and which impact it would have on wildlife. We used complementary approaches to document the emergence and dynamics of sarcoptic mange in wild carnivores in Switzerland: 1) we carried out a retrospective and prospective questionnaire survey among field partners (1980-2012; more than 3'000 filled forms obtained since 2002); 2) we screened the archived necropsy reports on wild carnivores (1958-2018; more than 1'000 reports) submitted to the Centre for Fish and Wildlife Health (FIWI); 3) we performed a review of published and grey literature; and 4) we evaluated camera-trap pictures of foxes shot in the framework of the Eurasian lynx monitoring (2005-2018; more than 60'000 pictures). In Switzerland, sarcoptic mange mainly affects red foxes, in which it was mentioned as early as 1835. The first case submitted to the FIWI was in 1959. Prior to 1980, sarcoptic mange occurred in non-adjacent hunting grounds or surveillance districts scattered all over the country. Following a decrease in the 1980s, sarcoptic mange re-emerged after 1995 and showed an epidemic spread through the 2000s, occurring in nearly the whole country in 2012. Since 1999, *S. scabiei* has sporadically been isolated from a few lynx and martens (*Martes foina*, *M. martes*), one badger (*Meles meles*) and one gray wolf (*Canis lupus*). The apparent prevalence of mange-like lesions in foxes estimated by camera trapping ranged from 0.1-10.3% depending on the study area and period. The dynamics of sarcoptic mange in wildlife is typically epidemic and associated with a high mortality when it emerges in naïve populations; it becomes endemic with time, likely as a result from a progressive host-parasite adaptation. The endemic phase is characterized by lower case incidence, increased host survival and a cyclic re-appearance. Our preliminary analyses suggest an unusual transition from an endemic to an epidemic situation of sarcoptic mange in red foxes in Switzerland in the 1990s. The nearly disappearance of the disease in the 1980s temporally corresponds to the demographic bottleneck that followed the rabies epidemic and associated control measures in the 1960-1980s. Although the epidemiological transition may be multifactorial, including also mite genetic variations, climatic factors and increasing fox densities, modifications of the genetic structure of the fox population may have generated a susceptible “naïve-like” population and have been the origin of the re-emergence of sarcoptic mange in the 1990s. This epidemic has been associated with occasional spill-over events on other wild carnivores as well as domestic carnivores and their owners. Although genetic analyses of the mites are needed to prove a relationship among these cases, epidemiologic evidence highlights the role of red foxes as a source of infection for other animals and humans.

## **Epidemiological study of *Dichelobacter nodosus* in free ranging Alpine ibex (*Capra ibex ibex*) and other potential hosts: identifying maintenance hosts and risk factors for infection**

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**Keywords:** foot rot, ibex, Switzerland

Foot rot is an economically important, painful, contagious bacterial disease caused by *Dichelobacter nodosus*. It is endemic in sheep worldwide and has also been documented as a fatal disease in free ranging Alpine ibex (*Capra ibex*) and mouflon (*Ovis ovis orientalis*). Benign and virulent strains have been identified in sheep presenting mild and severe foot lesions, respectively. By contrast, in Alpine ibex both the benign and virulent strains have been associated with severe lesions. A nationwide foot rot control program for sheep focusing on the virulent strain shall soon be implemented in Switzerland. However, interactions on alpine pastures between wild and domestic susceptible hosts are suspected to pose a serious risk of transmission. Healthy carriers have been identified among sheep, cattle and goats but there are currently no data on the prevalence of *D. nodosus* infections in Switzerland, neither in domestic nor in wild animals. Therefore, a nationwide prevalence study was recently initiated in both wildlife and domestic livestock to identify hosts potentially posing a threat to ibex colonies and to evaluate the reinfection risk for sanitized sheep. The study includes: (1) a cross-sectional study to estimate the prevalence of infection in selected potential wildlife and domestic hosts at national level, distinguishing between benign and virulent strains of *D. nodosus*; (2) a questionnaire survey to record the occurrence of foot rot outbreaks in ibex and contacts of affected and non-affected colonies with other wildlife and with domestic livestock. For the prevalence study, interdigital swabs of 1786 wild ruminants from four species (Alpine ibex, Alpine chamois *Rupicapra rupicapra*, roe deer *Capreolus capreolus* and red deer *Cervus elaphus*) and 3059 domestic animals (sheep, cattle, goats and South American camelids) are being sampled over two years (2017–2018) and analysed by Real-Time PCR. For the questionnaire survey, all Swiss game wardens with ibex colonies in their district of surveillance have received an online questionnaire to report past foot rot outbreaks in ibex. By the end of March 2018, almost half of the target sample size has been analyzed. One Alpine ibex without lesions harboured the benign strain, another one showing severe lesions was infected with the virulent strain. Both the virulent and benign strains were found in a significant number of asymptomatic sheep. The benign strain was additionally detected in numerous cattle as well as in a few individuals of other species. The questionnaire survey is on-going. Our preliminary results suggest that not only sheep but also cattle may represent a significant source of infection for ibex, while wild ruminants may be irrelevant for the maintenance of *D. nodosus*. Interactions between ibex and cattle, although less common than between ibex and sheep, have indeed been documented on summer grazing pastures. However, the total sample size and questionnaire results are required to draw sound conclusions. This study will be crucial to implement disease management measures relevant to both ibex and sheep.

## Disease risk analysis for the western barred bandicoot (*Perameles bougainville*)

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**Keywords:** Disease risk analysis, endangered, marsupial

The western barred bandicoot (WBB) is a small marsupial which became extinct on the Australian mainland in the 1940s. It survives on Bernier and Dorre Islands in Shark Bay, off the coast of Western Australia. Key identified threatening processes include predation by introduced cats and foxes, alterations in fire regimes and habitat destruction. The WBB is now listed nationally as Endangered with fewer than 3000 individuals remaining. Prior translocations to captive and wild destinations have occurred with mixed success, and proposals for future translocation of WBBs to various island and mainland (fenced) sites are currently being considered. Prior research has identified a number of disease hazards including, but not limited to, the WBB papillomatosis and carcinomatosis syndrome (BPCS), Q fever (*Coxiella burnetii*), and chlamydia (Bennett 2008, Woolford 2008). BPCS typically presents with lesions involving cutaneous and mucocutaneous surfaces. Smaller epithelial lesions resemble papillomas, whereas larger lesions are most commonly carcinoma-in situ and squamous cell carcinomas (Woolford et al. 2008). Lesions typically increase in size over time and involvement of the feet, eyes and mouth can lead to problems with ambulation, vision, and eating. Affected animals may die due to secondary infection or may require euthanasia on welfare grounds. The volume of existing knowledge of this disease in WBB, although now 10 years old, is substantial. However, uncertainties of prevalence, detection, transmission, and treatment complicate current understanding of disease risk. At a recent stakeholder meeting it was decided that a Disease Risk Analysis (DRA) should be undertaken to further examine the risk of BPCS and other disease. DRA is a process for identifying significant disease risks and proposes measures to mitigate these risks. DRA in Australia is most frequently carried out in association with conservation translocations but has also been applied to investigating the potential role of disease in native mammal declines (Pacioni et al. 2015; Reiss et al. 2015). We followed the IUCN (2014) Manual to undertake a DRA comprising a problem description, hazard identification, risk assessment, risk management, implementation and review. Risk communication was central to the success of the DRA. This DRA was unusual in that multiple possible source and destination environments existed. For this reason a one day workshop with key stakeholders was undertaken to formulate likely translocation pathways, including source populations, destination environments, and transportation methods, and also to clearly articulate translocation risks (e.g. genetics, expense, logistics) which may have a bearing on how disease risk is assessed. We presented the hazard list and justification to stakeholders, and engaged the group to prioritise hazards based on a likelihood and consequence matrix. We then developed a disease risk management strategy for WBB to be translocated from a yet to be determined source and destination population/s based on structured, evidence based analysis of current information. Results of the DRA comprising assessment of disease importance in light of other medium to high risk non-infectious hazards will be presented, highlighting the importance of a holistic approach to DRA for conservation translocations.

## Epidemiological analysis of Aujeszky disease in wild boar in Italy

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**Keywords:** Aujeszky's disease, wild boar, serology

Aujeszky's disease (AD) is a notifiable infection that causes substantial economic losses to the swine industry and has major economic impact due to trade implications and income losses for farmers. Pseudorabies virus (PRV), which belongs to the family of Herpesviridae, causes AD. Thus, the European general policy is to eradicate AD in order to support free intra-EU trade. In Italy, an AD national monitoring program was implemented in 1997. Extraordinary regional control plans were also applied in the Northern Italian regions with the highest concentrations of pig industry; these regions have recently been included in annex two of the EU Decision 2008/185/EC. PRV may infect a wide spectrum of mammals, although domestic and wild Suidae are the only hosts that can act as reservoir. Although PRV has been eliminated in domestic pigs in many European countries, AD is being continuously reported in wild boar populations; consequently, their possible impact on the application and success of AD eradication programs and the risk they pose to the PRV-free status should be taken into account. During 2016/2017 hunting season, a cross sectional study was conducted in order to evaluate the seroprevalence of AD in wild boar populations in different ecological areas in Italy. Overall 226 sera samples were collected from 245 hunted wild boars from four Italian ecological areas: 53 were collected from Alps, 25 from Northern-Apennine, 65 from Central-Apennine and 83 from Southern-Apennine. Sera samples were tested for anti-gE antibodies using an ELISA kit. In addition, variables regarding wild boars (age; sex) and hunting area were recorded. An overall seroprevalence of 28.32% (64/226) emerged. The seroprevalence was respectively 13.21% in Alps (7/53 95% CI: 5.48% - 25.34%), 56.00% in Northern-Apennine (14/25 95% CI: 34.93% - 75.60%); 41.54% in Central-Apennine (27/65 95% CI: 29.44% - 54.44%), 19.28% (16/83 95% CI: 11.44% - 29.41%) in Southern-Apennine. The difference between seroprevalence in the four areas is statistically significant ( $\chi^2=24.338$ , p-value < 0.001). Statistical analyses showed that seroprevalence was associated by gender ( $\chi^2=4.534$ , p-value=0.033) and age ( $\chi^2=27.236$ , p-value < 0.001); in particular seroprevalence was higher for females (31.96% 31/97) than males (18.07% 15/83); and it was higher for adult wild boars (43.48% 20/46) than young (0% 0/2) and sub-adult (14.71% 5/34). However, including all factors in a multivariate logistic regression models, only location resulted statistically significant (LRT = 24.079, p-value < 0.001), as age and gender dependent on ecological areas. Results of this study suggest that different ecological areas show different pattern of AD circulation, the structure of wildlife population may be influence the dynamics of AD transmission. The highest seroprevalence was recorded in areas with intense wild boar management. The epidemiology in wild boars should be continuously monitored and assessed. This study was funded by a National Research Project PRC2015001.

## **Plasticosis from intact and micronizing plastic in birds, reptiles and mammals, including humans**

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**Keywords:** *Plasticosis, plastic micronization, endangered species, birds, reptiles, biodegradable plastics*

Petroleum-based plastics and their toxins are increasingly contaminating our rivers, oceans and soil. Recent estimates suggest that 5.2 trillion pieces of plastic already contaminate the world's oceans. As plastic degrades it micronizes placing smaller fauna and/or juvenile animals (particularly endangered species) at risk of gastrointestinal impaction. The clinical implications of our team's research to document the health effects of plastic ingestion/exposure including clinical changes in affected species, pathologic lesions and systemic effects of plastic ingestion as well as our progress in replacing petroleum-based plastics with biodegradable alternatives will be discussed.

## An update on the puzzling world of human and animal pathogenic treponemes

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**Keywords:** *Spirochetes, Treponema, genetics*

Bacteria of the genus *Treponema* belong to a group of spirochetes that contains several pathogens of global importance for human and animal health. It includes nonpathogenic and pathogenic species, some of which are uncultivable. Difficulties in studying non-cultivable bacteria affected the availability of genetic data for these species. However, modern molecular and genomic techniques resulted in dozens of completely or partially sequenced genomes of treponemes. These data provide some deeper understanding on the complexity and evolution of this ancient group of pathogens. We discuss the different treponemes in the context of their natural ecosystems, their host specificity, and the clinical manifestations they cause. In humans, *T. pallidum* causes syphilis (subsp. *pallidum*), bejel (subsp. *endemicum*) and yaws (subsp. *pertenue*). The latter is currently subject to global eradication efforts and has reemerged in Western Africa, Southern Asia, and the Pacific region. Reports are accumulating that in Africa, nonhuman primates (NHPs) are infected with strains that are genetically and functionally highly similar to yaws-causing *pertenue* strains of human origin. The remarkable geographic overlap of historic and present endemic areas for human yaws and NHP infection with *T. p. pertenuae* argues for a spatial and temporal connectivity between multiple infected NHP species and humans. However, important questions on disease maintenance and possible transmission routes between humans and monkeys remain unanswered. While *T. pallidum* and *Treponema paraluisleporidarum* ecovar (TPe) *Cuniculus* are genetically distinct species, there is a high degree of similarity, relative to the clinical manifestations they produce. Since the first description of a syphilis-like infection in European brown hares (*Lepus europaeus*) in 1957, it has now been reported in several European countries. The infection, however, is not limited to the European brown hare since our newest data include strains isolated from mountain hares (*Lepus timidus*). Interestingly, TPe<sub>Lepus</sub> from mountain hares appears to be closer related to the ecovar *Cuniculus* than to the TPe<sub>Lepus</sub> which was found in the European brown hare. Analysis of the genomic structure and diversity of the agents of hare and rabbit syphilis could be of great importance in clarifying the evolution of syphilis treponemes and for the identification of virulence determinants that allow infection of the human and nonhuman primate hosts. Digital dermatitis is a globally occurring disease in domestic livestock. North American Elk (*Cervus elaphus*) infection has been linked to *Treponema medium*, *Treponema vincentii*-like, and *Treponema phagedensis*-like species. Surprisingly, related treponemes were described to infect the genitals of the critically endangered Gilbert's potoroo (*Potorous gilbertii*) in Western Australia. Further characterization of the treponemes is underway and we provide some updated information on the infection in potoroos. Our understanding of the biology of treponemes infecting humans, domestic animals, and wildlife will only improve when we understand the ecological landscape in which the diseases emerge as well as the genetic mechanisms that impact host specificity and virulence. Parts of this work have been funded by DFG KN1097/3-1, KN 1097/3-2, and KN 1097/7-1 (SK) as well by GAČR 18-23521J (DS).

## Does stress impair healthy aging? Relationship between stress and telomere length in roe deer

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**Keywords:** senescence, telomere, stress

With increasing age, most organisms experience progressive decline in performances, including immunity and health. The dysregulation of immune functions and increased risk of infectious diseases or parasites are essential in the deterioration of reproductive success and survival, i.e., senescence. In the natural environment, both predictable and unpredictable fluctuations of the biotic and abiotic conditions are common, this variability is a major constraint for individuals. Physiological adjustment to these fluctuations is expected to affect the senescence process, however the complex mechanism by which environmental conditions are perceived by individuals and converted into the physiology of aging is not clear yet. Identifying relevant indicators of this physiological adjustment are a key to understand variations of health with age. Glucocorticoid (GC) hormones (cortisol, corticosterone) are of particular importance in this context, as they serve diverse functions related to the maintenance of energy balance of an organism according to its needs. Hence, GCs are crucial mediators of individual phenotypic flexibility and are involved in coordinating adjustments to variation in climate, resource abundance, social and internal conditions. Among senescence mechanisms, telomere attrition is among the most generic biomarkers of individual physiological state and past experience. Telomeres are repetitive DNA segments at the chromosome tips. Telomeres vary in size throughout life: they are shortened at each cell replication, but may be maintained and lengthened by telomerase. Several recent findings suggest that telomere length (TL) serves as an early predictor of onset of disease and earlier mortality, and that stress plays a major role among the environmental and behavioural factors that affect TL and telomerase. In this study, we test the hypothesis that stress influences telomere erosion in a long-lived wild ruminant, the roe deer *Capreolus capreolus*. For this purpose, we analysed interannual variation of leucocyte TL from roe deer (*Capreolus capreolus*) captured in two French roe deer populations living in contrasting habitats. As part of the long-term survey of these populations, 44 individuals aged 8 months to 13 years old in 2016 were captured twice in 2016 and 2017. The age, sex and body mass were known for each capture. Moreover, we measured telomere length of leukocytes from peripheral blood, and the level of faecal glucocorticoid metabolites (FGM). FGM levels showed high repeatability, thus individual levels of stress were relatively stable between two consecutive years. As suggested by a previous descriptive study of TL in the same populations, telomeres of a given individual could either shorten or lengthen between two consecutive years. The difference in TL between the two years (2017-2016) was not related to age, sex, mass or population. However, this difference was negatively correlated to average FGM levels. These preliminary findings suggest that a high level of stress increases telomere erosion. Our future studies will test whether stress and/or sharp decrease in TL are associated to early decline in terms of immune parameters, parasite prevalence and health in general.

## **MHC class II DQA locus variation in the species of European Brown hare: pathogen-driven genetic differentiation.**

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**Keywords:** *MHC, DQA, genetic variation, local adaptation, pathogens*

The Major Histocompatibility Complex (MHC) has a primary role in the immune system of the vertebrates and its role is to bind fragments of antigenic proteins and transport them to the cell membrane. The MHC-antigen complex is there recognized by T-cell receptors (TCR), which initiate the cascade of immune responses. A specific domain of the MHC molecule is responsible for the antigen recognition and is called peptide-binding region (PBR) and the match between the PBR-antigen-TCR is required for the initiation of the cascade. MHC's gene family is comprised by several genes, usually organized in clusters. MHC gene family possesses a position among the most polymorphic genes and most of the variation is mapped in the PBR regions, resulting in the extended ability of recognizing a great repertoire of antigens and subsequent presentation to T-cells. In such a manner, populations are capable of resisting a variety of pathogens, affecting populations' adaptation and viability. Many associations between the variation of DQ genes (genes of the HLA complex) and susceptibility or resistance to disease have been conducted so far, reflecting the importance of maintaining high levels of variation at these particular genes. In the species of the European brown hare, there are studies correlating specific alleles of MHC genes with susceptibility to EBHS virus. The species of the European brown hare is the only small sized mammal in Europe which raises their young above the ground right from the day of birth, so there is a need for a very efficient immune system, which ensures the protection against any possible infectious factors. In this study, we use a marker of the MHC gene family and more specifically the exon 2 of DQA gene (MHC class II) that have been proven to be useful for studies of adaptive polymorphism. The populations under study came from both the lineages of the species, the European and the Anatolian, which are believed to have adapted and diversified during the Quaternary's Ice Age in distinct refugia (the Balkans peninsula and the Anatolia respectively). The genetic differentiation between the two lineages in the specific gene may reflect the different pathogens that each population had to face in its refuge, that favored specific alleles under the action of positive selection, while the susceptible alleles were removed by purifying selection.



## **Elephant Endotheliotropic Herpesvirus (EEHV) infection in Asian elephants (*elephas maximus*) possible correlated hereditary coagulation disorder**

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**Keywords:** *Elephas maximus, Elephant Endotheliotropic Herpesvirus, Coagulation disorder*

In North America and Europe, there are breeding facilities which are highly affected by the EEHV hemorrhagic disease (EEHV–HD) and others which are minimally, or not affected by this disease. The EEHV is responsible for the death of nearly one third of all captive born Asian elephants in Europe, shows an 80% mortality rate and is the greatest cause of fatality of calves, presenting a serious risk for the conservation breeding efforts made by the European Zoos. Since 75% of these deaths occur between 1,4-3,2 year of age, it further aggravates the replacement of future potential female breeders, and as the world population continues to decline, understanding which are the trigger elements and protective factors to slow down the fatalities caused by EEHV becomes critical. The disease manifests itself as an acute hemorrhagic syndrome due to vast endothelial destruction, causing sudden death - at physical examination we can find facial edema and severe cyanotic tongue, and the most common pathological findings at necropsy are intracranial hemorrhages, pericardial effusion, and micro hemorrhages in several organs. Recently, an Asian elephant bull was diagnosed with hemophilia, due to coagulation Factor VII deficiency, and three of his offspring were also reported to be carriers of this mutation. To better understand if a hereditary coagulation disorder is involved in the onset/fatal outcome of this disease in the European population of Asian elephants, we measured the coagulation time using fresh blood and studied the presence of coagulation FVII gene mutations in frozen samples. Although establishing a causative relation between Factor VII mutation and EEHV-HD is not yet possible, we here present our latest achievements and the preliminary results obtained during this investigation. This project is part of a doctoral study supported and co-founded by the European Association of Zoo and Aquaria's Elephant Taxon Advisory Group.

## Genomic response to experimental infestation with *Sarcoptes scabiei* in Iberian ibex.

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**Keywords:** *Capra pyrenaica*, gene expression, sarcoptic mange

Sarcoptic mange is a contagious skin disease caused by the mite *Sarcoptes scabiei*, affecting different mammalian species worldwide, including wild ungulates. Iberian ibex (*Capra pyrenaica*) is a medium-sized ungulate endemic to the Iberian Peninsula, whose populations have suffered mortalities over 90% due to sarcoptic mange. The aim of this study is to characterize the gene expression in Iberian ibexes experimentally infested with *S. scabiei*. Twelve Iberian ibexes from four different haplotypes of the major histocompatibility complex class II DRB1 gene were experimentally infested with *S. scabiei*, whereas other six ibexes with the same haplotypes were maintained as controls. Clinical signs were monitored periodically during the infestation period. At day 131 post infestation, whole blood samples were collected in commercially available tubes with RNA preservation buffer (PAXgene™ blood RNA tubes, QIAGEN). Skin samples were also obtained the same day in RNeasy® solution to stabilize and protect RNA. The samples were stored at -80°C until analysis to determine the systemic and local genomic response to sarcoptic mange. RNA was extracted using PAXgene kit (QIAGEN) for blood samples and RNeasy mini kit (QIAGEN) for skin samples. Gene expression was measured with microarrays using the Ovine Gene 1.0 ST array (Affymetrix). The samples were processed according to the following Affymetrix protocols: GeneChip WT PLUS Reagent kit (P/N 703174 Rev. 2) and Expression Wash, Stain and Scan User Manual (P/N 702731 Rev. 3) (Affymetrix Inc., Santa Clara, CA, USA). After processing, data were quality controlled and normalized. Linear models for microarrays (LIMMA) were used to detect differences in blood and skin gene expression. The clinical evolution of the infested ibexes showed different trends during the infestation period. Four of the infested ibexes developed a mild stage of the disease, with percentages of skin affected by mange below 25%. Three of these ibexes showed later a reduction of the skin lesions, and two of them even recovered completely. Conversely, other eight ibexes progressed continuously to severe stages of the disease. The genomic analysis results were compared between infested and control ibexes, among haplotypes, and according to the different clinical evolution of sarcoptic mange, in order to study the effect of this disease on gene expression. This knowledge will allow a better understanding of sarcoptic mange dynamics in the individual and therefore in Iberian ibex populations.

## High prevalence of heart anomalies of suspected genetic origin in a reintroduced Eurasian lynx (*Lynx lynx*) population, Switzerland

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**Keywords:** cardiac biomarkers, heart murmur, inbreeding

The Eurasian lynx was reintroduced to Switzerland in the 1970s, resulting in two genetically distinct populations (Jura and Alps), which are nowadays estimated to 200 individuals. In 2003 an adult male lynx from the Alps that was previously diagnosed with a heart murmur died of a severe cardiomyopathy characterized by myocardial fibrosis and arteriosclerosis of the coronary arteries. A retrospective histological study on 87 lynx (1987-2009) revealed a high prevalence of similar heart lesions in the Alpine population, in particular in adult males, and a few more fatal cases of cardiac insufficiency were recorded. In parallel, heart murmurs were detected in an increasing number of live lynx in the Alps but only very few of them could be examined post-mortem. Furthermore, genetic analyses revealed a reduced heterozygosity of Swiss lynx, particularly in the Alps. The present study aims at evaluating the clinical significance of heart murmurs in the reintroduced lynx populations of Switzerland and at assessing a potential genetic origin of clinical and histological cardiac anomalies. From 2000 to 2018 (19 years), 202 clinical exams including heart auscultation were performed on 101 free-ranging lynx aged from 3 months to 17 years. All examined lynx were blood-sampled at capture for a range of analyses, including genetics. Archived frozen serum samples from 71 individuals (2003-2018) were available to measure two cardiac biomarkers: cardiac Troponin I (cTnI) and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Data were analysed based on a case-control study design (with versus without murmur). Genetic analyses were performed using microsatellites. Lynx pedigrees were established using both genetic and field data. A heart murmur was detected in nearly 50% of the examined lynx from the Alps, mainly adult males, while it was a rare finding in the Jura. Preliminary data analyses hint at significantly higher values for both biomarkers in the case group than in the control group, with record values in the male lynx that died of cardiac insufficiency in 2003. First attempts to merge histopathological and clinical data with the pedigree of the lynx suggest an inheritance of the observed heart anomalies, with affected animals being more closely related than expected from a random population sample. The use of cardiac biomarkers has not yet been evaluated in lynx but these first data suggest that heart murmurs may be associated with a heart disease. Furthermore, the prevalence pattern of murmurs in lynx matches the pattern observed histologically, suggesting a link between clinical and post-mortem anomalies. In domestic cats, plasma concentration of cTnI is a predictor of cardiac death in animals with hypertrophic cardiomyopathy, and NT-proBNP concentration reliably discriminates animals with occult cardiomyopathy from normal animals. Currently, there is no indication of a decreasing population trend in the Alps, but data on individual lynx survival are lacking. The high prevalence of cardiac anomalies in the Alps together with the loss of genetic variability is alarming and suggestive of inbreeding depression. These data urge for genetic management of lynx in Switzerland and underline the value of long-term health investigations on reintroduced wildlife populations.

## Landscape immunology across the range of a widespread Neotropical bat species

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**Keywords:** *Desmodus rotundus, geographic range, leukocytes*

Understanding how the environment influences variation in host immune defense is important for wildlife conservation and for human and animal health. For hosts in non-optimal conditions, including in habitats at the limits of their geographic range, greater physiological stress could impair individual immune defenses and alter population susceptibility to endemic and emerging pathogens. Understanding the extent that immune phenotypes vary across landscapes is limited by the rarity of studies that measure immunity across the range of broadly distributed species. We here analyzed cellular immune profiles (e.g. relative number of the different circulating leukocytes) from 42 wild populations of the common vampire bat (*Desmodus rotundus*) across its wide range in Latin America. White blood cell profiles were spatially autocorrelated at both small and large distances, suggesting that local- and broad-scale environmental factors influence immunity. Bats from relatively colder, drier, and more seasonal habitats that are characteristic of the northern and southern limits of the species range limits had more neutrophils, monocytes, and basophils but fewer lymphocytes and eosinophils than bats in the core and more tropical regions of their distribution. Our findings suggest that populations at the edge of their species range may experience chronic stress, inflammation in response to acute infection, or greater investment in general innate immunity and Th1 adaptive immune responses. Deeper understanding of how species distribution limits and local environmental conditions shape host susceptibility will be important for predicting infectious disease risks in the context of anthropogenic disturbance, land conversion, and host invasions facilitated by climate change.

## Disease ecology of lyssaviruses in the greater mouse-eared bat from South Tyrol (Italy)

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**Keywords:** bats, *Lyssavirus*, serology

The increasing interest for bats as possible reservoir for EIDs recently led to the discovery of a great number of novel viruses whose zoonotic potential has not yet been fully assessed. Among these, rabies-related lyssaviruses (RRLVs) are the only proven zoonotic pathogens found in European bats, as they are able to determine in mammals an acute and almost invariably fatal encephalomyelitis indistinguishable from that caused by rabies virus. While rabies virus (RABV) in Europe is still mostly associated with non-flying mammals, at least five different RRLVs are reported in bats. The most widely distributed is European bat Lyssavirus 1 (EBLV-1), which has been associated with spillover events to humans, domestic as well as wildlife animals. Most cases of EBLV-1 have been specifically associated with *Eptesicus serotinus* and *E. isabellinus*. However, live sampling of chiropters across Europe constantly provides serological evidence for its circulation in other bat species. The lack of geographical structuring of EBLV-1 further suggests that other hosts might be involved in the ecology of this virus, which should have higher dispersal ability compared to the serotine bat. Within the frame of a research, funded by the Italian Ministry of Health, we investigated the role of greater mouse-eared bats (*Myotis myotis*) in the ecology of RRLVs in Northern Italy and their potential as source for human infection. In addition, we investigated the impact of RRLVs in this bat species, whose conservation status is vulnerable. In order to do so, we combined studies of host ecology and virus dynamics in five maternal colonies covering almost the whole Bolzano province up to the border with Austria. Of a great concern for a public health perspective, the colonies selected for the study are located in roofs of officiant churches. Serological evidence for the circulation of RRLVs dates back 2012 in this area. Fieldwork for this study took place between 2016 and 2017, and involved the live sampling of 30 individuals from each colony during two to eight sampling campaigns. Ecological studies included the assessment of population dynamics and genetic structure within the study area and relatively to other European countries. In particular, genetic analyses were performed on 11 nuclear microsatellites and two highly variable mitochondrial markers. Blood samples were analyzed for the presence of antibodies against RRLVs using a modified Rapid Fluorescent Focus Inhibition Test (RFFIT), using EBLV-1b as challenge virus, as suggested by preliminary cross-reactivity analyses. Genetic studies supported all maternal colonies of South Tyrol to be included in a single meta-population characterized by both reproductive connectivity and individual movements between roosts. Population dynamics remained constant throughout the whole study period and no rabies-related mortality was detected in any colony. Antibodies highly cross-reactive with EBLV1 were detected in almost all the sampling campaigns, with infection dynamics following a seasonal pattern across years and colonies. Altogether, these results support the maintenance of a EBLV1-like RRLV within each colony and across the whole area, and confirm that greater mouse-eared bats do survive the infection, so that virus circulation does not constitute a conservation issue.

## Vector-borne pathogens in tissues of bats from Central and Eastern Europe

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**Keywords:** Vector- borne, *gltA*, *Bartonella* spp.

Bats are the second largest order of mammals and include about 20% of all mammal species worldwide. *Bartonella* spp. are vector-borne, Gram-negative bacteria which infect a large number of mammals and are distributed worldwide. *Bartonella* spp. have been reported on many occasions, with different prevalence and a high genetic diversity in bats and in bat flies. However, the host range and geographical distribution of these pathogen in Europe is unknown. The aim of this study was to investigate the presence and genetic diversity of *Bartonella* spp. in tissues of bats collected in four different countries from Eastern and Central Europe: Austria, Czech Republic, Hungary, and Romania. A total of 435 bat carcasses were collected between 2001 and 2016 from different places, such as caves, mines and buildings. All bats were identified according to morphological keys and tissues (heart, liver, kidney) were stored in 96% ethanol, at -80°C (samples from Austria) or in a freezer until their necropsy. PCR was performed using universal primers targeting a portion of the *gltA* gene, followed by sequencing. The results of this study show for the first time the presence of *Bartonella* spp. in bats in Central and Eastern Europe. A total number of 6 samples were positive with a prevalence of 1.38%. The positive samples belonged to three different bat species: *Myotis* cf. *alcathoe*, *Nyctalus noctula* and *Pipistrellus pipistrellus*. The comparative phylogenetic analysis of multiple sequences of *Bartonella* spp. from bats and bat flies worldwide showed a high diversity and the existence of 6 major clades, clustering mainly on geographical basis. Our sequences were closely grouped with other European isolates.

## Passive surveillance of bat lyssavirus infections in the Netherlands, a three decade overview

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**Keywords:** bat, lyssavirus, Netherlands, EBLV, surveillance

**Introduction:** In the Netherlands, passive surveillance for European bat lyssaviruses (EBLVs) began in 1987. Since then over 5000 bats belonging to 11 native species were tested. Over four hundred serotine bats (*Eptesicus serotinus*) were found positive for EBLV type 1 whereas in 5 pond bats (*Myotis dasycneme*) EBLV type 2 was identified. So far no other lyssavirus types were found in the Netherlands. For all bats, data including species, sex, age, date and location found as well as general condition were recorded if possible. Also, in many cases the situation leading to the finding of the bat was recorded. Contacts with cats were the most reported (~33%), in around 14% of the cases direct contact with humans was reported. **Materials and Methods:** Bat brains were tested using the prescribed fluorescent antibody test (FAT). For conformation a real time PCR test on the N-gene was used. On the same nucleic acid isolation used for the real-time PCR N and G-gene sequencing was performed on an ABI sequencer according to the manufacturers protocol. From almost 200 of the positive cases at least partial N-gene sequences are available. Estimates of the rate of molecular evolution (substitutions per site per year) and the TMRCA for the alignments were inferred using a Bayesian MCMC method in the BEAST package. **Results and Discussion:** Since the start of the passive surveillance system in the Netherlands two strains of lyssaviruses have been detected in indigenous bats. By far the most abundant strain is EBLV-1, which is, at least in the Netherlands, exclusively detected in the serotine bat. This situation is similar to the results of comparable surveillance programs in other countries in mainland Europe. A molecular epidemiology study on a collection of EBLV-1 viruses that currently circulate in the Netherlands detected both subgroups EBLV-1a and EBLV-1b. Surprisingly the larger EBLV-1a group could be further subdivided into two phylogenetic groups that were consistent with topology. A most recent common ancestor analysis was performed which confirmed a distinct difference in origin of the two EBLV-1 subgroups. Whereas the EBLV-1a subgroup migrated into Europe via the east-west route, EBLV-1b apparently entered the continent from the south, most likely through the Iberian Peninsula. EBLV-2 is only detected in five pond bats in the Netherlands, the last positive case dating from 1993. Species identification of these five bats are beyond doubt and recently in a comprehensive study in which whole genome sequences of 29 EBLV-2 strains were compared it was shown that four of these five Dutch EBLV-2 cases indeed form a separate genetic cluster. It remains unclear why EBLV-2 seems to have disappeared from the Dutch territory. These data confirm the importance of a thorough surveillance programme for lyssaviruses in bats for human and animal health.

## **A passive surveillance for emerging viruses in bats in Italy implications for public health and biological conservation**

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**Keywords:** bats, viruses, passive surveillance

Bats are recognized as the reservoir hosts of emerging viruses with zoonotic potential in tropical regions; however, little is known on viruses which may be present in bats in European countries. Considering the poor eco-epidemiological data on the circulation of viruses in bats in Italy, a passive surveillance on bat populations for emerging viruses has been implemented since 2010. The survey involved the analyses of dead animals collected from bat rehabilitation centers or from known roost sites. Fresh carcasses were fully necropsied, and tissue specimens from different organs were analyzed. Fecal samples collected from injured and hospitalized individuals were also sampled and tested. Organs and fecal samples were examined through a broadly reactive PCR-based protocol for the presence of viral agents. Next generation sequencing (NGS), virus isolation techniques and negative staining electron microscopy were also used in order to detect newly-emerging viruses with a potential zoonotic importance or proving dangerous for bats. A total of 635 fecal and tissue samples from 12 different bat species (mostly pipistrelle bats) were collected and analysed between 2010-2017. We demonstrated that coronaviruses (COVs), both AlphaCoVs and BetaCoVs, are widespread among Italian bat populations. The whole-genome sequences of representative viruses belonging to the clade 2c of the genus BetaCoVs (MERS-like CoVs), clade 2b (SARS-like COVs) and to the genus AlphaCoVs were obtained and characterized. The survey also provided evidence that insectivorous bats carry a wide variety of Mammalian orthoreoviruses (MRVs), with members of the type 3 mostly represented and new reassortant strains belonging to serotype 1 and 2. A novel and previously unknown Rhabdovirus named Vaprio virus belonging to the genus Ledantevirus was isolated in cell culture from organs of an adult female of *Pipistrellus kuhlii* spontaneously dead in a wild animal recovery center. Positivity for astroviruses and adenoviruses were also detected by PCR respectively in faecal and organ samples. An adenovirus 2 strongly related to a virus previously detected from *P. pipistrellus* bats in Germany [JN252129] in 2007 was also isolated from the intestine of a young female of *P. kuhlii*. Molecular tests for rabies and related lyssaviruses performed on brain samples resulted constantly negative and no arboviruses have been so far detected in insectivorous bats, despite the endemic presence of Usutu virus and West Nile virus in the sampled areas since 2008. Results offer a preliminary dataset on the distribution of major viral infections in bats in Italy, an achievement so far not yet obtained, which improves our understanding on their spread and evolution and enhanced the methods for detecting the viruses that may emerge from bats. The knowledge of the high biodiversity of bats, the broad geographical distribution and the genetic diversity of bat-associated viruses is crucial for a comprehensive study from which viral discovery studies, viral disease prevention and biological conservation issues can benefit. This study was partially funded by the Italian Ministry of Health under the Research projects: WFR GR-2011-02350591 PGR201100; ISS PE-2011-02351681 PRF201130.



## Does bat diversity explain Ebola spillover?

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**Keywords:** *Biodiversity, public health, zoonotic disease*

Some of the world's deadliest diseases and greatest public health challenges are zoonoses from wildlife, such as Ebola (Ebolavirus spp.). It has been widely hypothesized that increasing human population densities and anthropogenic disturbance largely explain outbreaks of Ebola virus disease. While studies indicate that Ebola is likely hosted by bats (Chiroptera), their role in outbreaks of the disease remains unclear. We tested whether bat species richness (total and within bat families), human population density, and anthropogenic disturbance explain the occurrence of Ebola spillovers within Africa using point process generalized linear models and Maximum Entropy (Maxent) models. Our models consistently showed that spillover occurred in areas with high bat species richness, and particularly areas with high species richness within the Nycterid bat family and low human population density. Spillovers of Ebola have devastating effects on people and communities and our results provide an important step toward understanding how and where Ebola may spillover to human populations.

## **Is stress caused by anthropogenic changes responsible for virus spillover from bats to other species?**

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**Keywords:** *bats, viruses, spillover*

In recent years several viruses that cause no obvious ill effects in their natural bat host have spilled over into other animals, including man, causing serious and often fatal disease. These diseases include, among others, severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS) and porcine epidemic diarrhoea (PED) causing coronaviruses (CoV). The reasons for these spillovers are likely complex, but may involve anthropogenic changes that result in stressful alterations in bat habitat, reduction in food supplies, introduction of new pathogens etc. We have examined the possibility **that bat viruses in their natural hosts cause long-term, low grade persistent or latent infections and that a variety of stressors upset the delicate virus-bat relationship leading to increased virus shedding**– thereby increasing the probability of the spillover of the viruses to other species. Here we show that many (~30%) north American little brown bats (*Myotis lucifugus*) are persistently infected with a coronavirus, closely related to the virus responsible for causing PED, and most (80%) big brown bats (*Eptesicus fuscus*) are latently infected with a gammaherpesvirus. The levels of corona viral RNA in little brown bat intestines is low. However, co-infection with the fungus *Pseudogymnoascus destructans* causes a dramatic increase in virus replication. To elucidate the molecular mechanisms influencing stress-related changes in virus replication, we have established a long-term persistent coronavirus infection in big brown bat kidney cells. Most cells show low levels of viral antigens and RNA, with vastly increased viral proteins and RNA in a few cells. Studies to determine the molecular triggers that allow the virus to switch from low-level persistence to active replication are underway. Results of studies correlating gammaherpesvirus reactivation in free-living brown bats with stress-related changes will also be presented.

## Detection and characterisation of multiple herpesviruses in free-living Western European hedgehogs (*Erinaceus europaeus*)

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**Keywords:** *Molecular virology, wildlife disease surveillance, zoonanthroponosis*

The Western European hedgehog (*Erinaceus europaeus*) has been in significant decline in recent decades in Great Britain. This decline is considered multifactorial and suggested causes include habitat loss and fragmentation, food limitation due to the usage of pesticides, and road traffic accidents. It is unknown if infectious or non-infectious diseases are contributing to the decline. Sporadic cases of herpesvirus-associated disease have been reported in hedgehogs, but there has been little surveillance for, or sequence characterisation of, herpesviruses in *E. europaeus* to date. The aims of this study were to investigate the occurrence of herpesvirus infections in free-living Western European hedgehogs in Great Britain, to characterise these herpesviruses and those previously detected, and to assess the clinical significance of the identified herpesvirus infections in this host. A nested pan-herpesvirus polymerase chain reaction (PCR) targeting a region of the DNA polymerase gene was used to test pooled liver and brain tissues from 129 hedgehogs from across Great Britain, 2011-2016; 59 (46 %) of which were PCR-positive. No statistically significant relationship was detected between PCR result and sex, age class, year or season and infection appeared to be spatio-temporally widespread. In most PCR-positive animals (19/22) from which liver and brain were tested separately, both organs were PCR-positive. Sanger sequencing of amplicons revealed at least two novel viruses within Gammaherpesvirinae. Thirteen of the PCR-positive hedgehogs had liver and brain tissues screened for microscopic abnormalities, of which only one had lesions: non-suppurative meningoencephalitis, but neither intranuclear inclusion bodies nor herpesvirus virions (on electron microscopical examination) were identified. The clinical significance of the gammaherpesviruses detected remains uncertain and it is possible that infection is subclinical and not affecting individual or population health. Whether these viruses can cause substantial disease on initial infection or when reactivated, such as when the immune system is compromised, as can occur with some alpha-, beta- and gammaherpesvirus infections in humans, remains unknown. In addition, samples from two previously described cases of herpesvirus infection in *E. europaeus*, from Sweden and Switzerland, tested positive using the pan-herpesvirus PCR. Sequencing of the whole DNA polymerase gene confirmed two genetically different Human alphaherpesvirus 1 viruses. Although not proved to have caused disease in the Swedish and Swiss hedgehogs, the presence of virus particles, detected by transmission electron microscopy (virions) and/or histopathological examination (inclusion bodies), was closely associated with microscopic lesions and such a causal relationship seems likely. Our results highlight the zoonanthroponotic potential of Human alphaherpesvirus 1 and the need for people working with hedgehogs to practice good hygiene and biosecurity.

## Grey seal (*Halichoerus grypus*) predation on marine mammals in German waters

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**Keywords:** grey seals, predation, marine mammals

Since a couple of years the phenomenon of grey seal predation on marine mammals including harbour porpoises (*Phocoena phocoena*), harbour seals (*Phoca vitulina*) and other grey seals has been described in different areas of the North Sea. Retrospective, earlier cases might have been occurred but were most probably misinterpreted as e.g. bycatch with additionally human caused injuries, propeller wound or shark attack. In 2012, bite marks on harbour porpoise carcasses gave the first hint towards grey seal predation which was confirmed one year later by witnessing a predation of a grey seal on a harbour porpoise. From this point on, grey seal predation should be considered as differential diagnosis in marine mammal carcasses with large wounds especially in the neck area. As part of the German stranding network of Schleswig-Holstein, stranded marine mammals are regularly assessed for their health status and cause of death. During post-mortem examinations in 2017 eight cases (four grey seals, two harbour seals, and two harbour porpoises) were identified as “most likely” preyed by a grey seal. Further 22 cases were classified as “possible” grey seal predation victims. Classification was mainly made macroscopically by inspection of the wound location, pattern and its margin. All carcasses classified as “most likely” grey seal predation victims showed a large tissue defect in the throat/neck area which often circled around the whole neck and could extend to almost the whole body. The skin sometimes remained partly in skin flaps. The blubber in the wound area was either intact, showed punctual incisions or was almost completely removed. Wound margins are typically regular with some triangular-shaped tears. In one harbour porpoise carcass grey seal DNA could be detected within a bite lesion via a rapid loop-mediated isothermal amplification (LAMP) assay. Awareness of this grey seal behavior and the respective wound patterns will help to identify more predation victims. More information is needed to elucidate whether this is a specific behaviour of certain specialized individuals or if the use of marine mammals as prey resource is more widespread in grey seals. Furthermore, studies need to investigate the ecological relevance this feeding behaviour might have on the harbour porpoise and seal populations.

## **Antibiotic resistant *Klebsiella pneumoniae* causing illness in juvenile common seals (*Phoca vitulina*) on the East coast of England**

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**Keywords:** *seals, antimicrobial resistant, Klebsiella, human origin*

The Animal and Plant Health Agency (APHA) Diseases of Wildlife Scheme (DoWS) delivers, in collaboration with other agencies, national wildlife disease surveillance in England and Wales. DoWS working with the Royal Society for the Protection of Animals (RSPCA) Norfolk Wildlife Hospital, which specialises in rescuing free-living sick or stranded common seals, have together been monitoring bacterial pathogens and their antimicrobial resistance patterns, in rescued common seals off the East coast of England for 7 years. This popular holiday coast has the largest breeding colony of common seals in the UK. Preliminary results were published in 2016 (Duff et al.) and molecular characterisation using whole genome sequencing of *Klebsiella pneumoniae* from seal lesions, together with the analysis of antimicrobial sensitivity patterns and comparison with characteristics reported for human isolates, provided evidence that some bacterial pathogens of seals, are directly or indirectly derived from man. This work has continued and, in 2017, a *K pneumoniae* clinical isolate from a common seal was found resistant to an extensive range of antibiotics including, for the first time, to carbapenems, which are 'highest priority - critically important antibiotics' (HP-CIA). Carbapenems are important for treatment of human infections caused by multidrug-resistant Gram-negative bacteria, but are not licenced in Europe for animal therapeutics. The isolate produced an OXA-48-like carbapenemase. This finding indicates that highly resistant Gram-negative bacteria are contaminating coastal waters and environments and can cause disease in seals. Contamination of the marine environment through discharge of sewage into the sea presents the most likely route of exposure for seals. Ref: Duff et al (2016) *Klebsiella pneumoniae* of suspected human origin from free-living common seals on the east coast of England, *Veterinary Record*, 2016, doi: 10.1136/vr.104018

## Genome-wide screening and functional MHC components reveals susceptibility to lungworms in female striped dolphins

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**Keywords:** Heterozygosity Fitness Correlations, RADseq, marine mammals, lung nematode

We investigated Heterozygosity Fitness Correlations (HFC) for a marine mammal known to be subject to epizootic episodes, the striped dolphin (*Stenella coeruleoalba*). Dolphins included in the study varied with respect to their parasite burden of a highly pathogenic lung nematode (*Skrjabinalius guevarai*). Genetic diversity assessed for nuclear SNP loci (from RADseq), microsatellite DNA and exon-2 of the MHC Class II DQB1 locus was measured to evaluate associations with fitness as reflected in *Skrjabinalius guevarai* burden. Microsatellite markers showed no association with respect to parasite load. For the higher resolution RADseq data a clear association was seen, but only for female dolphins for which the more heterozygous individuals had lower *Skrjabinalius guevarai* burden. At the MHC locus the effect was again only significant for females, this time associated with specific alleles and with the charge properties of the residues in the peptide binding region. One RADseq SNP was a strong outlier for association with parasite load (heterozygous in all uninfected females, homozygous for 94% of infected females), found in an intron of the Citron Rho-Interacting Serine/Theonine Kinase (CIT) locus (associated with milk production in mammals). These data provide insight into the mechanisms that determine the impact of inbreeding on fitness in natural populations of this species.

## **So why do they strand? What we know from 25 years of marine mammal surveillance in the UK**

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**Keywords:** *Cetacean, pinniped, marine, surveillance, strandings, long-term data*

Monitoring health in marine mammal populations is critical to identify and quantify the role of disease, trauma and other significant factors affecting these populations. It is also often required to meet statutory obligations and inform strategies for effective population management. Apex species, such as many marine top predators, act as sensors for the health of the ecological communities they depend on. They are sentinels of population and ecosystem health, integrating the impacts absorbed by the many species that comprise the food webs and ecosystems they bestride. In particular, the impact on marine mammal species from environmental pollution, climate change and other anthropogenic activities can be assessed through examination of beach-cast carcasses and, in many cases, can provide data or samples unobtainable by other means. Systematic and coordinated surveillance of marine animal strandings around the UK coast has been in operation since 1992. We present a summary of these long term studies and highlight what we have, and have not, been able to conclude based on these opportunistic data. Funded by the UK governments, the schemes collate reports of marine animal strandings and a subset of cases are necropsied to establish causes of mortality. In the 27-year period between 1991 and 2017, 19,186 marine strandings were reported; 14,927 cetaceans, 4152 pinnipeds, 75 basking shark and 37 marine turtles. Systematic gross necropsy and, where applicable, ancillary histopathological, microbiological, diet, life history and contaminant burden analysis have been undertaken on 4285 cases during this period. From these data, we outline the common direct anthropogenic drivers of mortality including by-catch and/or entanglement (n=718) and ship strike (n=35) and compare this to mortality due to interspecific aggression (n=388). We show other factors for mortality, including infectious disease related mortality (n=664), live stranding (n=506), starvation and debilitation cases (n=448, of which 141 were neonatal starvation cases). We show the apparent incidence of several emerging bacterial pathogens, such as *Brucella* sp and *Photobacterium* sp, in order to highlight the importance of robust infectious disease diagnostics in ascribing accurate cause of death. In a time when budgets are under exceptional pressure and drivers for, and strategies of, wildlife surveillance are under increasing scrutiny, we feel it is particularly important to contextualise the benefits and constraints of long-term datasets such as those that derived from the strandings record. To conclude, we discuss the importance of a complete and detailed necropsy, comprehensive ancillary testing and appropriate multifactorial analysis on individual cases in order to effectively understand the health of, and threats to, at-sea populations.

## Toxoplasmosis in free-ranging Eurasian Beavers (*Castor fiber*) from Switzerland

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**Keywords:** *Eurasian Beavers, Toxoplasmosis, Switzerland*

The Eurasian beaver (*Castor fiber*) was successfully reintroduced to Switzerland in the 19th century. A decade of thorough, systematic pathological examinations did not reveal serious health concerns in the beaver population. However, the brain had rarely been investigated as the preservation of skulls for museum or private collections was a priority. In 2012, an apparently increasing number of animals with abnormal behavior was reported by field partners, and from then on, the brain was examined by histology, revealing frequent inflammatory lesions partly associated with parasitic cysts consistent with *Toxoplasma gondii*. The aim of this study was to describe the occurrence of brain lesions and estimate the relevance of *T. gondii* infection in beavers in Switzerland. From 2012 to 2017, 154 beavers submitted to necropsy were included in the study. Sections of the brain stained with hematoxylin-eosin were examined by light microscopy. Brain samples of 45 beavers with inflammatory lesions with and without visible parasitic cysts were tested by PCR for the presence of *T. gondii* DNA. Brain lesions were observed in 97 beavers (63%) originating from the Swiss Plateau with a majority of adults of both sexes. Among them, 17 cases were shot because of abnormal behavior. The brain lesions consisted mainly of a non-suppurative, lymphoplasmacytic meningoencephalitis or encephalitis. In 28 of the affected animals (29%), the inflammation was associated with the presence of parasitic cysts. Intralesional cysts were 5-70 µm in size, had a thin wall and contained several bradyzoites. The presence of *T. gondii* DNA was confirmed in 20 animals with presence (n=14) or absence (n=6) of intralesional cysts. In most cases brain lesions were mild to moderate and toxoplasmosis was not considered the cause of death (road kills, diverse infectious and non-infectious causes). In seven cases toxoplasmosis was the main diagnosis and considered as the main contributor to the beaver's death; in these cases the brain lesions were moderate to severe. Of these, three had a systemic toxoplasmosis with inflammation and intralesional cysts in other organs. Our findings indicate that *T. gondii* infection is widespread in Eurasian beavers in Switzerland and may be associated with disease ranging from various degrees of inflammation in the brain up to fatal systemic disease. Toxoplasmosis has been reported as a health issue in several marine mammals. Its occurrence in beavers shows that it is also an issue in the fresh water environment and underlines the risk posed by free roaming domestic cats to aquatic mammals. We suspect that toxoplasmosis did not emerge in 2012 in beavers in Switzerland but it was most likely previously not recognized due to restrictions concerning the head examination. We recommend to systematically examine the brain of dead beavers in the framework of health surveillance programs and to consider *T. gondii* as a possible differential diagnosis of brain disease even in the absence of visible parasitic cysts.



## Surprisingly Long Lungworms from Common Seals of the Dutch North Sea

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**Keywords:** *Parafilaroides gymnuris*, lungworm, eastern Atlantic common seal

Lungworms are responsible for the foremost disease of eastern Atlantic common seals, *Phoca vitulina vitulina*, in the Dutch North Sea. This parasitic bronchopneumonia is caused by two nematode genera, *Parafilaroides* and *Otostrongylus*. A recent sharp increase in lungworm infections in this location and initial observations that the *Parafilaroides* sp. adults were unusually long, prompted us to ask whether these worms were as expected, *P. gymnuris*. These nematodes are usually small and delicate, making them difficult to extract from the host tissue and there is often difficulty in accessing fresh specimens from seals for morphological study. The large size of the North Sea worms and the accessibility of specimens from numerous animals, as provided by the facilities at the Seal Centre, Pieterburen, The Netherlands, enabled the description and measurement of many intact specimens (N=64) from numerous host animals (N=20). In addition to describing these parasites morphometrically, we characterized them by sequencing the ITS-2, D3 expansion and COI regions of DNA. Although these worms were consistent with the majority of the features described for *P. gymnuris*, the mature females were surprisingly long for the species in common seals. Our females were significantly longer than the *P. gymnuris* females previously described from eastern Atlantic common seals ( $P \leq 0.001$ ) from France and also than those described from western Atlantic common seals, *Phoca vitulina concolor* ( $P \leq 0.0001$ ) from Canada. Our mature females were also significantly longer than mature female *P. gymnuris* described from other seal host species in the literature, including harp seals, *Phoca groenlandica*, from St. Brides, Newfoundland, Canada ( $P < 0.05$ ). However, they were not significantly longer than the *P. gymnuris* females previously described from harp seals collected in Les Escoumins, Quebec, Canada. Several morphological characters showed an individual host animal effect in our specimens, including the length of the mature females ( $P < 0.05$ ). We determined that there was a strong plasticity in body length of mature female *P. gymnuris* between different seal host species, geographic locations (western Atlantic versus eastern Atlantic), individual host animals and over time in the eastern Atlantic common seal (current time compared to the 1899 description). We speculate that intraspecific genetic differences in *P. gymnuris* and the environment within the host could play a role.

## Clinical Significance of *Uncinaria* spp in Orphan Mediterranean Monk Seal Pups in Greece

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**Keywords:** monk seal, *Uncinaria* spp, anaemia

With fewer than 700 individuals remaining, from which approximately 350 live in Greece, the Mediterranean monk seal (*Monachus monachus*) is classified as “Endangered” by the IUCN. The knowledge about the biology and health problems of the species is still limited and poorly understood. Therefore studying the effects of any parasitic disease on Mediterranean monk seal populations is of utmost importance. The main aim of the present study was to determine for the first time the prevalence of *Uncinaria* spp. and its clinical significance in stranded Mediterranean monk seal pups rehabilitated in Greece. Since 1990, 30 stranded monk seal pups (16 males and 14 females), have been rescued and rehabilitated in MOM’s Rehabilitation Facility, in cooperation with the School of Veterinary Medicine, AUTH; their age ranged from 1 week - 2.5 months, and their weight from 9kg - 18kg. On presentation, severe dehydration, malnutrition, mild to severe skin lesions, and mucosal pallor were noticed. After oral rehydration, fecal and blood samples were collected from all monk seal pups. Eggs of *Uncinaria* spp. were found in the rectal feces (collected late August to late November) of all animals, whereas in 7 animals adult hookworms were also recovered. Parasite eggs were found in feces using a flotation parasitological technique and the identification of the adult worms was based on their morphology according to standard identification keys. The findings (range and median value, respectively) of the haematological evaluation were as follows: Packed Cell Volume (9.0%-58.0%; 36.5%), haemoglobin (2.7-18.8 gr/dl; 11.5 gr/dl), erythrocyte (0.78-4.72x10<sup>6</sup>/μl; 3.0x10<sup>6</sup>/μl), leukocyte (11.7-37.0x10<sup>3</sup>/μl; 21.4x10<sup>3</sup>/μl) and platelet counts (194-962x10<sup>3</sup>/μl; 537x10<sup>3</sup>/μl). Body condition, malnutrition and PCV in monk seal pups were directly related to the number of hookworms present. Pups in poor condition and anaemia (9.0-28.6) had significantly higher numbers of hookworms than the pups in good condition. All animals were treated with fenbendazole po at a dose of 10mg/kg for 3 successive days. Seventeen animals died and 13 survived and were safely released back in the wild after reaching a certain age and weight. Hookworms (*Uncinaria* spp.) are common parasites in otariids. The relationship between the hookworm infection and the clinical hookworm disease remains unclear, but it appears that the clinical appearance is directly related to the number of hookworms present. Hookworm disease can have a detrimental impact on juvenile pinnipeds. Considering that nearly half of animals died shortly after entering the rehabilitation program, we conclude that parasitological examination along with haematological and biochemical evaluation upon admission, are of utmost importance for the health screening and prognosis of rehabilitated monk seal pups.

## Vomiting in seals indication of bycatch?

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**Keywords:** *seals, food aspiration, bycatch*

Within one week, between November 5 and 11 2017, a local die-off of adult grey seals (*Halichoerus grypus*) took place in Zeeland, the Netherlands. Six animals were found stranded; all had died recently and were in good body condition. Veterinary pathologists at the Dutch Wildlife Health Centre (DWHC) performed necropsy on four of these animals: three males and one pregnant female carrying a male fetus. Dietary investigation was performed at Wageningen Marine Research, toxicological analysis on stomach contents was done at Ghent University and RIKILT. All animals were found to have fed shortly before death and showed strong esophageal dilation by partially digested food extending into the oral cavity, trachea and bronchi. One seal had acute intraocular hemorrhages, another acute systemic petechial hemorrhages. Diet consisted of varying amounts of herring (*Clupea harengus*), sprattus (*Sprattus sprattus*), and sand smelt (*Atherina presbyter*). The herrings, sprats and sand smelts were considered small prey when compared to predator size. Toxicology of stomach contents excluded presence of phosphate-esters, carbamate-esters, strychnine, tetrodotoxins, saxitoxins, domoic acid and anatoxins. Combination of good nutritional condition, recent feeding and exclusion of other causes of acute death lead to strong suspicion of bycatch as cause of death. Digested food in the esophagus indicated vomiting, resulting in food aspiration during agony and acute death. In our experience, esophageal distention by stomach contents and/or agonal food aspiration is an uncommon necropsy finding in wild seals. Here, four food aspiration cases were associated with strong suspicions of bycatch. Therefore, we suggest that food aspiration in seals may be added to the list of indications of bycatch.

## Changing ecosystem dynamics: Increasing evidence of direct lethal, indirect lethal and non-lethal interactions between grey seals and harbour porpoises

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**Keywords:** *inter-species interaction, marine mammals, causes of death*

In the southern North Sea, hundreds of mutilated harbour porpoise (*Phocoena phocoena*) carcasses are found each year. In 2014, the grey seal (*Halichoerus grypus*) was identified as the perpetrator based on DNA evidence and it was confirmed that mutilated porpoises are the remains of predation by grey seals. Apart from an acute death, harbour porpoises may also escape from a predatory attack. Here, direct lethal, indirect lethal and non-lethal interactions between these two top predators are identified with the aim of establishing a frequency of occurrence of such events, and the potential impacts of these interactions on the harbour porpoise population in the North Sea are discussed. A retrospective study of post mortem photos dating back to 2003 helped to characterize grey seal induced wounds and indicated that grey seal predation is one of the main causes of death of harbour porpoises that strand in the Netherlands. Approximately 20% of all necropsied porpoises directly died as a result of inflicted injuries. Additional to acute deaths, bite marks with associated inflammation were also present on harbour porpoises that died of infectious causes, including bacterial septicaemia. The bite wounds may have acted as the port of entrance. In 2016 and 2017, 13/110 (12%) necropsied harbour porpoises that demonstrated sub-acute or chronic bite wounds died of associated infectious disease or emaciation and general debilitation. Cultures of these bite wounds and organs of the same individuals revealed infections with, among others, *Salmonella* spp., *Streptococcus* spp., *Staphylococcus hyicus*, *Neisseria animaloris* and *Photobacterium damsela* as determined by MALDI-TOF; some of which are possibly originated from grey seal oral cavities. Finally and in addition to lethal and sub-lethal interactions, non-lethal interactions also occur. In at least four harbour porpoises belonging to the small resident population in the semi-enclosed tidal bay ‘the Eastern Scheldt’, the Netherlands, bilateral tailstock lesions and additional body scarring that matched descriptions of lesions induced by grey seals were identified. These lesions appeared to be completely healed and sightings of these scarred animals in multiple years demonstrates complete recovery from the attacks. The extensive investigation of the interactions between grey seals and harbour porpoises, revealing both direct and indirect lethal cases, as well as cases of survival, resulted in an increase in the diagnose of grey seal induced mortality in necropsied harbour porpoises. It may be concluded that the grey seal could be considered a significant factor in mortality of harbour porpoises. This has the potential to affect ecosystem dynamics, yet currently to an undefined extent. Behavioural adaptations in harbour porpoises, aiming to prevent detection, encounter, and, eventually capture by grey seals, can be expected, especially considering the increasing numbers of grey seals in the North Sea region.

## **Dead useful; Separating the Signal from the Noise through Marine Mammal Strandings Surveillance**

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**Keywords:** *marine mammals, strandings, surveillance, cetaceans, pinnipeds, marine ecosystems*

The value of health data derived from wildlife populations is often challenged due to the perception of biases associated with opportunistic surveillance and the complexities of inference from the individual to the population. This is particularly prominent for the surveillance of marine ecosystems, where even the most basic population data are largely hidden from direct observation. Marine mammals are considered useful indicators of wider ecosystem health. Nevertheless given the large heterogeneity of the marine environment and large range and high mobility of marine mammal species; population studies on living animals are notoriously difficult and logistically challenging, and crucial metrics of population health are largely to be inferred from stranded animals. There are several biases associated with the strandings process and observed mortality through strandings surveillance can be seen as a function of the biological signal of interest, along with noise created by a number of environmental and social factors. An animal needs to make landfall in order to be recorded, which is influenced by a number of environmental confounders such as wind, currents, tides, and coastal topography. It then has to be found and reported, hence data quality is highly variable and dependent on public engagement and awareness. Nevertheless the long term accumulation of continuously collected stranding data and samples makes analysis of trends and patterns possible, providing means to define a baseline for mortality as well as biological metrics of the stranded population. This allows the assessment of the role of disease, trauma or other significant factors affecting these populations, and assists in identifying change, pressures and threats. In Scotland the coordinated surveillance of marine mammal strandings is done by the Scottish Marine Animal Stranding Scheme (SMASS) since 1992. The scheme collates reports and approximately 70 animals per year are subjected to necropsy to establish a cause of death. We show how this dataset has allowed us to establish a baseline in stranding frequencies of harbour porpoises, and how this facilitates the identification and quantification of unusual mortality events. Secondly we discuss how improvements to the surveillance strategy provides means to reduce the bias associated with the currently opportunistic method of data collection. In 2014 SMASS implemented a collaborative citizen science strategy through establishing a trained volunteer network, which already yields data of higher quality and from a wider geographical extent than was possible before. With technology maturing this initiative is currently further progressed by the development of a mobile phone app which allows recording of searching effort which, in combination with sophisticated statistical methodologies, has the potential to quantify sampling biases and thereby generate estimates that account for the non-standard way strandings data are obtained. As with many other opportunistic wildlife data, information derived from strandings usually cannot be gathered by other means, thus tools to improve and expand the utility of these data will be of significant benefit.

## **Marine debris and plastic pollution – an emerging non-infectious threat to aquatic wildlife and human health**

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**Keywords:** *Plastic pollution, Marine debris, Marine mammals*

Ocean plastic pollution is a widely recognised problem of increasing environmental and public health concern. Plastics degrade slowly and macro pieces are usually grinded into smaller micro and nano particles. Plastics and their chemical derivatives also enter the human food chain mainly through ingestion by aquatic organisms and subsequent bioaccumulation, but also through the use of sea and river water. According to recent studies, between 4.8 – 12.7 million metric tonnes of plastic leak into the world's oceans every year. It is known that the highest marine debris input comes from land-based sources. Up to 94% of the total plastic load is transported through rivers from cities into the oceans. This plastic pollution poses a threat to the marine environment and its flora and fauna. 56% of all cetaceans, from small fish-eating porpoises to the huge filter feeding baleen whales, have been recorded to ingest marine debris, which they have most likely mistaken for prey. Entanglements can cause severe physical trauma and are of major animal welfare concern, while ingestion can lead to death through internal injuries or have long-term ecological effects. It was found that especially micro plastics are capable of adsorbing chemical pollutants and releasing them together with plastic additives inside organisms which thus leads to bioaccumulation. This has been linked to developmental problems, immunosuppression and reproductive failure. Records of 1622 harbour porpoises (*Phocoena phocoena*), harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) examined by the Institute for Terrestrial and Aquatic Wildlife Research between 1990 and 2014 along the German coasts revealed 14 entanglements and 17 cases of debris ingestion. The detected objects comprised fishing-related debris (64.9%) as well as general debris (35.1%), and associated pathologies included suppurative ulcerative dermatitis, perforation of the digestive tract, abscessation, suppurative peritonitis and septicaemia. Furthermore, large amounts of marine debris (250 fishing-related items and 72 pieces of general debris) were found in nine out of 22 sperm whales (*Physeter macrocephalus*) that had stranded along the North Sea coast in early 2016. While the debris was not responsible for the sperm whales' death, the findings clearly demonstrated the risk of severe consequences due to the quantity (24 kg net in one individual) and the quality (fishing net of 13.01 m length; a broken hard plastic bucket; 68 × 23.5 cm large engine cover of a ©Ford SUV) of the ingested items. Both studies match other assessments, showing the evidently high level of top predator species exposure to marine debris and the imminent threat of associated health and welfare risks.

## National-wide survey for vector-borne pathogens in wild and domestic canines and associated ticks and fleas in Chile

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**Keywords:** *carnivores, vector-borne, shared diseases, tick, flea, South-America*

Canine vector-borne pathogens (CVBP) comprise a group of disease agents transmitted by arthropods infecting dogs worldwide. Wild canids are increasingly recognized as potential reservoirs for many of these pathogens, that may in turn affect the viability of populations of endangered species. Chile hosts three species of foxes: two widely distributed and abundant species (the Andean or culpeo fox *Lycalopex culpaeus*, and the chilla or gray fox *L. chilla*), and a menaced species with a restricted distribution area (the Darwin's fox *L. fulvipes*). The country hosts about 4 million dogs. Those inhabiting the countryside, that are prone to share ectoparasites and pathogens with foxes, use to roam free and rarely receive veterinary care. The information about the presence and importance of CVBP in Chile was very limited to date, and almost no information at all existed for foxes. Besides, the characteristic geography of Chile makes the country an ideal scenario for the study of CVBP and their vectors. Since 2014, by a combination of active and passive surveillance, we have surveyed 650 rural dogs and 250 foxes (130 Andean, 40 chilla and 80 Darwin's) from seven different bioclimates, from the high steppe in the North to the oceanic climate in the South, including the tropical Easter Island and the Mediterranean central region. *Amblyomma tigrinum* was the tick species most frequently observed in Andean foxes surveyed alive, though *Rhipicephalus sanguineus* was also identified. In contrast, dogs were almost exclusively infested by the latter species. The presence of ticks was restricted to the warmer seasons. In Andean and chilla foxes, fleas were far more frequent and more widely distributed than ticks. The most prevalent flea in foxes belonged to *Pulex* spp., followed by *Ctenophalides felis*. This latter species was the most frequent in dogs. Almost no ticks or fleas have been retrieved from Darwin's foxes. *Anaplasma platys* was detected in foxes and dogs from all study areas, with the exception of the southernmost regions and the Eastern Island, with diverse prevalence depending on the region and the host species, and resembling the known distribution and abundance of its putative vector, *Rh. sanguineus*. In contrast, *Ehrlichia canis* was not confirmed in any sample. We described for the first time the presence of *Hepatozoon* sp. in Chile: a sequence closely related to *H. felis* was detected in a high proportion of foxes from the central region. *Hepatozoon* sp. was also detected in dogs but with markedly lower prevalence. Hemotropic mycoplasmas, including *Mycoplasma haemocanis* (Mhc) and *Candidatus M. haematoparvum*, were confirmed in all the species, and beyond the southern limit of the distribution of its putative vector, *Rh. sanguineus*. Mhc was fairly common in the Darwin's fox, showing significantly higher prevalence in this species than in sympatric rural dogs. We confirmed the infection of Andean foxes and rural dogs with *Trypanosoma cruzi*, the causal agent of Chagas' disease. We are expanding the knowledge about the distribution and importance of CVBP and their vectors in Chile and the southern cone of South America.

## A survey of tick-borne pathogens in ixodid ticks and their wild boar hosts in the Barcelona metropolitan area

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**Keywords:** hard ticks, *Rickettsia* spp., *Sus scrofa*

Wild boar (*Sus scrofa*) populations have been steadily increasing in the highly populated Barcelona metropolitan area (BMA, NE Spain) for the last 35 years. Since wild boar in BMA are commonly infested by ixodid ticks, the zoonotic pathogens transmitted by these ticks represent an increasing public health concern. Our aim was to describe the species and relative abundance of ticks hosted by wild boar and to determine the presence of selected tick-borne pathogens (TBP) in ticks and their wild boars hosts in BMA. From 2013 to 2016, we examined 486 wild boar hunted or captured and euthanized for population management purposes in the Collserola massif and peri-urban/urban areas within BMA. We collected 2,235 ticks and 167 spleen samples from 261 of these animals. Ticks were morphologically identified and pooled according to species and individual host. A selection of 180 tick pools and all the spleen samples were screened by reverse line blot hybridization assay and/or conventional, nested and real-time PCR for *Borrelia burgdorferi* sensu lato, *Coxiella burnetii* and members of the genera *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Theileria* and *Babesia*. Amplification products were sequenced to clarify the identity of the species detected. Four species of tick were encountered on wild boar, namely *Hyalomma lusitanicum* (51%, 1143), *Rhipicephalus sanguineus* sensu lato (25%, 558), *Dermacentor marginatus* (24%, 533) and *Rhipicephalus bursa* (<1%, 1). Infestation prevalence was 54% (261/486), with a median abundance of five and a range of 1 to 70 ticks per boar. One-tick species infestation predominated (67%, 175/261), *H. lusitanicum* parasitizing most of the infested wild boar (56%, 146/261). *D. marginatus* and *R. sanguineus* s.l. appeared in nearly 45% (117/261) and in 33% (87/261) of the infested wild boars, respectively. *Rickettsia* spp. DNA was amplified in 90/180 tick pools, *D. marginatus* (76%, 56/74) and *R. sanguineus* s.l. (72%, 31/43) displaying significantly higher ( $p < .001$ ) detection rates than *H. lusitanicum* (5%, 3/62). The sequencing of a citrate synthase gene fragment (*gltA*, n=62) revealed three different *Rickettsia* species. *Rickettsia massiliae* was found in *R. sanguineus* s.l. tick pools (28/28) while *Rickettsia slovacica* (24/34) and *Rickettsia raoultii* (9/34) were both detected in *D. marginatus* pools. *R. slovacica* was also identified in the one *H. lusitanicum* pool sequenced. Wild boar spleen samples were negative for *Rickettsia* spp. No evidence of the presence of other TBPs was obtained. To our knowledge, this is the first description of *R. slovacica* in *H. lusitanicum* ticks. In addition, we demonstrate circulation of pathogenic spotted fever group *Rickettsia* species in ticks parasitizing wild boar roaming urban and peri-urban areas and inhabiting a highly visited natural area in BMA. Tick-borne rickettsiae are considered to be emerging and wild boar might play a role in dispersion of infected ticks given the increasing trends of wild boar abundance and presence in urban areas.



## Landscape and invasive species effects on infection dynamics of *Bartonella* in indigenous rodents from southern Africa

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**Keywords:** *Strain spillover, zoonotic potential, rodents*

South Africa has a rich rodent biodiversity with more than 50 species occurring in this country, many of which are indigenous. In addition, at least four invasive rodent species are also known to occur. Despite recognized epidemiological links between rodents and a range of infectious diseases of animal and public health concern, and their key role in perpetuating ectoparasite life cycles, baseline information on rodent-borne diseases is presently lacking for the southern African region. *Bartonella* species are vector-borne bacterial pathogens known to infect wildlife, livestock and humans. Rodents have been reported to play a significant role as reservoirs of over 22 rodent-associated *Bartonella* species. In this study, we contrast prevalence and diversity of *Bartonella* infections in 268 small mammals representative of three terrestrial rodent genera, namely *Aethomys*, *Rattus* and *Rhabdomys* and one subterranean mole-rat species (*Bathyergus suillus*). The latter were sampled in close proximity to an informal settlement, whereas the afore-mentioned murid rodent genera were sampled across a range of landscapes (natural, urban, peri-urban and rural settings) from three provinces (Gauteng, Limpopo and Western Cape) in South Africa. Molecular estimates of *Bartonella* infection rates were determined through multi-gene PCR assays targeting the citrate synthase (*glcA*) gene and NADH dehydrogenase gamma subunit (*nuoG*) or beta subunit of bacterial RNA polymerase (*rpoB*) genes. Of the indigenous species evaluated, *Aethomys* spp. had an infection rate of 85%, whereas invasive *Rattus* sampled from the same locality had an infection rate of 7%. *Bartonella* sequences obtained from *Aethomys ineptus* clustered within three lineages of which one was closely related to zoonotic *B. elizabethae*. The latter species is associated with *Rattus* hosts worldwide and has been linked to cases of human endocarditis. Thus detection of this species in invasive *Rattus* spp. and in indigenous rodents from the same locality suggests spillover from invasive species to endemic species, and the potential for establishment of zoonotic strains in endemic fauna and their associated ectoparasites. Interestingly, this was previously observed in *Micaelamys namaquensis*, a highly adaptable species, which like *Aethomys* is capable of utilizing natural and modified landscapes. Our data further reveal that spillover from invasive *Rattus* is not only limited to terrestrial small mammals, but possibly also to at least one subterranean mole-rat species, *Bathyergus suillus*. Molecular assessment of the latter vertebrate hosts revealed that, in addition to hosting species-specific *Bartonella* lineages, that spillover between surface-dwelling invasive *Rattus* and indigenous *Rhabdomys* to *Bathyergus* may explain the presence of terrestrial rodent *Bartonella* strains in subterranean small mammals. Our results further indicate that the level of anthropogenic transformation is significantly correlated with *Bartonella* prevalence, with *Rhabdomys* sampled from rural settings having infection rates of 36% versus 0% in a nearby urban setting. This study has identified a number of small mammal reservoirs of *Bartonella* in South Africa, including those shown to host zoonotic *B. elizabethae* and *B. rochalimae*-like strains.

## Understanding wildlife disease requires good host data: wild boar and ASF risk

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**Keywords:** *Sus scrofa*, species distribution model, wild boar, ASF, citizen science

Emerging and re-emerging diseases in wildlife are a growing concern, with a list of 33 diseases in Europe (as defined by WiREDS) that includes many mammal taxa. For mammals in particular we have very limited data on the host abundance, distribution and density across Europe. Having good quality data on host abundance and distribution are vital to understanding the risks of disease spread, and effects of management. Where is the host most abundant? Which areas are likely to have a density that permits disease spread? If management is through bait distribution (e.g. vaccines), how many baits are required in different areas? If management is through culling, how much effort or how many animals must be removed to reduce density to stop disease spread? Models of disease spread require data on the distribution and density of the host. Such models are also useful to simulate management options and compare the output of management with expectations, to provide an adaptive management approach. Here, I report on an ongoing project (ENETWILD) that is currently looking at the distribution and abundance of wild boar in Europe because of the emergence of African Swine Fever (ASF) in Eastern Europe. This is of international concern, as shown by the recent Bulgarian Presidency of the EU taking a strong interest in wildlife disease in general, and ASF in wild boar in particular. I will present the three types of available data (occurrence, density and hunting statistics) and show how these can be used to produce refined maps of wild boar distribution and abundance. With examples, I will demonstrate why more occurrence and density data are required to provide the best estimate of host abundance and present a road-map of how we should collate the available data and collect future data to provide the best information to make future decisions on emerging diseases in wildlife.

## **Pathogenic potential of Bagaza virus in three phasianid species: Red-legged partridge, grey partridge and common pheasant**

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**Keywords:** *Bagaza virus, experimental inoculations, game birds*

Bagaza virus (BAGV), synonymous to Israel turkey meningoencephalomyelitis virus (ITV), is a mosquito-borne epornitic flavivirus. Before 2010, the circulation of BAGV had been demonstrated only in sub-Saharan Africa, Israel and India. In 2010, it was detected for the first time in Europe as the etiological agent of an outbreak that affected game birds (red-legged partridges and common pheasants) in Southern Spain. The detection of specific neutralizing antibodies in 2011-2012 in game birds from the same area suggested sustained circulation of the virus in Southern Europe. The main objective of this study was to assess if the BAGV strain isolated from this outbreak was able to reproduce, under experimental conditions, the disease observed in the field. For that, we experimentally inoculated the virus in the species affected during the outbreak: red-legged partridges (*Alectoris rufa*) and common pheasants (*Phasianus colchicus*). Moreover, another relevant game bird species, the grey partridge (*Perdix perdix*), was also included in the study. A complete clinical, virological and pathological assessment of the disease was made and the susceptibility and potential role as natural reservoir of the three species was evaluated. In three independent experiments, 10 adult red-legged partridges, 10 grey partridges and 10 pheasants were inoculated with an infectious dose of BAGV. In all cases, 4 non-inoculated birds were caged together with the inoculated ones as contact group and an additional sham-inoculated group was maintained as control group. Birds were observed daily for clinical symptoms and a follow-up of viral load in blood, feathers, oral and cloacal swabs (by real-time RT-PCR), and neutralizing antibody titres (by VNT) was made up to 15 days post-infection (dpi). Viral inoculation triggered a systemic infection in all birds, although important differences in morbidity and mortality rates were observed depending on the species. The red-legged and grey partridges were the most severely affected by the infection, suffering a remarkable weight loss (up to 23% in red-legged and 13% in grey partridges) and a mortality rate between 30% and 40%. By contrast, common pheasants only suffered a mild weight loss and no mortality was registered. The highest viral loads in blood, feathers and swabs were detected in red-legged partridges, followed by grey partridges and pheasants. Based on viremia results, red-legged partridges were considered competent hosts for BAGV while the role in virus transmission was limited for grey partridges and pheasants. Direct (non-vectored) transmission of the virus was only observed in red-legged partridges as the 4 contact birds became viremic and seroconverted. In conclusion, the three species were susceptible to the infection although the clinical outcome was species-dependent: severe in red-legged and grey partridges and mild in pheasants. The host competence capacity also varied among species, being the red-legged partridge the species with the highest transmission capacity.

## Past and current features of Usutu virus circulation in Hungary and Austria

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**Keywords:** *arbovirus, flavivirus, Usutu virus, Turdus merula, blackbird, Austria, Hungary*

Following the first contemporary detection of Usutu virus (USUV, Flaviviridae) circulation on European soil in 2001 in Austria, the virus soon also emerged in Hungary in 2005. A sequence of Europe wide USUV emergence events followed between 2005 and 2011 (Italy, Switzerland, Spain, Germany, Czech Republic) with further expansion in 2016 signalled by wild bird mortality in Germany, France, Belgium and the Netherlands. In addition to intermittent targeted research efforts and reliance on mortality events reported by the public, the passive surveillance scheme of wild bird mortality cases established in the wake of the 2005/2006 H5N1 avian influenza epidemic provided a steady background for the parallel monitoring of Avian Influenza Virus, West-Nile Virus (WNV) and USUV activity in the region. This report presents the results of passive monitoring of USUV between 2010 and 2017 (Austria 2016). Sporadic cases of USUV related wild bird mortality were diagnosed between 2010 and 2015 in Hungary (6 cases: 5 blackbirds (*Turdus merula*) and 1 fieldfare (*Turdus pilaris*)), followed by a significant increase in summer and autumn of 2016 (12 cases: 10 blackbirds, 1 Eurasian jay (*Garrulus glandarius*) and 1 European starling (*Sturnus vulgaris*)). Further USUV activity was detected during 2017 (5 cases: 4 blackbirds and 1 Eurasian tree sparrow (*Passer montanus*)). USUV infection was identified in two blackbirds in 2016 in Austria. Phylogenetic analysis based on complete genome and partial NS5 protein gene sequences established that the 2010-2015 USUV strains from Hungary are closely related to the virus that emerged in Austria in 2001 and then in Hungary in 2005. However, one Hungarian sequence from 2015 and all sequences from Hungary and Austria from 2016 clustered with USUV sequences reported from Italy between 2009 and 2010. Our results of the study indicate a persistent USUV circulation in the region and the exchange of USUV strains between Italy, Austria and Hungary. This evidence is congruent with the implications of epidemiological and genetic features of identified following the westward expansion of USUV in recent years. Epidemiological and evolutionary patterns of USUV in Europe seem to exhibit similar features to those of WNV on the same continent. Understanding USUV epidemiology and environmental factors shaping its endemicity and intermittent outbreak patterns are ever-more intriguing in the light of recent studies indicating that USUV possesses a much higher zoonotic significance than so far perceived.

## Seasonal longevity of the West Nile virus vector mosquito *Culex pipiens*

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**Keywords:** *biodemography, aging in wild, vector biology*

The epidemiological importance of the age structure and longevity potential of wild populations of mosquito disease vectors has been known for over 60 years. However, no routine method currently exists that provides reliable insights into the population age dynamics of this medically important group of insects. In this paper we use a technique originally developed for studying wild fruit fly populations to study the post-capture longevity dynamics in populations of the West Nile virus mosquito *Culex pipiens* in Greece. This approach, referred to as the captive cohort method, analyzes and interprets the longevity trends in wild-caught *Cx. pipiens* to infer demographic changes in their field population. Approximately 10 adult females were captured each day from June through November, housed in individual cages in the laboratory, and their remaining longevity recorded. Strong differences were observed in the mean, variation, and extremes of post-capture longevity. Early season (June–July) mosquitoes lived the shortest and late season the longest with a clear transition period in September. The mean levels of post-capture longevity were quite high at over 2 months in early season to over 85 days in late season when the vast majority of adults were nulliparous and likely preparing for hibernation. Implications for both basic and epidemiological research are discussed.

## Magpies and West Nile Virus: Reservoir or Sentinel?

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**Keywords:** *Magpie, West Nile Virus lineage 1 and 2, reservoir*

West Nile virus (WNV), a zoonotic pathogen naturally transmitted by mosquitoes whose natural hosts are birds, has spread worldwide during the last few decades. Resident birds play an important role in flavivirus epidemiology, since they can serve as reservoirs and facilitate overwintering of the virus. Magpies (*Pica pica*) are abundant corvids in Europe that thrive in peridomestic urban and rural habitats. They have been suggested to play an important role in West Nile virus epidemiology. Integrating field and experimental data, we try to elucidate this role further. For this we carried out an experimental infection of magpies with a lineage 1 (NY-99) and a lineage 2 (SRB Novi-Sad/12) West Nile virus. Magpies were highly susceptible to WNV infection, with similar low survival rates (30% and 42.8%) for both lineages. All infected magpies developed viremia detectable at 3 days post-infection with titers above those necessary for successful transmission of WNV to a mosquito. Neutralizing antibodies were detected from 7 to 17 days post-infection. WNV genome was detected in the brains and hearts of all magpies that succumbed to the infection, and, in some of the surviving birds at the end of the experiment (D17). WNV-RNA was amplified from swabs (oral and cloacal) at 3, 6 and 7 days post-infection and feather pulps, from 3 to 17 days post-infection, of infected animals. Infectious virus was recovered from swabs up to 7 days post-infection and from feather pulps up to 10 days post infection. Sham-infected control animals were negative for viremia, viral RNA, and antibodies. These results suggest that the magpie, could represent a source of WNV transmission for birds and humans. None of 343 magpies tested in the field were shedding WNV on the day of capture, but 61 (17.8%) were positive against WNV in a commercial ELISA. Nevertheless only one of these (0.3%) was confirmed to have WNV antibodies by VNT while another was VNT seropositive against Usutu virus. This data confirms that magpies are exposed to and infected by different Flaviviruses that crossreact with WNV and USUV and may modulate the epidemiology of WNV in this host.

## **First report of massive deaths in wild birds with neurological signs due to West Nile Virus infection during an epizootic outbreak in Peloponnesus, Greece, 2017**

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**Keywords:** Greece, West Nile Virus, Wild birds

West Nile Virus (WNV) is a mosquito-borne flavivirus causing fatal encephalitis in humans, horses, domestic and wild birds. Wild birds play an important role as reservoir hosts and in the introduction of the virus to new unaffected areas. Sentinel birds are typically used for arbovirus surveillance and the detection of WNV activity in a specific area. The largest European outbreak took place in Greece, with more than 624 confirmed cases of human infection and 79 deaths reported from 2010 to 2016. In 2017, a total of 48 laboratory confirmed cases of WNV infection were reported to Hellenic Centre of Disease Control and Prevention (HCDCP). Among the WNV disease cases, five deaths were reported in patients over 70 years of age. Since June 2017, one month before human cases occur, dead wild birds, more specifically Eurasian magpies (*Pica pica*) and Hooded crows (*Corvus corone cornix*), were reported in different areas of Argolida regional unit, in the Peloponnese region. Until mid-July, a noticeable reduction of the native wild bird population, especially Eurasian magpies, was mentioned by locals. Moreover, presence of wild birds with neurological signs was verified near village of Inachos; affected birds were lethargic, unable to fly, stayed low to the ground and had no reaction to external stimuli (i.e. human presence). During July and August 2017, dead Eurasian magpies were collected in the study area for WNV molecular detection. We report the first RT-PCR positive results for WNV RNA in Eurasian magpies (*Pica pica*) with neurological signs that were found dead during the latest re-emergence of the disease in the region of Argolida, Peloponnesus, Southern Greece. RT-PCR was performed using different primer pairs targeting various regions of WNV genome and viral RNA was detected in brain and tissue pools. Sequence analysis confirmed the detection of WNV that was subsequently clustered with lineage 2 strains. Moreover, we collected serum samples from 190 free-living chickens (1 to 10 months of age), from 40 farms in the study area; antibodies against WNV were detected in 32 samples from 17 farms. Surveillance in various wild and domestic animals and vectors allows the timely detection of WNV local circulation and implementation of appropriate measures such as mosquito population control, to reduce the disease incidence in humans and animals.

## Combining long-term testing and ecological data to improve the accuracy of disease diagnosis: An example from a long-term study of tuberculosis in wild badgers in the UK

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**Keywords:** *Diagnosis, Probability, Tuberculosis*

**Background:** Accurate diagnosis of many infectious diseases in wildlife is challenging owing to imperfect diagnostic tests. The cryptic nature of many wild animal populations adds an extra layer of complexity because they are often unobserved for long periods and may be difficult to catch and sample. This poses a problem because infectious diseases in cryptic populations can be immensely important: the majority of emerging infectious diseases originate in wildlife. Methods for detecting infection in such populations need to try to account for hidden or uncertain processes if they are to reveal the true dynamics of disease and help us to develop effective disease control interventions. **Study question:** How can we combine ecological and epidemiological data, including repeated measurements from the same animals over time, to improve the accuracy of disease diagnosis? **Objective:** To estimate the probability that any individual badger is truly infected using information from the whole of its capture history and any combination of previous diagnostic test results. **Population and data studied:** Seven years of data (July 2006 to October 2013) from a longitudinal field study of wild European badgers (*Meles meles*) naturally infected with *Mycobacterium bovis* in south-west England. **Research protocol:** A multi-event capture-recapture approach was used to model imperfect observations (the results of three imperfect diagnostic tests) of true epidemiological states (the probability of whether an animal was truly infected). We developed a method for interpreting potentially disparate results from individual badgers sampled multiple times over an extended period. Given that each badger's true infection status was unknown, we used the set of diagnostic test results to infer the true infection status of the individual. This was achieved by combining the individual capture histories with the probabilities of capture, being infected at first capture, becoming infected (transitioning from uninfected to infected) and of obtaining each combination of diagnostic test results. In doing so, we moved diagnosis away from the traditional binary classification of apparently infected versus uninfected to a probability-based interpretation which was updated each time an individual is re-sampled. **Key results:** The probability of capturing individual badgers varied significantly according to sex (males more likely than females to be caught) and infection status (uninfected animals more likely than infected animals to be caught). The infection status of 30% of badgers could not be ascertained with more than 90% certainty, indicating that the infection status of a sizeable proportion of the population may have been incorrectly diagnosed using traditional approaches. Since the estimates for the probability of infection were updated each time an individual was caught and sampled, certainty about infection status tended to increase for animals caught on more than one occasion. **Conclusions and implications:** This novel approach to combining ecological and epidemiological data has the potential to aid disease management decision-making by providing a framework for the integration of multiple diagnostic test data with other information. The approach used could improve the reliability of decisions made to manage disease in populations where diagnostic test results are available from the same animals over a period of time.



## Temporal and spatial distribution of antibodies against *Mycobacterium bovis* in wild boar (*Sus scrofa*) in the Basque Country (Northern Spain)

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**Keywords:** *Mycobacterium bovis*, wild boar, ELISA

Animal tuberculosis (TB) is a worldwide zoonotic disease caused principally by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex that infects a wide range of livestock and wildlife. Because of its impact on public health and economic losses in livestock industry, eradication programs have been implemented in Europe through the last decades. However, the implication of wild reservoirs such as wild boar (*Sus scrofa*), among other factors, has prevented the complete eradication of TB in many countries. In The Iberian Peninsula, most studies performed on the epidemiology of TB in wild boar have been carried out on South-Central Spain, where artificial management of game species has increased its density and aggregation. However, in Northern regions, where wild boar density and aggregation are lower, fewer studies have been carried out. The aim of this study was to describe the temporal and spatial distribution of antibodies against *M. bovis* in wild boar from the Basque Country, Northern Spain. Serum samples from 1894 hunter-harvested wild boar were collected between 2010 and 2016. The presence of antibodies against *M. bovis* was determined by using an in house enzyme-linked immunosorbent assay previously validated. The relation between antibody prevalence and quantitative variables (sex, age, hunting-season, and region) was determined by Chi-Square Test and Fisher's Test, using the R Software 3.4.4. Overall, 17% of wild boars (317/1894; 95%CI, [15.1%–18.5%]) showed antibodies. Significant differences were detected among hunting seasons ( $p < 0.0001$ ), with the highest seroprevalence detected in 2012-2013 (37.9%; 39/103; 95%CI, [29.1%–47.5%]); and regions ( $p < 0.0001$ ), where the highest prevalence was detected in Tolosaldea (33.6%; 80/238; 95%CI, [30%–39.8%]). No significant differences in prevalence of antibodies were observed between sexes or ages. On the other hand, low antibody titers were the most representative among seropositive animals (60.3%; 191/317; 95%CI, [54.8%–65.5%]) ( $p < 0.0001$ ). Nevertheless, the highest antibody titers were mainly observed in young animals (75%, 21/28; 95%CI, [56.6%–87.3%]), ( $p = 0.01$ ). The overall antibody prevalence detected in wild boar is higher than previously reported in Northern Spain. Moreover, detection of higher antibody titers in young animals rather than in adults could be related with more extended lesions and consequently a higher excretion of mycobacteria. Hence, further studies are needed to better understand the role of wild boar in the epidemiology of TB in Northern Spain and consequently, its relevance when developing control strategies. Work funded by The Gipuzkoa Provincial Council- "Health and environment research" Agreement, "La Caixa" Foundation and Spanish National Institute for Agricultural and Food Research and Technology (INIA) (RTA2014-00002-C02-02 grant).

## Assessment of BCG and inactivated *Mycobacterium bovis* vaccines in an experimental tuberculosis infection model in European badger (*Meles meles*)

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**Keywords:** Badger, *Mycobacterium bovis*, vaccination

Animal tuberculosis (TB) is a complex animal health problem that causes disruption to trade and significant economic losses. TB transmission involves a multi-hosts system in which European badger (*Meles meles*) play a role. In the United Kingdom and Ireland, badgers have been identified as an important reservoir for TB. Vaccination of badgers is a promising tool for controlling the TB infection. Preliminary protective results have been obtained in badgers with live BCG vaccine, both by intramuscular injection and oral administration. However the use of a live vaccine at protective doses is only licensed by intramuscular route and the use in baits is challenging. Inactivated vaccines are in this sense attractive for field delivery because they are expected to be more stable in baits, especially under high environmental temperatures, and safer in the field conditions. The aims of this study were to confirm an experimental TB infection model in badger with a Spanish *Mycobacterium bovis* field strain (SB0339) isolated from a TB diseased wild boar, and to evaluate the efficacy of two oral vaccines against TB in badgers: the live-attenuated *M. bovis* BCG vaccine (Danish strain) and a heat-inactivated *M. bovis* (HIMB) vaccine. Badgers were separated in three treatment groups: oral vaccinated with live BCG (108 CFU, n=5), oral vaccinated with HIMB (107 CFU, n=7) and unvaccinated controls (n=12). All twenty-four badgers were experimentally challenged with *M. bovis* by the endobronchial route targeting the right middle lung lobe (103 CFU). Throughout the study, clinical, immunological (IFN-gamma and ELISPOT), pathological and bacteriological parameters of infection were measured. Badgers were successfully infected and all except two badgers (one BCG-vaccinated and one HIMB-vaccinated) developed gross TB lesions in the respiratory system. Both vaccines conferred considerable protection against experimental TB in badger, as measured by a reduction of the gross lesion volumes and of spreading to locations beyond the respiratory system. Based on these data, HIMB vaccination appears like a promising oral vaccine candidate for badgers, pending further development in large scale production, incorporation in baits, minimum protective doses, higher number of animals and assessment in field conditions. Funding: This study was funded by a grant from Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), reference number RTA2014-00002-C02 (FEDER co-funded).

## **TB maintenance community: investigation on the role of red foxes**

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**Keywords:** fox, tuberculosis, *Mycobacterium bovis*, epidemiology

In Europe, bovine tuberculosis (TB) is a zoonotic disease regarded not just as a problem for cattle but as a concern for multi-host communities that include wildlife species such as wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and badger (*Meles meles*). The red fox (*Vulpes vulpes*) is usually considered as a spillover host of TB as the likelihood of excretion is considered low and only a sparse numbers of infected foxes had been found in highly prevalent TB regions such as in Great Britain. However, in the last years in Spain or Portugal high prevalence estimations of TB in red foxes (14% and 26.9% respectively) were reported in endemic regions where TB circulates in a multi-host species system. Until recently the number of infected foxes since the earlys 2000s in TB endemic areas in France was low. However, during a punctual and local initiative in 2015, 4 out of 6 red foxes were found infected by *M. bovis* in a municipality of a well-recognized TB-endemic area in Dordogne (south-western France), where cattle, badgers, wild boar and even roe deer and red deer are affected by the disease. None of the infected red foxes exhibited TB gross lesions although *M. bovis* fecal excretion was established in all of them. One red fox also showed urine and oropharyngeal mucus excretion. In parallel, a recent study demonstrated that in Burgundy, a region where TB is prevalent both in cattle and wildlife, red fox is the species that visits cattle environments the most. In this context, a two-year study was launched in 2017 in a 525 km<sup>2</sup> area in Dordogne to investigate TB prevalence, pathology, and routes of infection and excretion in red foxes to assess the role played by this species in the local TB multi-host system. 188 red foxes were sampled the first year in the 25 municipalities of the study area. Eleven were positive to *M. bovis* (P=5.9%; CI95% 2.9-10.2%), with 2 municipalities disclosing > 50% of cases. 3 foxes showed infection in respiratory lymph nodes (LN), 4 in mesenteric LN and 4 in both types of LNs. Whereas none of the infected foxes presented TB-like gross lesions, all foxes exhibited *M. bovis* in their faeces and 2 in oropharyngeal mucus. The infection rate observed in foxes is similar to that observed in badgers in the same area (P=7.1%; CI95% 4.7-9.5%). These first results strongly suggest that foxes could play a role in the maintenance community of *M. bovis* raising a worrying question in terms of surveillance and control. A second sampling is ongoing to better investigate the routes of infection and excretion.

## The big bad wolf helping Spanish farmers - a tale on predation and tuberculosis

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**Keywords:** *Animal tuberculosis, Disease control, Sanitary police, Selective predation*

Animal tuberculosis (TB), caused by infection with members of the Mycobacterium tuberculosis complex (MTC), is a widespread multi-host infection. On the Iberian Peninsula, infection is maintained in a complex network of domestic and wild hosts, including abundant wild ungulates such as the Eurasian wild boar (*Sus scrofa*). Predation may contribute to disease control in multi-host systems if it reduces the maintenance host population and thereby reduces pathogen transmission rates, or if it selectively targets severely infected individuals. Asturias, in northern Spain, is a region with a well-preserved wolf population and where TB is one of the main concerns of cattle farmers. Hence, Asturias is a region in which to test the impact of wolf predation on TB prevalence in wildlife reservoirs (particularly wild boar) and in the TB official control target species, cattle. We used field observations and modelling to test the hypothesis that wolves may contribute to TB control in wild boar and cattle. More specifically, we used available observational information to test if wolf presence affects wild boar demography and wild boar TB prevalence; and if wolf presence predicts cattle TB herd prevalence and trends. Wolf population data, wild boar harvest data and cattle TB data were available from the Asturias government. A total of 1,051 wild boar sera were tested for antibodies against MTC. To underpin and extend the observational findings we developed a model of the wild boar – TB system that includes predation by wolves. The model can be used to assess the long-term effects of wolf predation on disease control for a range of scenarios of wolf density change. Field observations show that wolf presence is stable in 2/3 of Asturias. In the remaining third of the region, wolves are only sporadically recorded. The mean annual wild boar harvest grew less in areas with wolves than in areas without wolves ( $p < 0.001$ ). Wild boar MTC antibody prevalence declined 77% in sites with wolves ( $p < 0.001$ ), while the difference was not significant in sites without wolves. Cattle TB remained almost stable in areas with wolves, but increased by 56% ( $p < 0.01$ ) in areas without wolves. Model insights suggest that predation can control virulent infection in a prey species and thereby reduce the subsequent level of environmental contamination. Predation can therefore reduce the chance of spillover from reservoir prey species to other hosts. The model shows that restrictions to predator growth may have only minor impacts on prey density but a major detrimental impact on the prevalence of infection in prey species.

## Host heterogeneity and TB dynamics in badgers

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**Keywords:** *heterogeneity, behaviour, disease*

The management of disease transmission amongst livestock and wildlife populations is a major global challenge. Bovine tuberculosis is the most serious endemic disease of livestock in the United Kingdom (UK) where its management is confounded by the presence of infection in the European badger (*Meles meles*). The persistence and spread of infection in badger populations is the collective result of badger physiology, behaviour, demography and interactions with their environment. Understanding how these processes influence patterns of disease risks for badgers and cattle across the landscape, and predicting the responses of both populations to management interventions is critical to the development of effective disease control strategies. An intensive capture-mark-recapture study of a badger population in south-west England has provided the opportunity to study how individual heterogeneity amongst badgers may relate to disease transmission risks, and to explore the potential for certain individuals to play a more pivotal role in disease persistence and spread. This long-term study has consistently identified behavioural correlates of *Mycobacterium bovis* infection in badgers, relating to ranging and foraging behaviour. Recent research on social networks, foraging behaviour, life history strategies and TB progression in badgers have provided evidence for the importance of individual heterogeneity as a driver of disease risk, which may inform the development of practical and sustainable approaches to disease control in badgers and cattle

## A compartmental dynamic model for *Mycobacterium bovis* transmission between badger and cattle in south-western France

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**Keywords:** bovine tuberculosis, compartmental model, badger-cattle interface

In France, bovine tuberculosis (bTB), mainly due to *Mycobacterium bovis*, is a complex problem despite the officially free status obtained in 2001: a few areas can be considered enzootic, with persistence of the infection in cattle and some wildlife species such as red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) wild boar (*Sus scrofa*) and badger (*Meles meles*). For this last species, the infection level observed by the national bTB wildlife surveillance is of concern. Focusing on south-western France, we designed a compartmental stochastic model operating in discrete time (monthly) to simulate bTB transmission within the badger-cattle system. The badger population was represented at the social group level with home ranges based on sett locations using Dirichlet tessellations, and groups composed of adults, sub-adults and cubs. The cattle population of the study area was considered at the farm level with known size, trade movements, herd management practices and pasture locations. The model simulated the infectious process (i) within and between (trade and pasture neighborhoods) cattle farms, (ii) within and between (home ranges neighborhood) badger groups and (iii) between cattle farms and badger groups (pasture-home range neighborhood). The cattle part of the model was adapted from the within-herd model of Bekara et al. (2014). The model accounted for the specific bTB detection and surveillance systems in the study area. Surveillance data produced between 2007 and 2015 in cattle (65 farms detected infected by 16 molecular types) and in badger (38 social groups detected infected by 2 molecular types) was used for parameter estimation, each molecular type being considered as an independent epidemic. Six transmission parameters were estimated using Adaptive Approximate Bayesian Computation (Lenormand et al. 2013): (i) two accounted for the between-herd transmission, (ii) two for badger transmission (within and between groups) and (iii) two for the interspecies transmission (from badger to cattle and from cattle to badger) which corresponded to indirect contacts allowed by the mycobacterial survival within the pasture environment. Results highlighted a strong involvement of badger population in the bTB dynamic within the study area, although direct transmission routes between cattle farms also play a significant role. Designing efficient control measures against bTB in the study area requires taking into account this multi-factorial aspect. **References** Bekara MEA, Courcoul A, Bénét J-J, Durand B (2014) Modeling tuberculosis dynamics, detection and control in cattle herds. PLoS ONE 9.: doi: 10.1371/journal.pone.0108584 Lenormand M, Jabot F, Deffuant G (2013) Adaptive Approximate Bayesian computation for complex models. Comput Stat 28:2777–2796. doi: 10.1007/s00180-013-0428-3

## Surveillance of wildlife tuberculosis in Catalonia (Spain), a low prevalent area of bovine tuberculosis, 2012-2018.

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**Keywords:** Tuberculosis, wild boar, wildlife, Catalonia, Spain

Animal tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC), is a multi-host disease that requires holistic control approaches focused on its main hosts and their shared interfaces. In this regard, we report herein the results of wildlife TB follow-up in Catalonia during five hunting seasons (from September 2012 to March 2018) conducted in the framework of the Wildlife Health Surveillance Plan. A total of 7283 sera from wild ungulates (4121 wild boars, 479 red deer, 198 fallow deer, 409 roe deer, 607 Iberian ibex, 1199 chamois, 67 feral goats and 203 mouflon) were analyzed by MTBC-specific enzyme linked immunoabsorbent assay (ELISA). Presence of TB lesions in tissue samples was also evaluated in 1649 wild ungulates and 293 badgers. Animal culling and sampling was intensified in areas where a TB focus was detected. Mycobacterial isolation and identification were conducted from all suspected lesions and from a fraction of samples without visible TB lesions. One hundred ninety-seven out of the 7283 serum samples (2.7 %) were positive to ELISA. In one hand, 158 (3.8 %) wild boar sera were classified as positives to ELISA, 32 of them (21 %) were samples obtained from a TB focus related to infected cattle in a National Game Reserve of Southern Catalonia. On the other hand, 9 red deer (1.9 %), 2 fallow deer (1 %), 13 Iberian ibex (2.1 %), 3 feral goats (4.5 %), 12 chamois (1 %) and 2 mouflon (1%) sera were classified as positives to ELISA, whereas no seropositive roe deer were detected. TB compatible lesions were observed in 42 wild boar (3 %) and one red deer (1.1 %). No TB lesions were found in the other wild ungulates nor badgers. *M. avium* was isolated in four out of the 34 positive cultures obtained from wild boar samples. Interestingly, within the other 30 isolates, 23 (77 %) belonged to *M. caprae*, whereas only 6 (20%) belonged to *M. bovis* (most likely related to 3 bovine TB outbreaks), and the other one (3%) to *M. microti*. Moreover, two *M. bovis* isolates were also obtained from red deer, both of them most likely related to a cattle TB outbreak. The wild boar is considered the main TB wild reservoir in the Iberian Peninsula. Accordingly, the low proportion of both seropositivity and presence of TB lesions found in these five hunting seasons is consistent with the low prevalence of bovine TB in Catalonia reported throughout this period (< 0.5 %). However, the fact that *M. caprae*, the main causative agent of caprine TB in Catalonia, was identified from over 3/4 of wild boar isolates, mostly in areas without cases of bovine TB, suggests the existence of alternative cycles of TB maintenance in wildlife, such as direct or indirect transmission between domestic small ruminants and wild boars.

## Quantitative characterization of the community of tuberculosis-infected hosts in the Iberian Peninsula

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**Keywords:** bovine tuberculosis, wildlife, livestock

Quantitative characterization of the community of tuberculosis-infected hosts in the Iberian Peninsula Abstract Animal tuberculosis (TB) is a disease of economic importance to the livestock industry, subject to eradication programs in cattle. In 2016, the cattle herd prevalence in the Iberian Peninsula was 2.3%, with large regional variations. Despite the overall long-term success of cattle TB control, the trend in the recent years has been of a slight increase, despite an annual investment of over 30 million € in control. Epidemiological evidence points to wildlife and livestock species other than cattle as the origin of part of the cattle TB breakdowns. Furthermore, observational and experimental studies support the multi-host nature of TB in the Iberian Peninsula. Our goal was to quantitatively characterize the community of infected hosts in the Iberian Peninsula by estimating the number of infected wildlife and livestock. This characterization was performed for those species suggested to be maintenance hosts for the Mycobacterium tuberculosis complex (MTC): cattle, goat, sheep, free-range pigs, farmed cervids, wild boar, red deer, fallow deer and badger. True prevalence was estimated based on apparent prevalence, sensitivity and specificity of the diagnostic tests and combined with host population data in a bayesian framework to obtain the posterior distribution of the number of infected hosts. The model was informed by published data on TB surveys with random sampling, eradication programs, sensitivity and specificity of diagnostic tests, and host populations. A geographically structured approach was used for goats and wildlife species due to the large regional differences in prevalence or host populations previously described in these species. We estimate the number of MTC-infected animals in the Iberian Peninsula to be 195,178 – 952,764. Cattle comprise 0.02-8.9% of the community of infected animals, livestock species other than cattle 15.7-81.2% and wildlife 18.0-83.8%. The estimates of infected non-cattle species exceed that of infected cattle, with a ratio of 90.8 (9.8-4,551). These results support the view that in the Iberian Peninsula TB is a true multi-host disease maintained by a community of domestic and wild hosts. Disease control programs need to address this multi-host nature of TB to significantly progress towards eradication. Research on innovative control tools and the combination of multiple approaches to decrease infection prevalence in the main host species will need to be strengthened, as started with the Spanish PATUBES strategy.



## Progress towards the development of an oral vaccine against TB in badgers

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**Keywords:** *Badger, Mycobacterium bovis, vaccine*

Tuberculosis caused by *Mycobacterium bovis* remains one of the most important infectious diseases in cattle, and in spite of routine test and slaughter policies, the eradication of the disease can be hindered by the persistence of infection in wild animal reservoirs. European badgers (*Meles meles*) play an important role in the transmission cycle of bovine tuberculosis in the UK and Ireland, and possibly in other European regions. Oral vaccination of badger populations is considered as a promising tool to control the risk of transmission of *M. bovis* from badgers to cattle, by reducing the level of TB infection (prevalence and severity of disease) in badger vaccinated populations. An oral bait (HPO-PT) has already been developed; it is attractive for badgers and the titre of the live vaccine (BCG) remains stable inside the baits for several days following delivery. The protective effect of live BCG delivered orally in HPO-PT baits, or manually inside the mouth, was quantified in a series of six vaccine efficacy studies including a total of 114 wild badgers. All the animals were experimentally infected with *M. bovis*, and the severity of the TB lesions observed at post-mortem was scored using a collation of different macroscopic and microscopic measures of disease described as the Disease Burden Score (DBS). The reduction of DBS in vaccinated badgers involved in each study was compared with non-vaccinated controls by an analysis of variance. The analysis took account of uncontrolled differences between individual badgers (sex, age, origin, weight at vaccination and pre-sensitisation of animals to *M. avium*) that could affect the DBS. Despite differences between studies in the effect of oral vaccination, in particular in association with the dose of BCG delivered to animals, there was significant evidence of protection overall in reducing the development of TB lesions experimentally induced. This result supports the continuation of the programme for developing an oral vaccine bait for badgers against tuberculosis.

## **Mongolian khulan (*Equus hemionus hemionus*) are exposed to multiple influenza A strains**

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**Keywords:** *Influenza, Emerging diseases, Wildlife*

Influenza A viruses are widespread in their natural avian reservoirs and also in several mammalian species, such as equids, swine, and dogs. Equids can be infected with two main influenza viruses: Influenza A/H7N7 or equine-1, which is considered extinct, and Influenza-A/H3N8 or equine-2, which circulates worldwide. H3N8 has recently jumped to canines in the United States, to camels in Mongolia, as well as pigs in Asia. Mongolia, a country with a large population of domestic horses, is also home to the largest community of Asiatic Wild Asses (*Equus hemionus hemionus* or khulan) and the Przewalski horses (*Equus ferus przewalski*). In a 2015 expedition to the Southeast Gobi Desert, Mongolia, we immobilized 21 khulans and collected blood, serum, and nasal swabs. Using a protein influenza antigen microarray, we tested the serum samples against 32 different haemagglutinin proteins. We detected antibodies against multiple strains in seven animals. Low titers for H5, H8, and H10 and moderate titers for H7 and H1 were detected. Five khulans reacted to equine H3 antigens, an influenza strain known to circulate in the area. These findings suggest, that although equine-1 (H7N7) is considered to be extinct in the region, it could be still circulating at low prevalence among equids in Central Asia and that H3 strains co-circulate in wild and domestic equids. The additional antibodies against other influenza strains may indicate susceptibility of equine species to a wider spectrum of influenza strains than previously thought. Horses express sialic 2,3-linked receptors in their upper respiratory tract, which are similar to those in birds, increasing their susceptibility to bind avian-like viruses. This biological trait in combination with ecological factors, such as the increase of the wildlife-domestic animal interface, through sharing pasture and waterholes, warrant the need for further studies on the influenza epidemiology in wild equids. Determination of possible neglected ecological niches for influenza viruses and alternative disease transmission routes will be required to understand the full dynamics of influenza virus maintenance and transmission.

## **Bufonid Herpesvirus 1-associated proliferative dermatitis in free-ranging common toads (*Bufo bufo*)**

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**Keywords:** *Herpesvirus, common toad, dermatitis*

Starting in 2014, it was observed an increasing number of free-ranging common toads (*Bufo bufo*) with skin changes characterized by dark brown patchy coloration of their integument associated with skin thickening (hyperplasia). Toads with skin lesions were observed in different regions of the Swiss territory during the mating season, between the late winter and early spring. Affected toads were observed either in good or poor body conditions, but always alive. Differently, in 2017 a mass mortality event was associated with the presence of the brown skin patches in about 25% of the dead toads. The brown skin patches, observed in the affected toads since 2014, corresponded to prominent epidermal hyperplasia often associated with hyperkeratosis. Histological examination revealed the presence of intranuclear eosinophilic to amphophilic inclusions in the keratinocytes of the affected skin with only a minimal or no associated inflammatory infiltrate. Electron microscopy showed the presence of herpesvirus-like particles in the hyperplastic keratinocytes. Molecular analyses, including the sequencing of the genome, were consistent with the presence of a novel herpesviral agent. Phylogenetic analysis positioned the tentatively named Bufonid herpesvirus 1 (BfHV1), as a new member of the genus *Batrachovirus* in the family *Alloherpesviridae*. The lesions associated with BfHV1 appear to be confined to the integument, although PCR test revealed the presence of herpesviral DNA also in the brain of one of the affected toads. The role of BfHV1 in amphibian disease ecology and its potential nature as amphibian pathogen is unknown. This is the first herpesvirus ever characterized in common toads.

## Serological survey of hepatitis E virus in hunted wild boar (*Sus scrofa*) in Gipuzkoa (Northern Spain)

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**Keywords:** *Hepatitis E virus, wild boar, seroprevalence*

Hepatitis E virus (HEV) belongs to the genus *Hepevirus* within the family *Hepeviridae*, which is responsible for an enteric viral hepatitis infection causing human liver disease of worldwide distribution. To date, reported outbreaks in developed countries have been associated with inadequate water treatment. However, sporadic and non-travel related HEV cases have increasingly been described in European countries linked to zoonotic transmission, where the infection may occur by consumption of undercooked or raw meat of domestic pigs or certain wild species. Although wild boar has been addressed as the main HEV wild reservoir in those countries, there is no data available for the situation in Northern Spain. The aim of this study was to determine the HEV seroprevalence in wild boar (*Sus scrofa*) and its spatial and temporal distribution in the province of Gipuzkoa (Northern Spain). Serum samples (N=1504) were collected between 2010 and 2016 during each hunting season (September to February) in 7 different areas. Detection of HEV antibodies was accomplished using a commercial indirect enzyme-linked immunosorbent assay. The relation between HEV antibody prevalence and quantitative variables (sex, age, hunting-season, and area) was determined by Chi-Square Test in SAS® Enterprise Guide 7.1 software. The overall seroprevalence was 5.52 % (83/1504; 95%CI: 4.47-6.79) and it was higher in adults (9.50%; 40/421; 95%CI: 7.06-12.68) than in young specimens (4.98%; 27/542; 95%CI: 3.45-7.15) (p=0.0062). However, no significant differences were observed between sexes. Significant differences were also detected among the studied areas (p<0.0001) and hunting seasons (p<0.0001), with the highest prevalence being recorded in Deba Garaia during 2013-2014 (28.21%; 22/78; 95%CI: 19.43-39.03). Additionally, whereas only one positive sample was detected in a single area in 2010, positive animals were identified in 5 out of 7 studied areas in the following hunting seasons. Despite the low global prevalence recorded, HEV seems to be well established in the wild boar population from Gipuzkoa. Therefore, further research is needed to gather information about the possible impact of HEV on hunters and on those who consume wild boar meat. Work funded by The Gipuzkoa Provincial Council- "Health and environment research" Agreement, "La Caixa" Foundation.

## **Lagovirus europaeus GI.2 (Rabbit Hemorrhagic Disease Virus 2) in an isolated population of mountain hares (*Lepus timidus*) expands the host range of GI.2.**

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**Keywords:** Rabbit Hemorrhagic Disease Virus 2, *Lepus timidus*, emerging disease

In the 1980s, Rabbit Hemorrhagic Disease (RHD) and European Brown Hare Syndrome (EBHS) were first described in European rabbits (*Oryctolagus cuniculus*) and hares (*Lepus* spp.), respectively. Both are caused by lagoviruses of the family Caliciviridae, and until 2010, the causative viruses were considered to be genus-specific. However, this changed in 2010 when *Lagovirus europaeus* GI.2, also known as Rabbit Hemorrhagic Disease Virus 2 (RHDV2), emerged. In addition to causing disease in rabbits, infection and clinical disease have been reported in the Sardinian Cape Hare (*Lepus capensis mediterraneus*), the Italian Hare (*Lepus corsicanus*) and the European Brown Hare (*Lepus europaeus*). The mountain hare (*Lepus timidus*) is native to Sweden. Although it once ranged throughout the country, its distribution is gradually decreasing and mountain hares have disappeared entirely from the far south mainland. In addition to mainland populations, isolated populations of mountain hares are found on numerous islands. In the fall and winter of 2016/2017, a mortality event in mountain hares was reported on the small island of Hallands Väderö. Three carcasses collected from November to March were examined by necropsy and microscopically. The main finding was acute hepatic necrosis consistent with pathogenic lagovirus infection and viral capsid antigen was demonstrated within lesions using immunohistochemistry. Genotyping and immunotyping of the virus independently confirmed infection with *L. europaeus* GI.2/RHDV2. GI.2 has been documented in Sweden since 2013 and in 2016, there was a widespread epizootic in wild and domestic rabbits. Phylogenetic analyses of the VP60 capsid gene grouped the mountain hare strains together with a rabbit strain from July 2016, collected approximately 50 km away. This suggests that the virus on Hallands Väderö was introduced from the adjacent mainland. This outbreak is the first documented infection of GI.2 in mountain hares and represents a further expansion of GI.2 host range. Although the distribution of mountain hares overlaps with that of wild rabbits and/or European brown hares in many areas of southern Sweden, mountain hares are the only leporid species on Hallands Väderö. The outbreak of GI.2 in mountain hares persisted for at least 4.5 months in the absence of rabbits, which would have required virus circulation among mountain hares, environmental persistence of the virus and/or repeated introductions. Further research is needed to explore these hypotheses and evaluate mountain hares as a competent reservoir host for GI.2. Additionally, because island populations of mountain hares are prone to population crashes, we propose that GI.2 incursion could serve as a stochastic event triggering such a decline. Mountain hares are the fourth *Lepus* species known to succumb to GI.2 infection, suggesting that susceptibility may be common in *Lepus* species and measures to minimize the spread of GI.2 to vulnerable *Lepus* populations are prudent.

## The occurrence of the moose nasal bot fly (*Cephenemyia ulrichii*) in Norway—an emerging disease?

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**Keywords:** moose, *Cephenemyia ulrichii*, emerging disease

The nasal bot fly (*Cephenemyia ulrichii*, Diptera: Oestridae) is a parasitic fly dependent specifically on moose (*Alces alces*). After the fly deposits its larvae in the nostrils of the moose, the larvae migrate up the nasal passages to grow for several weeks to months before being eventually coughed or sneezed out as third instar larvae (L3: 30mm+) to complete the final stages of development. In the past decades, *C. ulrichii* has been spreading in Fennoscandia. It was first reported in northern Norway in 1987 but was not reported again until 2003. Since 2003, sporadic observations in moose have been reported through Norway's cervid health surveillance program. This program relies largely on reported observations of abnormalities by hunters. However, *C. ulrichii* larvae are small during hunting season (L1: 1-3mm), and, thus, easily overlooked. In 2015 the Norwegian Veterinary Institute (NVI) performed a specific investigation, whereby 18/30 (60%) of investigated moose collected during the hunting season from south-eastern Norway were infested. This high prevalence indicates that this parasite is likely more widespread than previously known in Norway. However, the national distribution and the effect of this parasite on the health of the Norwegian moose population is still unknown. The purpose of the present study is to evaluate the prevalence and the intensity of the nasal bot fly infection in moose collected from several areas within Trøndelag (mid-Norway). The occurrence of the parasite will also be related to the age and sex of the hosts. During the moose hunting season September-November 2017, over 60 moose heads were collected and frozen. To find larvae, the heads are skinned and cut in half using a bandsaw. After removing the nasal septum, the pharynx, ethmoid processes, nasal passages, and sinuses are opened and rinsed thoroughly with water. The water is passed through a series of sieves with a final sieve size of 180µm. Larvae are then counted and staged. Preliminary results from the first 23 moose investigated show a prevalence of 73% and an intensity ranging from 1-17 first instar larvae. Final results and sex/age analysis are pending. This data in combination with data from NVI's previous survey indicate a high prevalence of this parasite in the moose population in mid- and southern Norway. Reindeer are reported to display violent sneezing, head shaking, rubbing the nose in the ground and kicking in response to oestrid attacks. These behavioral responses lead to considerable energy demands on the animals and less time is spent foraging, with reduced fitness as a potential outcome. Mortality and injuries from aberrant migration of larvae and/or suffocation due to larval blockage of the airways has also been reported. This may also apply to moose, but remains to be investigated.

## Serological screening for viral pathogens among semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*) from eight reindeer herding districts in Norway

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**Keywords:** reindeer, serology, viral pathogens

In Norway, 214 000 semi-domesticated reindeer were registered in the reindeer herding year 2016 – 2017. It has been shown in previous studies that pestivirus, alphaherpesvirus and gammaherpesvirus are all enzootic in reindeer in Finnmark County (northern Norway). The reindeer alphaherpesvirus, cervid herpesvirus 2 (CvHV2), has been identified as the primary transmissible agent of infectious keratoconjunctivitis (IKC) in reindeer. In cases of IKC, CvHV2 infections are often accompanied by secondary bacterial infections, such as *Moraxellabovoculi* and others. It has also been suggested that CvHV2 may cause respiratory infections and that the virus can be vertically transmitted from mother to fetus via placenta. IKC outbreaks may affect many animals in a herd, most often calves and young animals. Antibodies against gammaherpesvirus, malignant catarrhal fever virus (MCFV) group, have been detected in reindeer in Finnmark. A single animal was diagnosed with malignant catarrhal fever, displaying bilateral corneal oedema and opacity, swollen eyelids and fibrinopurulent ocular discharge, as well as hair loss, thickening of the skin and crusts. Gammaherpesvirus (ovine herpesvirus 2) DNA was identified by PCR in tissues from brain and from swab samples of mucosal membranes (conjunctiva, nose, tonsil). No clinical disease or pathology has so far been associated with Pestivirus infections in semi-domesticated reindeer, but there is evidence that a pestivirus is circulating in semi-domesticated reindeer herds in Finnmark County. Except for the screenings conducted in Finnmark County, little is known about the exposure of reindeer to these viruses in the reindeer herding regions in other parts of Norway (approximately 67 000 animals). The aim of this study was to investigate semi-domesticated reindeer representative of all reindeer herding regions of Norway, for antibodies against these viruses. Blood samples were obtained over a three-year period (2013 – 2015) from 20 animals per year from eight herds (Tana, Karasjok, Tromsø, Lødingen, Hattfjelldal, Fosen, Røros and Filefjell) (n = 480). Antibodies against alphaherpesvirus CvHV2 were found in all eight herds, with a total prevalence of 53 % (min-max: 23-75 %). Antibodies against gammaherpesvirus (MCFV) were also found in all the herds, with a total prevalence of 9.5 % (min-max: 3-15 %). Antibodies against pestivirus, using a commercial serological test (ELISA) with antigens from Bovine viral diarrhoea virus (BVDV), were detected in five of eight herds, with a total prevalence of 14 % (min-max: 0-55 %). Two herds had notably high seroprevalence against pestivirus, (55 % and 32 %, respectively), indicating that these herding districts could be investigated further to determine if this virus causes disease in reindeer as in other ruminants. This study confirms that these viruses indeed are enzootic throughout the geographical reindeer herding regions of Norway. Further studies are needed to address their potential impact on reindeer health and herding economy.

## **Peste des petits ruminants (PPR) as a great threat to wild ungulates in the Asian Continent**

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**Keywords:** *Peste des petits ruminants, PPR, wild ungulates*

Peste des petits ruminants (PPR), is a highly infectious and devastating viral disease of small ruminants in Asia and Africa. The causative agent is a single stranded – negative sense RNA virus (PPRV) with four identified lineages. The lineage IV dominating in Asia, but recently the virus has extended its range southward in Africa and northward in Asia. In 1999 the PPRV reached Turkey, the eastern gateway to Europe. PPRV has emerged as a global animal health concern as it threatens the small-scale sheep and goat farming of more than 300 million low-income families, and the survival of endangered populations of a variety of wild ungulate species. The global strategy for eradication of PPR virus by 2030 (OIE-FAO-2017) does not consider wildlife. Although the current evidence does not support maintenance of PPRV in wildlife, there are examples of vectoring of the virus by wildlife species, which might also occur across borders or control zones. The eradication plan is challenged by a lack of thermostable vaccines, weak vaccination efforts or unavailability of vaccines in some areas resulting in persistence of infection in domestic animals, uncontrolled cross border animal movements, unavailability of an oral-baits vaccine for wildlife and limited knowledge on potential reservoirs in other mammalian species. There is increasing evidence of spill-over of PPRV from domestic to wild hosts. Several outbreaks had significant impacts on free-ranging populations of bharal (or blue sheep), Siberian ibex and argali sheep in China (2007-2016); wild goats and urial sheep in Iraq's Kurdistan (2010-2011) and Iran (2014-2017); and saiga antelope, argali sheep, Siberian ibex and goitered gazelle in Mongolia (2016-2017). In addition, the disease has been suspected or confirmed in captive markhor, Sindh ibex, Laristan mouflon, rhim (sand) gazelle, dorcas gazelle, Nubian ibex, Barbary sheep, Thomson's gazelle, springbok, grey duiker, gemsbok, bushbuck, impala and urial. The growing number of domestic livestock across the West and Central Asia results in pasture sharing and thus increases the risk of transmission of infectious agents in general. PPRV may threaten of extinction the relatively small populations of susceptible wildlife, typically exposed to other threats and living in widely separated and often fragmented habitats. The authors see a need to foster an integrated approach at both the agency and ministry levels to further disease research and management across the regions with an emphasis on understanding the epidemiology and ecology of PPR in livestock and wildlife populations. We envisage the development of disease risk and transmission models to further understanding and identify critical control points and subsequently allow for multiple stakeholder engagement and long-term multi-sectoral collaborations, a key step toward the elaboration of integrated management strategies to benefit both livestock and wildlife and secure the livelihoods of millions of people.



## **Chronic Wasting Disease in Norway: An update on surveillance, research and disease development**

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**Keywords:** Surveillance, CWD, disease development

Norway Chronic Wasting Disease (CWD) is an invariably fatal neurodegenerative disease of cervids, belonging to the group of transmissible spongiform encephalopathies. The disease is endemic in some areas in North America, with natural infection in different cervid species. Prior to 2016, CWD had never been reported outside North America, except in captive deer in South Korea introduced by import of CWD-infected animals. The surveillance for CWD in Europe has been limited. In Norway, approximately 2 100 cervids were tested for CWD in the period 2004-2015. Less than 20 of these were free-ranging reindeer (*Rangifer tarandus tarandus*). Norway is the last refugium for wild tundra reindeer in Europe that live in fragmented sub-populations in remote alpine regions of South Norway. In March 2016, a young adult female wild reindeer was diagnosed with CWD, representing the first detection of CWD in Europe and the first case of natural infection in reindeer worldwide. Since 2016, through enhanced surveillance, 18 free-ranging reindeers have been diagnosed in Norway along with three cases in moose (*Alces alces*), and one case in red deer (*Cervus elaphus atlanticus*) from a total of 39,496 cervids tested. Molecular analysis cannot differentiate the CWD-prions detected in reindeer from findings in North America, while prions found in Norwegian moose and red deer have an atypical character. This raises important questions concerning the disease itself (origin, pathogenesis and transmissibility) as well as its future management. CWD research conducted at the Norwegian Veterinary Institute is currently focusing on disease progression and pathogenesis, diagnostics, epidemiology and genetics. We will present and update on the surveillance and disease status two years post discovery, along with future perspectives and a brief overview of the current research projects.

## The background for the management of CWD in Norway – scientific reasoning with high degree of uncertainty

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**Keywords:** *chronic wasting disease, Norway, management*

It is an understatement to say that the diagnosis of chronic wasting disease (CWD) in a wild Norwegian reindeer in March 2016 was unexpected. The shock increased when two moose with CWD were found in May and June - 300 km away from the index case. Already in the end of June the Norwegian Food Safety Authority and the Norwegian Environment Agency asked the National Scientific Committee on Food Safety to provide a scientific opinion. Phase 1 in this opinion should almost immediately supply the authorities with knowledge about food safety, zoonotic potential, disease transmission between animals and the origin of the disease. Phase 2 was given a longer working period and should provide an opinion on 1) which risk factors relevant to disease transmission that should be included in different management strategies, how these factors affect disease transmission and what the beneficial and non-beneficial consequences of reducing the risk factors were, and 2) which management strategies, e.g. strategies used in CWD-infected areas in North-America could be relevant to implement in Norway, and how do choice of strategy depend on the occurrence of CWD in a given population. In such a situation a scientist should be able to form an opinion despite great uncertainty, as the potential consequences of delaying management decisions until more information is available are enormous. The current presentation describes the work of the scientific panel providing Phase 2 of the opinion. While the first screenings of Norwegian cervid populations were performed, two additional cases were found in reindeer and the clinical, pathological and epidemiological picture of the disease emerged. During this time, the panel searched the literature on CWD and gathered knowledge about the affected populations, other cervid populations in Norway, environmental factors and potential measures. One important observation was that the epidemiology and pathology of the moose cases seemed to deviate from, while the reindeer cases seemed relatively consistent with, the “classic CWD” described in North-America. It could consequently not be excluded that the moose cases were a form of “atypical CWD”. Based on the knowledge gained, the panel identified risk factors and formulated strategy options for management of the disease. In case of “classic CWD”, one could choose to not intervene, but this would likely cause increased prevalence, increased distribution range, increased risk of spillover to other cervid species, and increased exposure for other species. With time, one would expect population declines of the affected population(s). Another option, feasible in a confineable population, was to depopulate the affected area, let it fallow for at least five years, and thereafter reintroduce new stock. A third option, feasible in a continuous population of cervids with home range behaviour, was to initiate a spatially targeted harvest. If the moose findings really represented “atypical CWD”, less contagious than “classic CWD”, the panel expressed that no action should be taken regarding these cases, but emphasized that there was great uncertainty about this strategy. In addition, the panel strongly emphasised the need for large-scale surveillance to decrease uncertainty about disease occurrence.

## **“Working in Silo” & “Path Dependence”: Relevance for a global Management of Wildlife and Health**

Mark Artois

*VetAgro Sup*

**Keywords:** *wildlife, health, management, public policy*

“Working in silo” have been a frequent metaphor used to describe the isolation of institutions dealing with multifactorial problems. Similarly “path dependence” relies on the use of traditions and internal culture of working when facing such situations. The management of health crisis situations pose peculiar problems when wild plants or animals are deemed source of a disease of the humans or their livestock. Such context (more familiar under the denomination of “wildlife disease”) was explored by students in political science. These students at the National school for veterinary services (ENSV<sup>1</sup>) in France, were in charge of an inquiry on the social and political dimensions of these crisis situations. After reviewing the recent history of health events linked with wildlife in this country, a focus was made on four case studies: allergies due to ambrosia (*Ambrosia artemisiifolia*), Lyme disease management, HP avian influenza during the years 2005/2007 and finally a recent outbreak of two cases of human brucellosis linked to infection of a free living ibex (*Capra ibex*) population. The students led 17 semi-structured interviews of various involved people: stakeholders, heads of associations, government workers, professionals..., to collect their views on the management of health in this context. The purpose of the report was to draw recommendations for an appropriate and proportionate management of the health in epidemiological situations leading to the emergence of diseases resulting from direct or indirect exposure to wild plants or animals in a natural environment. The report have suggested that in crisis situations, stakeholders compete to monopolise the management of those complex situations in order to confirm their previews of the problem, leading to the creation of opposed “camps” which embed themselves in useless and counterproductive controversies. By comparison, complex situations may evolve positively in practical solutions which are not controversial when stakeholders cooperate, share information, and adopt a wide view of the determinants including a large expertise of professionals and citizens.

<sup>1</sup> Cf : <http://www.ensv.fr/wp-content/uploads/2017/10/Gepp-Faune-et-Flore-Sauvages-V2.pdf> (in French)

## Antimicrobial resistance in wildlife species: the potential for sentinel surveillance in a ONE HEALTH perspective

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**Keywords:** antimicrobial resistance, arctic, norway, one health

**Background** Antimicrobial resistance (AMR) is an emerging problem worldwide, and is widely spread in many environments. The use of antibiotics and other drivers for AMR development in humans, livestock or agriculture may have a direct impact on wildlife, and wild animals may provide a biological mechanism for the spread of antibiotic resistance genes. The wild reservoirs of resistance remain poorly understood, even though its origins and mechanisms are of paramount relevance to human health. We have conducted several studies with different wildlife species in Norway to monitor AMR and understand the potential for these to both work as sentinels/bio indicators for AMR spread, and help explain the dynamics and driver for resistance in the environment. **Methods** Between 2013-2017 faecal samples were collected from different wild species in continental Norway: wild birds (ducks and gulls) (n=357); red foxes (n=387); wild reindeer (n=265); roe deer (n=301). In addition faecal samples were collected from 27 wild reindeer from the arctic archipelago of Svalbard. Resistant bacteria were identified by culturing on selective media and/or by susceptibility testing of randomly chosen *E. coli* from the samples. Microbroth dilution were conducted to assess phenotypic resistance. **Results** Wild birds: *E. coli* resistant to extended-spectrum cephalosporins (ESC) were detected in 31 samples (8.7%) and quinolone resistant *E. coli* in 74 samples (5.6%). Additional resistance to one or more agents was found in 45.9% of the isolates. Red foxes: The occurrence of AMR was 9.2%, 6.3% and 14.7% in areas of low, medium and high human population density, respectively. A statistically significant difference in AMR occurrence was observed between medium and high population density areas. Resistance to fluoroquinolones occurred in 76 foxes (14.4%) with a statistical relevance between density areas. Roe deer and wild reindeer: *E. coli* was isolated and susceptibility tested from 274 out of 301 (91%) roe deer, and from 230 out of 265 (86.8%) wild reindeer. Resistance to streptomycin was detected in 1.7% of wild reindeer and in 5.1% of roe deer. The overall occurrence of *E. coli* resistant to ESC was 0.3% in roe deer. This is the first finding of an ESBL from a wild cervid in Norway. Svalbard reindeer: Fifteen isolates (55%) were resistant to more than one antimicrobial agent. Resistance regions with high homology to plasmid regions previously described in bacteria from swine in the Netherlands and poultry in China were identified. **Conclusion** Results from these studies demonstrate that AMR is present in the environment even in pristine ecosystems such as the Arctic. Longitudinal and spatial broad studies should be prioritized in order to better understand this problem and elucidate the role of wildlife species in the spread of AMR in a one-health perspective.

## New tools to face old problems: Whole genome sequencing for a better insight into an outbreak of *Salmonella* Serovar *Choleraesuis* var. *Kunzendorf* in wild boar

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**Keywords:** *Salmonella, Choleraesuis, wild boar, Whole Genome Sequencing*

**Background** Investigation of disease outbreaks in wildlife due to pathogens that raise concerns for livestock and/or human health is particularly challenging. Besides pathogens ecology and impact in the affected wild populations, further questions might arise such as the origin of the pathogens (e.g. endemic vs introduced); the biological basis of their clinical impacts (e.g. higher virulence acquired by an endemic pathogen vs new pathogen in a naïve population; imbalance of the host-pathogen equilibrium) and their related scenarios (e.g. extinction vs persistence; skip to livestock or humans). Here we describe a severe outbreak due to *Salmonella Choleraesuis* occurred in wild boar from 2012 to 2015 in North-Eastern Italy. Most of the animals analyzed (58 out of 75 totally infected) died due to septicemia caused by *S. Choleraesuis*. The aim of this work was the characterization of this epidemic strain and its zoonotic potential for hunters and wild meat consumers, by Whole Genome Sequencing (WGS). **Materials and methods** A subset of 30 from 75 *Salmonella Choleraesuis* isolates in wild boar during the outbreak was selected for WGS. Among this subset, 15 were isolated from wild boars dead due to septicemia or showing clinical disease in the field, while 15 were isolated from wild boar regularly hunted, without any clinical or pathological sign. **Results** All isolates were classified by in silico serotyping as *S. Choleraesuis* var. *Kunzendorf*, the most common biotype associated to outbreaks in pig farms. Concerning genes and plasmids associated to virulence, no significant difference was detected among all isolates analyzed. No genes or mutations associated to antimicrobial resistance were found in wild boar isolates. Comparing the 30 *S. Choleraesuis* isolates from septicaemic and healthy wild boar, virulence profile did not differ, while lower genetic variability was found in the first group. **Discussion** Although a lower number of isolates (19/75) came from hunted healthy wild boar compared to diseased ones in the study area, the results of WGS analysis support the hypothesis of a common origin. The absence of genes/plasmids typically encoding for antimicrobial resistance suggests an endemic wild cycle of *S. Choleraesuis*, although the possibility of an external source cannot be excluded, also considering the still existing practice of releasing wild boar of uncertain origin. For the purpose of tracing persistence and possible evolution in terms of virulence, antimicrobial resistance and in general host-adaption of the *S. Choleraesuis* detected clone, a follow-up of the situation in wild boar would be advisable. It should be also considered that *S. Choleraesuis* may be dormant in wild boars as well as in domestic pigs, being activated in response to multiple infectious (such as porcine circovirus or porcine reproductive and respiratory syndrome virus infections) and non-infectious stress factors. Thus, whenever possible, parallel investigation should be performed at the host-side, at individual and population level. This information would also be useful to evaluate the zoonotic risk of *S. Choleraesuis* for humans.

## Invasive species as a risk for public health: raccoon dogs and raccoons in the Netherlands

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**Keywords:** raccoon dog; raccoon; zoonoses

The raccoon dog and the raccoon are increasingly sighted in the Netherlands. Reproduction of both species has been reported and it is expected that both species will colonize large parts of the Netherlands in the future. This recent invasion of these species may imply a public health risk, as it may be associated with the risk of (re-)introduction and change of the epidemiology of (endemic) zoonotic pathogens. Zoonotic pathogens of interest are *Echinococcus multilocularis* (fox tapeworm), *Trichinella* spp. and *Francisella tularensis* in raccoon dogs, and *Baylisascaris procyonis* in raccoons. To assess whether these zoonotic pathogens are present in raccoon dogs and raccoons in the Netherlands, two studies have been performed in recent years. In the first study (2013-2014), raccoon dogs were tested for *E. multilocularis* and *Trichinella* spp. Raccoons were not included in this study, but were investigated on an ad-hoc basis in collaboration with the Dutch Wildlife Health Center. In the second study (2016-2017), both raccoon dogs (*E. multilocularis*, *Trichinella* spp. and *F. tularensis*) and raccoons (*B. procyonis*) were tested. In the first study, nine raccoon dogs, mainly road kills, were collected for necropsies. One tested positive for *Trichinella spiralis*, which has not been reported in wildlife since 1998 and thus far was not found in wild carnivores in the Netherlands. One raccoon dog tested positive in the qPCR for *E. multilocularis*. It was collected in the central part of the country, which is not a known endemic region for *E. multilocularis*. Two raccoons, found dead in 2014 in the eastern part of the Netherlands, tested positive for *B. procyonis*. In the twelve raccoon dogs collected in the study from 2016-2017, no *E. multilocularis*, *Trichinella* spp. or *F. tularensis* were detected. *Baylisascaris procyonis* was found in one out of five raccoons. This raccoon originated from the south of the Netherlands. Despite the small raccoon dog and raccoon populations that are present in the Netherlands and the limited number of animals that were tested, three out of the four zoonotic pathogens were detected. The epidemiological consequences of these findings differ, e.g. for *E. multilocularis*, the long roaming distance of raccoon dogs may increase the predicted spread of this endemic pathogen, that is currently only present in two areas. In contrast, *B. procyonis* is a new pathogen for the Netherlands. The contribution of raccoon dogs and raccoons in the number of human patients with these zoonoses remains to be determined, but is currently likely very low due to their small populations. However, this may change when their populations increase and they colonize large part of the Netherlands. Both public health professionals and the general public will need to be aware of this potential risk and preventive measures that can be taken.

## **NON-INVASIVE BLOOD SAMPLING METHODS IN ZOO ANIMALS WITH USE OF MEDICINE LEECHES (HIRUDO MEDICINALIS)**

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**Keywords:** *non-invasive blood sampling, medicine leeches, zoo animals*

Blood sample collection in zoo animal collections is challenging clinical procedure with anesthesia needed in most of the cases. Manual restraint is possible; however, it is connected with high risk of injuries and produces significant level of stress. Objective of this study was to evaluate usefulness of medicine leeches (*Hirudo medicinalis*) from family Hirudinidae in non-invasive blood sampling in various animal species in ZOO Ljubljana. In total, seven animal species represented by 25 animals from one zoological collection were included in this study. Medicine leeches were manually applied on animals. In the meantime, venipuncture of vena jugularis was used to acquire control blood samples in all animals. Hematology analyzer HM5 and chemistry analyzer VS2 (Abaxis, USA) were used for hematological and biochemical analysis in clinical field conditions. Medicine leeches were able to draw up to 20.0 ml of blood in about 23 minutes. Time, success of withdrawal and blood volume were dependent on external conditions including temperature and light. The results of hematology and biochemistry analysis performed on groups of blood samples taken by two different sampling methods were not significantly different. However, in four animals, some of the parameters were not measured, due to the marked hemolysis as reported by analyzer. Blood withdrawing using medicine leeches showed promising results. It is a good alternative to other more complex and invasive methods and can provide significant advancement in blood sampling for preventive medicine in zoo animals.

## Pathogen screening of Norway rats (*Rattus norvegicus*)

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**Keywords:** Norway rat, pathogen screening, zoonosis

Rats are reservoirs for various pathogens with zoonotic potential, e.g. *Leptospira* spp., Cowpox virus and *Streptobacillus moniliformis*, as well as for pathogens with no or unknown zoonotic potential such as *Rattus norvegicus* polyomavirus 1 (RnorPyV1) and rat hepatitis virus. The zoonotic potential of rat-associated hepatitis E virus (ratHEV) is controversially discussed. Similarly, extended spectrum  $\beta$ -lactamase producing enterobacteria and human pathogenic norovirus were detected in rat feces, but rats are most likely not involved in their transmission. For pathogen detection in wild rats and rats in breeding colonies the network Rat-borne pathogens was established. Rats were investigated by various pathogen-specific RT-PCR/PCR and serological assays, multiplex serology and isolation approaches. An initial survey of 299 Norway rats originating from five different European countries and different habitats resulted in the molecular detection of leptospires, ratHEV and RnorPyV1. *Leptospira* DNA was detected in 41 of 293 (13.9%) investigated rats. PCR-based typing resulted in the identification of *L. interrogans* type 17, serogroup Icterohaemorrhagiae. RnorPyV1 DNA and ratHEV RNA were also frequently detected (24.8% und 13.9%, respectively). Orthopox virus (OPV) DNA was not detected, but OPV-specific antibodies were found in three from 268 investigated animals. In pest Norway rats from zoological gardens in Germany (n=127) *Leptospira* DNA, *Rickettsia* DNA, RnorPyV-1 DNA and ratHEV RNA were detected. *Leptospira* spp. were found in 19 of 127 analysed rats, whereas OPV-specific DNA and antibodies were absent. From three rats which were positive for rickettsia DNA, two animals were also positive for leptospiral DNA. RnorPyV1 was detected in 30 of 127 animals (23.6%). In seven of 108 rats ratHEV-RNA was found. In addition to the detection of ratHEV in pest rats, a Syrian brown bear showed a spillover infection with ratHEV and HEV seroconversion. Phylogenetic analysis of the obtained ratHEV sequence showed a close similarity to other ratHEV sequences obtained from wild rats trapped in the same zoo. In feeder rats, however, ratHEV infections were absent. In a pilot study 61 Norway rats from three breeding colonies of rats originally taken from the wild were investigated. In none of the 61 investigated rats *Leptospira*-DNA was detected. *Acinetobacter baumannii* was isolated from tracheal samples of six rats from 61 analysed animals. *Streptobacillus moniliformis* was detected by PCR in 10 of 61 investigated rats. A single rat hepatitis virus RNA positive animal (1/60) could be detected. PCR-based investigations resulted in the detection of 20 RnorPyV1-DNA positive animals from 28 rats tested (71.4%). Multiplex serology analysis for 21 pathogens showed the presence of antibodies reactive with rat parvoviruses, murine pneumonia virus, rat rotavirus, *Streptobacillus moniliformis* and *Mycoplasma pulmonis*. In conclusion, the results presented here confirm the added value of a network approach to develop a workflow incorporating pathogen-specific assays, multiplex assays and open-view methods for pathogen screening. Monitoring of wild rats at vulnerable points may help to develop an early warning system for the incursion of novel pathogens with zoonotic potential.



## Chlamydiaceae in wild, feral and domesticated pigeons in Switzerland

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**Keywords:** Chlamydiaceae, wild pigeons, Switzerland

Feral pigeons, common wood pigeons and Eurasian collared doves are the most common representatives of the Columbidae family in Switzerland and are usually found in highly populated, urban areas. They are known carriers of various chlamydial species including *Chlamydia* (*C.*) *psittaci*, *C. avium*, *C. abortus*, *C. pecorum* and *C. trachomatis*. Taking into consideration that *C. trachomatis* primarily infects humans and that both *C. abortus* and *C. psittaci* are zoonotic agents, pigeons may present a human health hazard. Previous studies showed considerable differences in the prevalence of *C. psittaci* in Swiss feral pigeons (3.3% to 41.7% depending on city). In Italy, a study showed a higher *Chlamydia* prevalence in Eurasian collared doves (61%) compared to feral pigeons (14.42%). However, the sample size of these studies is rather small and little is known about the occurrence of *C. psittaci* and other *Chlamydia* species in Swiss wild pigeons. In this study, 406 pigeons (324 feral pigeons, 29 domesticated pigeons, 28 Eurasian collared doves, 25 common wood pigeons) from several geographic locations in Switzerland were investigated. Pooled swab samples (choana, cloaca) were taken from 185 live pigeons. Liver samples were obtained from 52 pigeons during necropsy. Additionally, from another 169 pigeons, both swab and liver samples were taken. DNA from all samples (n=575) was extracted and screened using a family specific 23S ribosomal RNA (rRNA) Chlamydiaceae real time PCR. Positive samples (n=92) were further analysed by a species specific 23S rRNA Arraymate microarray assay. Samples that revealed single infections with *C. psittaci* (n=49 pigeons) were not further processed. The remaining positive tested samples were identified as *C. avium* positive (n=6), *C. avium* and *C. psittaci* positive (n=1), or could not be further classified (n=36). For all of these, the 298 base pair amplicon of the Chlamydiales 16S rRNA signature sequence was amplified and sequenced. Preliminary results confirmed three of the six *C. avium* positive samples. The mixed infection and eight of the unclassified samples were identified as *C. psittaci* positive. Seven samples gave no clear result, but were confirmed as *C. psittaci* positive by a specific *C. psittaci* real time PCR targeting the outer membrane protein A (*ompA*). The sequencing results for the three *C. avium* positive samples and the 21 unclassified samples are still pending. In total, 16.0% (n=92) of all samples were positive for Chlamydiaceae, of which 64 could be identified as single infections with *C. psittaci*, three as single infections with *C. avium* and one as mixed infection with *C. avium* and *C. psittaci*, 24 samples are still pending. The highest infection rate could be found in domesticated pigeons (6/29; 20.7%) and feral pigeons (65/324; 20.1%), while common wood pigeons (2/25; 8.0%) and Eurasian collared doves (1/28; 3.57%) showed lower infection rates. The high prevalence of *C. psittaci* in domestic and feral pigeons not only elucidates the infection risk for pigeon fanciers, but shows that pigeons may present a reservoir of infection to wildlife and livestock especially during competitions.

## Measuring the distribution of tick-borne encephalitis virus circulation in low endemic areas using serological screening of roe deer (*Capreolus capreolus*)

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**Keywords:** Tick-borne encephalitis virus, roe deer (*Capreolus capreolus*), geographic distribution

Tick-borne encephalitis virus (TBEV) is a tick-borne flavivirus that occurs in large parts of Eurasia, and causes (meningo)encephalitis in more than 12,000 humans annually. There are indications that the virus is spreading to non-endemic areas, putting more people at risk. Serological screening of roe deer has been successfully used to identify novel foci of tick-borne encephalitis virus (TBEV) circulation. It enabled the first finding of TBEV in ticks and TBE patients in The Netherlands in 2016 when TBEV neutralizing antibodies were detected in 6/297 roe deer samples from 2010. Most of the positive samples (5/6) were from one location: the National Park (NP) the Sallandse Heuvelrug. Here, we investigated whether TBEV had a more widespread distribution in the Netherlands than was found previously. The aim of the current study was to obtain insight into the distribution of roe deer with TBEV neutralizing antibodies in 2017, in view of identifying new TBEV foci. This study was performed in parallel to serological studies in humans, to obtain a comprehensive view of the human risk of TBE for health guideline decisions. The sampling strategy used in 2010 was set to obtain a relatively random sample of roe deer blood samples through hunters throughout the Netherlands. The approach took into account that buck hunting occurs after doe and calf hunting. The strategy used in 2017 was largely the same as in 2010, but the aim was to obtain higher numbers of samples. Serum samples were first screened by an ELISA test for TBEV-reactive antibodies. Positive and borderline specimens were subsequently tested in a TBEV serum neutralization test (SNT). In addition, this ‘random sample’ set was complemented by another smaller sample set obtained through purposive sampling during the buck hunting season, mainly to intensify sample collection in areas where does or calves had been shown to have TBEV neutralizing antibodies. All samples were also tested by RT-PCR for presence of TBEV RNA. In total, 640 roe deer samples were tested, corresponding to around 1% of the estimated roe deer population. Eighty-eight of the samples tested positive or borderline in the ELISA, and 22 of these tested positive in the TBEV SNT. The serum samples that tested positive occurred in four provinces. None of the blood samples was positive by TBEV RT-PCR. The study shows that roe deer serum-neutralizing antibodies are found more widespread than the NP the Sallandse Heuvelrug, supporting the existence of other TBEV foci. Collection of ticks in the identified areas may confirm the presence of the virus. Modeling of the 2010 and 2017 data is on-going to distinguish disease emergence from sampling intensity, and the roe deer data is currently being compared with the human sera data for health guideline decisions. Regardless of the outcome of the latter, the roe deer results indicate that general awareness of the public about the presence of the disease and precautionary measures should be raised.

## Jackals' rabies outbreak, Israel 2017-18: Has the ORV failed?

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**Keywords:** rabies, jackals, ORV

Following a dramatic rise in sylvatic rabies in 1998 the Israeli authorities implemented an oral rabies vaccination (ORV) campaign. The campaign focuses on red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) as target species. Starting at a limited scale, the ORV program expanded gradually, until reaching its present state of almost full coverage of the country. The results were encouraging, numbers of rabid animals went down, and the disease appeared to have been almost eradicated from the fox population. Although common and in some regions overabundant, only few cases of rabid golden jackals were documented. Between 2005 to 2010 we faced a rise in the urbanic rabies, originating from dogs that crossed the border from Syria. Yet the sylvatic type of the disease remained moderate, and appeared under control. Toward the end of 2017 a dramatic outbreak of rabies emerged in north-east region near the town of Beit She'an, , restricted only to jackals. The outbreak spread westward reaching 68 documented cases towards the end of April 2018. The first cases were detected in October, right before we launched the yearly ORV campaign. Primary results of the tests to detect tetracycline in the bones, a biomarker for the consumption of the ORV baits, were quite disappointing. We increased the number of baits distributed in the area along with taking measures to reduce the extremely dense jackal population. After 6 months the outbreak seems to have faded. We raised two hypotheses to explain the outbreak: (1) the bait is unattractive to the local jackals because of the huge amount of fish available from local fish ponds. We rejected this hypothesis after field tests we conducted that indicated jackals in this region readily eat the bait. (2) Timing of the campaign is problematic - overabundance of the jackals in this region resulted in a high number of young unvaccinated jackals recruited into the population after the previous ORV. However, earlier deployment of the bait, i.e. during August-September, is problematic as well due to high daytime temperatures that are detrimental to the vaccine. We are in the process of collecting and analyzing the data to better understand of the outbreak and to plan our future activities so we will avoid similar outbreaks in the future.

## Oral Vaccination of Wildlife against Rabies in Europe; Past, present and future

Ad Vos | Ferenc Kovacs

*IDT Biologika*

**Keywords:** *rabies, oral vaccination, SPBN GASGAS*

Forty years ago, in 1978, chicken heads containing a vaccine-loaded blister pack were distributed for the first time in Switzerland. These baits were intended for foxes with the purpose of vaccinating these animals by the oral route against rabies; a zoonotic disease with the highest fatality rate of any infectious disease known. Since these first field trials, the rabies situation in Europe has changed dramatically; from more than ten thousand reported rabies cases annually in the early 1980s to possible elimination of fox-mediated rabies from the European Union (EU) by 2020. However, this means not that Europe is rabies-free and control methods like ORV can cease. Re-infection cannot be ruled out since rabies is still endemic in several neighbouring non-EU countries. Furthermore, 4 new lyssaviruses all capable of inducing rabies have been identified in different bat species within the EU since the mid-1980s. Also, rabies cases as a result of imported pets are regularly reported here. Although, the risk of a sustained spill-over infection from bats and pets to nonvolant wildlife reservoir species is relatively small it cannot be ruled out. Especially considering that besides the red fox potential new reservoir species like raccoon dog, golden jackal, small Indian mongoose and raccoon are increasing not only in numbers but also their distribution range in the EU, including urban areas. Therefore, vaccination belts along borders and potential emergency oral vaccination campaigns will remain part of the rabies control program in the EU for many years to come. Since the first field trials, the concept of oral vaccination has been optimized in many aspects. For example, the chicken head baits have been replaced by industrial manufactured baits and the laborious distribution of baits by hand has been replaced by aerial distribution. Remarkably, what hasn't changed are the first generation vaccines initially used. They are still the most widely distributed oral rabies vaccines in the EU; although highly efficacious several safety concerns are associated with these vaccines. For example, these vaccines do not contain a clearly defined virus strain but are made up out of a mixture of variants. Also, the low residual pathogenicity of these live replication-competent virus vaccines has resulted in several reported vaccine-induced rabies cases. Hence, a new equal efficacious but highly attenuated oral rabies virus vaccine has been developed; SPBN GASGAS (Rabitec). As a result of site-directed mutagenesis, an existing first generation oral rabies vaccine virus, SAD B19, has been attenuated by genetically modifying a pathogenic site within its genome (position 333). Furthermore, another site (position 194) has also been altered reducing the risk of reversion to virulence as a result of a potential known compensatory mutation at this position. Hence, in contrast to the 1st generation vaccines the genotype determines the phenotype of this 3rd generation oral rabies virus vaccine. This newly developed oral rabies vaccine with an enhanced safety profile is better suited for ORV, especially for the end-phase of the rabies elimination program in EU and beyond.

## **Invasive turtles as a threat for environment and source and vector of animal and human pathogens**

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**Keywords:** alien turtles, environmental hazards, eradication, health hazards, pathogens, prevalence

Invasive turtles tackle biodiversity and compete for food and territory with autochthonous, protected European pond turtle. They might carry pathogens for other animals and humans. Interdisciplinary project aimed at measuring possible environmental and epidemic threats. Epicrates Turtle Traps were used for life-trapping of invasive turtles. Project description was communicated to local communities. Altogether, 134 turtles collected between 2015-2017 represented *Trachemys scripta* (78 red-eared sliders, 32 yellow-bellied sliders, 12 Cumberland sliders), 4 false map turtles, common snapping turtle, and different species hybrid. Several alien non-invasive species were obtained: 3 river cooters, 2 Florida redbelly turtles, and Chinese stripe-necked turtle. Seventy-eight turtles trapped and 56 captured and delivered by community members show possible eradication methods via active trapping and community awareness rising. Physical examinations proved good condition and health status: captured animals were aggressive (scaring, biting) with good locomotion and muscle tension. Thirty-six animals showed no clinical symptoms, whereas others showed mostly carapace, plastron or integument disorders. Active ovaries and eggs ready for lay in some of prevailing females indicated breeding possibilities. Generally, alien turtles showed perfect adaptation to non-native environment of Poland. GPS/GSM monitoring of home-range (signal transmission varying from few days to several months) indicated that 4 sliders did not change the location. Three individuals moved up to 4,2 km downstream the rivers. Migration was interrupted with periods of stopovers, including wintering. Quarantine and post-euthanasia samples were tested for bacteria, viruses, parasites, fungi. Multiple *Salmonella* serovars possibly incriminated for reptile associated salmonellosis were found in 26,1% of turtles. *Salmonella* spp. found in reproductive tract point out possible vertical transmission while gall bladder indicate permanent colonisation and spread into digestive tract and further into the environment. Of possible turtle pathogens *Chlamydia* spp. was observed in 16,4% of the individuals, either in pharyngeal or cloacal swabs and less frequently in respiratory tract, cloacal tissue and reproductive system. Sequencing of relevant genes clustered some samples into presumably new *Chlamydia* species related to human pathogen *C. pneumoniae*. *Citrobacter* spp. isolates could be linked with clinical disorders and possible turtle diseases. Sliders were confirmed possible vector for fish pathogens (*Aeromonas* spp., *Pseudomonas* spp., *Shewanella* spp.). Multiple fast-growing *Mycobacteria* and numerous other bacteria contribute to determination of microbial flora in alien turtles. Scarce parasite findings might be justified with interruption of infection cycle and no co-introduction of alien parasites into Poland. Simultaneously, no native parasites were transferred from European pond turtle. Low virus occurrence (single turtle-specific iridovirus and adenovirus detection) might be explained with testing of clinically healthy sliders. Multiple yeast (*Trichosporon* spp., *Cryptococcus* spp., *Rhodotorula* spp., *Candida* spp.) and mold fungi (*Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp.) found on shell and integument should be considered commensals rather than pathogens. In conclusion, invasive and alien turtles are well-adopted to Polish climate. Multiple epidemiological and environmental threats are confirmed, but overall epidemiological relevance is still far from elucidation. Targeted trapping, communication and collaboration with local communities are essential for alien turtles eradication. Study supported by National Science Centre Grant No. 2013/11/B/NZ7/01690 (Invasive turtle species as a source and vector of animal and human pathogens)

## Comparison of three methods to characterize bushpig visits into crop fields in an African swine fever endemic area of Uganda

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**Keywords:** Wildlife-livestock interface, bushpig, African swine fever

Bushpigs (*Potamochoerus larvatus*) are considered a nuisance for farmers because of their crop raiding habits. Also, through their incursions into farmlands, they may interact with free-ranging domestic pigs and potentially allow transmission of infectious diseases such as ASF. The role of the bushpig in the epidemiology of ASF is poorly known and one of the gaps of knowledge is precisely the nature of interaction between bushpigs and domestic pigs (and thus the potential for ASF transmission). In this study, we aimed to investigate the frequency of bushpig visits to crop fields in rural communities at the edge of a wildlife protected area in northwestern Uganda where ASF is endemic. We used three different methods to assess bushpig visits to farmland – questionnaires, observations for tracks and camera-trapping – implemented concurrently in 28 farms during wet and dry seasons. The results gathered by each of the three methods were analyzed by generalized linear mixed models. Potential risk factors including crop type, season and landscape characteristics related to bushpig ecology were tested as explanatory variables. A fourth model, gathering the results obtained by the three methods was performed including the method in addition to the drivers tested previously. Consistency among the methods was also tested using Kendall test. Seventy-five percent of interviewed farmers reported visits from bushpigs in 29.6% of assessed crops (n=145), whereas a frequency of 0.014 +/- 0.05 visits per night was obtained through camera-trapping. Bushpig tracks were detected in 36% of sessions of observation. Cassava (*Manihot esculenta*) and groundnut (*Arachis hypogaea*) gardens were the most visited, and visits were more common during the rainy season. Distances from crop fields to the boundary of the protected area and to the river also influenced visit frequency. Camera-trapping was the least sensitive method while questionnaires and track observations presented quite consistent and complementary results to characterize spatially and temporally visits of bushpig into the crop fields. If used in combination, these methods could provide useful data to improve our understanding of the interactions between African wild and domestic pigs at the interface.

## **Radiographic Evaluation of Thoracic Girdle Fractures in Wild Passerine Cadavers after Presumptive Window Collisions**

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**Keywords:** radiology, coracoid, H-view

Window collisions represent a common cause of injury for both wild and companion birds, and often result in fractures to the bones of the thoracic girdle. Clinical diagnosis of these fractures can be difficult, as the bird may show few clinical signs and usually requires radiographic evaluation. Radiographs were performed on 103 cadavers comprising 31 species of Passeriformes in each of 7 projections (V/D, D/V, lateral, 45° cranioventral-caudodorsal oblique, 45° caudoventral-craniodorsal oblique, 45° craniodorsal-caudoventral oblique, and 45° caudodorsal-cranioventral oblique). Following radiographs, a gross necropsy was performed focusing on thoracic girdle evaluation. Radiographs were evaluated in a randomized order, and the results were compared to the necropsy data to determine diagnostic success rates for each radiographic projection. From the necropsy data, 54% of the cadavers had at least one fractured thoracic girdle bone. The radiographs resulted in an overall accuracy of 89% for correct identification of fractures in each image. The average accuracy across all bones for each radiographic projection was as follows: V/D – 90%; D/V – 88%; lateral – 87%; 45° cranioventral-caudodorsal oblique – 91%; 45° caudoventral-craniodorsal oblique – 90%; 45° craniodorsal-caudoventral oblique – 91%; 45° caudodorsal-cranioventral oblique – 87%. The scapulas had the highest fracture frequencies and were also the most commonly missed fractures. There was a high level of accuracy across all projections, with the highest accuracy noted for the 45° cranioventral-caudodorsal and 45° craniodorsal-caudoventral oblique projections.

## Pregnancy diagnosis of Muskoxen (*Ovibos Moschatus*) in high arctic conditions

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**Keywords:** *Ovibos moschatus*, reproductive tract, ultrasound.

**Abstract** Pregnancy diagnosis of muskoxen is difficult in arctic conditions. Reproductive ultrasound examination is challenging due to anesthetic time constraints, rapid battery depletion at low temperatures and limited access via traditional rectal or transcutaneous approaches. Reliable blood based pregnancy diagnoses often require more than one sample, and can be difficult to interpret. Fifteen muskoxen (*Ovibos moschatus*) were examined in North East Greenland during general anesthesia to evaluate gestation and reproductive health as part of a project on muskox population dynamics.<sup>2</sup> The ultrasounda batteries were transported in heated isolated boxes, allowing a scanning time of approximately 20 minutes per battery. Intravaginal scanning with a human obstetric probe resulted in excellent quality images, providing an alternative to clipping. Blood samples from pregnancies determined via ultrasound were included in a larger study, and allowed the evaluation of two commercially available enzyme-linked immunosorbent assays (ELISAs)<sup>b</sup> to detect pregnancy-associated glycoproteins (PAGs). PAGs are expressed by the ruminal placenta, making their detection in blood an accurate indicator of pregnancy.<sup>1</sup> The photometric ELISA showed a sensitivity and specificity of 93.7 % and 100.0 %, respectively. The visual ELISA required subjective interpretation, resulting in a slightly reduced sensitivity of 81.2 %, but still was adequate as an in-house tool when limited laboratory equipment is available. In conclusion, ultrasound of the muskoxen reproductive tract is feasible in arctic environments. Pregnancy may be reliably determined using an adequate ultrasound technique or using a simple blood sample. a Acuson P500, Siemens Medical Solutions USA, Inc. Mountain View, CA 94043 USA; b IDEXX Bovine Pregnancy Test, IDEXX Rapid Visual Pregnancy Test IDEXX Europe B.V., 2132 LR Hoofddorp, The Netherlands **ACKNOWLEDGMENTS** The authors thank Siemens Health and TJ imaging for providing the ultrasound equipment in the field. Eva Maria Greunz is supported by a grant from Annie and Ottos Johs. Detlefs' Foundations. **LITERATURE CITED**

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## Defining animal welfare standards for hunting based on body mass and flight distance

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**Keywords:** animal welfare standards, hunting, wounding

In many parts of the world hunting is necessary to keep wildlife populations at numbers that do not exceed available food resources and space needs and that minimize wildlife impacts on human infrastructure and production. Hunting is also regarded as an important leisure activity of great cultural value. In addition, hunting can provide food and/or income for the hunters and their families. However, there is growing concern about the animal welfare impact of hunting. One of the most critical points for animal welfare during hunting is the killing of the animal. However, it is inherently difficult to objectively and quantitatively measure animal welfare in the hunting situation, and only few efforts have been made. An ideal kill during hunting should cause the “least animal welfare harms to the least number of animals”. It should consequently be characterized by the shortest possible duration and the lowest possible intensity of suffering. Intensity is subjective and difficult to evaluate, which leaves only duration as the key assessor of animal welfare. In this presentation we describe the development of a theoretical model which uses body mass, allometric scaling, wound ballistics, and comparative physiology to estimate time to incapacitation for animals killed by hunting rifles. This time span is, however, difficult to measure for a hunter because animals can be difficult to follow after shooting and the hunter should be focused on the flight behaviour of the animal he shot at. A more convenient measure of animal welfare is flight distance - the actual distance traveled by an animal from where it was hit by the first bullet to where it fell incapacitated. We combined linear kinematics and measured flight distances from actual hunting situations where animals were shot according to codes of best practice (i.e. hit by a bullet of appropriate calibre perpendicularly to the thoracic wall and penetrating central parts of both lungs). Based on this we converted and calibrated the theoretical model, so that we based on body mass alone can calculate flight distances consistent with optimal killing with respect to animal welfare. Similarly, by using observed maximum flight distances (mfd) for animals killed according to codes of best practice, we developed an equation calculating theoretical values for mfd relative to body mass (M):  $mfd = 4.92M^{0.73}$ . Acceptable animal welfare is then characterized by flight distances shorter than mfd, whereas flight distances longer than mfd should be characterized as wounding, i.e. the animal is not killed as quickly as possible and hence experiences more harm than necessary. We believe that this model is a feasible tool to monitor and evaluate the quality of hunting and killing of terrestrial mammals. This tool may be used by individual hunters who wants to ensure and improve their ability to cause least animal welfare harms or stakeholders and authorities that want to ensure good animal welfare during hunting and management culling- It may also be a tool for producers of rifles and ammunition that want to develop and market products that facilitate improvement of animal welfare.

## Unraveling the potential causes of secondary hyperparathyroidism in free-living nestling white storks (*Ciconia ciconia*) from Central Spain

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**Keywords:** *White stork, Secondary hyperparathyroidism, nutrition*

Since 2014 severe bone decalcification, deformation and structural fractures were observed in white stork (*Ciconia ciconia*) nestlings in the colony of Cabañeros National Park in central Spain. The lesions are characteristic of secondary hyperparathyroidism, a metabolic disease caused by an imbalance of the calcium and phosphorus ratio, usually due to a calcium deficient diet. Many nestlings died, while others had to be euthanized. The aim of this ongoing study is to confirm secondary hyperparathyroidism and to determine possible causes. In the 2017 breeding season we used comparative photo-trapping between affected and unaffected nests to detect incubation problems. We compared body condition and biochemical parameters among affected and apparently healthy nestlings sampled in 2015 and 2016. We analyzed pathogens that could have affected intestinal calcium absorption by affecting intestinal mucosal integrity and motility such as *E. coli* and *Salmonella*, and the presence of antibodies to West Nile virus. In peripheral blood, we studied the exposure of affected and healthy nestlings from 2016 to contaminants that could either compete for intestinal absorption of calcium (heavy metals) or affect the metabolism of calcium (organic pesticides and cantharidin). We performed necropsies of six deceased or euthanized chicks and collected tissues for histopathology, pathogen detection and the stomach contents. In five stomach contents we analyzed the presence of pesticides and cantharidin. We identified prey remains in the stomach contents of two affected nestlings. Based on the results we captured beetles and grasshoppers, in which we determined the presence of pesticides, cantharidin and their calcium and phosphorus content. Using camera traps we did not identify significant disturbance during incubation. Nestlings with lesions compatible with secondary hyperparathyroidism (12 of 63, 19% in the 2015-2017 seasons) presented worse body condition and a reduction in alkaline phosphatase enzyme activity in their peripheral blood. No significant differences were detected in the levels of persistent organic pollutants (PBDEs, PCBs and DDTs) and heavy metals (Cd, Hg and Se) between the affected and healthy nestlings, being levels similar to the concentrations detected in other natural areas of Spain. The affected chicks had higher levels of lead associated with lower alkaline phosphatase, but within very low concentrations. This suggests that the lead levels could be a consequence of mobilization of calcium and lead deposits from the bone. Cantharidin and other pesticides were absent in grasshoppers and beetles, and the average concentration of calcium (0.23% in both groups) and phosphorus (0.47% grasshopper and 0.20% beetles) was low relative to dietary requirements. Thus we confirmed secondary hyperparathyroidism in the affected nestlings, and ruled discomfort during incubation and the studied contaminants out as possible causes. Although the data suggest an imbalance of calcium and phosphorus in the diet, further comparative study of the diet is needed. In addition, inclusion of samples from other years will increase the sensitivity and robustness of the study. Future activities include the analysis of cast pellets of healthy and affected nestlings, capture of prey items, and radio-tagging of adults from affected nests to identify foraging areas, potential risks and prey availability.

## **Cattle Egret as a potential reservoir of avian pathogens in South-West France.**

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**Keywords:** *Cattle egret, Mycoplasma gallisepticum, poultry*

Recent outbreaks of highly pathogenic avian influenza (HPAI) in Europe had strong impact on wild birds populations and poultry production. In South-west France, 2015-2016 and 2016-2017 HPAI outbreaks had dramatic consequences on the economy of French duck industry while wild birds seemed relatively spared. In response to these outbreaks, biosecurity of duck and other poultry farms has been questioned and studies have been implemented to better understand the drivers of local dissemination of highly pathogenic avian influenza viruses (HPAIV) and other avian pathogens. Indeed, while many studies have been undertaken to assess the role of migratory birds in the long distance spread of HPAIV, few studies have focused on the role of commensal wild birds in the epidemiology of those avian pathogens. Migratory birds, mainly anseriformes and charadriiformes, whose can introduce HPAIV in a new geographical area are seldomly in contact with domestic birds and we can assume that other birds species can be bridge hosts linking those two epidemiological compartments. Cattle Egret (*Bubulcus ibis*) is a gregarious species which nests in colonies in wetlands and feeds by following cattles in pastures. In South-west France, where the species is well implanted for more than ten years, birds are commonly observed feeding among domestic ducks in farms and thereby are suspected to play a role in both introduction and dissemination of avian pathogens, including AIV. In order to assess the epidemiological role of Cattle Egret in the epidemiology of AIV and other major avian pathogens among duck and poultry productions in South-west France, a longitudinal study were undertaken from 2016 through 2017. During this period, three nesting colonies located in the proximity of poultry farm infected with HPAIV were regularly monitored. For this purpose, fresh fecal swab (n=30 for each session, 265 in total) were sampled from the ground of colonies (Cattle Egret nests in trees from 1 to 20 m high). Swabs were pooled by five and after RNA and DNA extraction, quantitative PCR targeting AIV M gene, Newcastle Disease Virus (NDV) gene M and *Mycoplasma gallisepticum* (MG) 16s RNA gene were performed. Fecal samples of cattle egrets tested negative for both AIV and NDV nucleic acids. By contrast, MG was detected in 4% of pools, that was confirmed by sequence analysis. Absence of detection of AIV and NDV genomes is consistent with previous studies suggesting that, while Cattle Egret is highly susceptible to HPAIV, they usually shed low to extremely low virus loads. Moreover, short persistence of both viruses in their host makes their detection often challenging. Conversely, pathogens like MG can infect chronically birds, making them better marker of pathogens exchange at the wild / domestic interface. Thus detection of MG in cattle egrets would indicate possible role of this species in pathogens dissemination in commercial poultry.

## Comparison of Rev.1 live vaccine innocuousness between Alpine ibex (*Capra ibex*) and domestic goats (*Capra hircus*): an unexpected strong species effect

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**Keywords:** wildlife reservoir, brucellosis, vaccination

In 2012, unexpected bovine and human brucellosis cases due to *Brucella melitensis* biovar 3 occurred in the French Alps. Epidemiological investigations evidenced a reservoir population in Alpine ibex (*Capra ibex*) from the Bargy Massif. After a mass ibex culling implemented in 2013 failed to reduce brucellosis prevalence, Rev.1 vaccination, which has proved really efficient in domestic sheep and goats, was put forward as a possible additional tool to control brucellosis in this wild and protected population. Nevertheless, before implementing vaccination, it appeared necessary to ensure that this live vaccine was as safe in ibex as it is in domestic small ruminants. The present study thus aimed at comparing the innocuousness of a commercial Rev.1 conjunctival vaccine (OcuvevÒ, CZV, Spain) between ibex and domestic goats (*Capra hircus*). The study included 24 animals divided into 4 groups: 6 males or 6 non pregnant females of each species. Ibex (captive-born) and goats were housed in two different settlements, males and females being kept separately. Within each group, five animals were vaccinated whereas one unvaccinated contact animal was further introduced 3 to 24 h post vaccination (pv). Half of the vaccinated animals were euthanized and necropsied at day 45 pv while the remaining animals (i.e., including contacts) were necropsied at day 90 pv. Eight organs and three swabs were sampled systematically during necropsy for bacteriological examination according to the same protocol for all individuals. Additional samples were collected in vivo at day 20 and 68 pv in order to further explore the kinetics of bacteria shedding (whole blood, eye, nasal and genital swabs) and antibody production (Rose Bengal, iELISA, and Complement Fixation tests). Culture results from vaccinated animals were analysed accounting for the vaccine strain dissemination: the proportion of culture-positive organs and the bacterial load in infected organs. The serological and bacteriological status of unvaccinated contact individuals was also considered. *Brucella* distribution and antibody (Ab) profiles were highly contrasted between the two species. While higher and more lasting Ab titres were observed in ibex, the proportion of infected samples was significantly higher in ibex compared to goats (OR ibex/goat=4.184, 95% IC [2.311; 7.574]). This effect was even higher when focusing the organs of the pelvic region (OR ibex/goat=7.222, 95% IC [2.192; 23.799]). In infected organs, the bacterial burden was higher in ibex compared domestic goats (P ibex/goat=+2.410, 95% IC [+1.409; 3.411]). Two ibex males showed culture-positive genital swabs or urine samples from day 20 to 65 pv. As a probable result, the contact male ibex seroconverted (between 45 and 68 days pv) and was found culture-positive at necropsy at day 90 pv. Neither excretion nor transmission to contacts was observed in the other groups. In this experiment, differences between species remained the main source of variation, with low impact of other individual factors (age, sex, or sanitary status). The multiplicative and shedding capacity of the Rev.1 vaccine was indeed much higher in ibex compared to domestic goats. Consequences for vaccine application in nature are further discussed.

## **Few wild birds - many domestic outbreaks of highly pathogenic avian influenza H5N8 during the 2016-2017 epizootic: the French paradox**

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**Keywords:** Avian Influenza, Epizootic, France

The 2016-2017 H5N8 highly pathogenic avian influenza (HPAI) epizootic started in Europe with a first case reported in October 2016 from a wild bird in Hungary. French authorities had already set up a reinforced AI surveillance towards wild and domestic birds, after the FAO alerted on a new H5N8 HPAI outbreak first detected in May 2016 in the Tuva Republic, concerning wild birds and threatening to spread to Western Europe along migratory bird corridors. In November 2016, the first French H5N8 cases were detected in dead decoy ducks in Northern France. At the same time in neighbouring European countries, many tufted ducks (*Aythya fuligula*) were dying from H5N8 HPAI in the Netherlands, Germany and Switzerland, close to the French border. Two gulls (*Laridae*) were soon confirmed positive in France on the Lac Leman shore, near to the Swiss cases. Only few other cases were further detected in France here and there. Surprisingly, wild bird cases remained mostly sporadic and dispersed in France during this outbreak contrary to many other member states. On the other hand, H5N8 HPAI quickly spread in the poultry sector in South-western France, far from wetland areas and the two main migratory routes, and contaminated unexpected bird species. By the end of January 2017, after the end of the cold period, only two clusters of mortality cases were reported in wild birds. The last wild case in France was declared in March 2017. No further cases have been detected in France in wild birds until now while many H5N8 cases were still being regularly reported in Italy, the British Isles, Switzerland, Germany and the Netherlands (among other European countries). The contrasted situation observed in wild birds between France and the neighbouring countries, led to questioning the efficacy of our country's passive surveillance system. Nevertheless, the number of birds (focusing on waterfowl) collected for AI testing had been multiplied by 20 during the last epizootic by our surveillance network (SAGIR): 913 wild birds had been analysed, of which only 90 were found H5N8 positives, in 52 either isolated or grouped mortality cases. In order to better assess the efficacy of the passive surveillance system, a specific task force has been deployed by the French Hunting and Wildlife Agency consisting in systematically searching for dead birds in 10 main wetland areas at risk during the winter. More than 825 000 live birds were observed, but only 35 dead birds were found, all were negatives. These results suggest the efficiency of our passive surveillance system to detect AI cases. Such a low incidence level is probably related to the behaviour of migratory birds. Beyond this episode, wild bird infection remains a permanent threat as a source of introduction of AI viruses in France. Therefore, the French authorities consequently imposed enhanced biosecurity measures both in the farms and during transportation to manage the risk of introduction and spread of AI viruses, as well as specific surveillance measures for waterfowls.

## Virulence and excretion dynamics of H5 highly pathogenic avian influenza virus of the Goose/Guangdong lineage in experimentally infected wild ducks

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**Keywords:** *Avian influenza virus, Anatidae, Virology, Pathology, Poultry disease, Wildlife reservoir*

Highly pathogenic avian influenza (HPAI) is essentially a poultry disease. Wild birds have traditionally not been involved in its spread, but the epidemiology of HPAI has changed in recent years. After its emergence in southeastern Asia in 1996, H5 HPAI virus of the Goose/Guangdong lineage has evolved into several sub-lineages, some of which have spread over thousands of kilometers via long-distance migration of wild waterbirds. In November and December of 2014, the HPAI H5N8 GsGd virus (clade 2.3.4.4, group A, Buanlike) was detected in wild birds and poultry in various countries of Asia, Europe, and—for the first time—North America. The global spread of HPAI H5N8 virus in 2014/2015 raises the question whether this H5 virus was better adapted to wild birds (i.e., increased virus replication and transmission combined with decreased virulence, resulting in better virus survival in wild bird populations) than the HPAI H5 virus from before 2014/2015. In this study, we questioned whether the HPAI H5N8 virus clade 2.3.4.4 group A detected in Europe in 2014 had different virulence in different species of wild waterbirds than the HPAI H5N1 virus detected in Europe before 2014. We experimentally infected four species of wild ducks: Eurasian wigeon, common teal, mallard, and common pochard with an avian isolate of HPAI H5N8 virus from Europe obtained in 2014. We chose ducks because they are abundant, migratory, and an important group in the epidemiology and the ecology of influenza A viruses in the wild. We chose these particular duck species because of their abundance, preference for freshwater habitats, geographical distribution, and migratory pattern spanning Asia, Europe, and Africa. We also compared virus excretion and disease severity with historical data of the HPAI H5N1 virus infection from 2005 in the same four species. Our results showed that excretion was highest in Eurasian wigeons for the 2014 virus, whereas excretion was highest in common pochards and mallards for the 2005 virus. The 2014 virus infection was subclinical in all four waterbird species, while the 2005 virus caused clinical disease and pathological changes in over 50% of the common pochards. In chickens, the 2014 virus infection caused systemic disease and high mortality, similar to the 2005 virus. In conclusion, the evidence was strongest for Eurasian wigeons as long-distance vectors for HPAI H5N8 virus from 2014. The implications of the switch in species-specific virus excretion and decreased disease severity may be that the HPAI H5 virus more easily spreads in the wildwaterbird population.

## *Poster presentations*

### **Investigating the genetic diversity of lagomorph infecting treponemes and their relatedness to human pathogenic *Treponema pallidum***

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**Keywords:** *Treponema paraluisleporidarum*, lagomorphs, syphilis

The rabbit infecting *Treponema paraluisleporidarum* ecovar Cuniculus (TPeCuniculus) and the hare infecting orthologue TPeLepus belong to the group of uncultivable nonhuman pathogenic treponemes. Similar to human syphilis, rabbit treponemal disease is typically sexually transmitted and results in erythema and edema of the anogenital area, often followed by skin ulceration and crusting of the lesions. Infection of the nose, eyelids, lips, and paws can also occur. So far, limited information is available on the diversity, clinical relevance and occurrence of TPeCuniculus infection in wild rabbits and almost nothing is known about TPeLepus infections in hares. Based on serological results, infection of hares is widespread in the European area. However, infection seems to be mostly sub-clinical (no ulcerative skin lesions reported) compared to the frequency of seropositivity. Yet, the impact of infection on hare populations is currently unknown. Based on newly obtained genome data from treponemes infecting mountain hares (*Lepus timidus*), phylogeny suggests that the causative agent of hare syphilis is similar but distinct from the agent of rabbit syphilis. However, the genetic relatedness of strains infecting the two hare species *L. europaeus* and *L. timidus* is currently unsolved. We outline a study that aims to investigate whole genomes of uncultivable treponemes with a greater number of newly collected and characterized TPeCuniculus and TPeLepus strains from wild lagomorphs in Europe, Africa, and Australia. Trapping and sampling of live rabbits and hares is anticipated if samples cannot be obtained through collaborations or from dead animals. Sampling of dead animals and initial processing of samples will be followed by serology, DNA extraction and quantification of DNA via qPCR. Furthermore, a newly established and validated Loop-Mediated Isothermal Amplification (LAMP) will be performed under field conditions to rapidly test for treponemal DNA. Multi-locus strain typing analysis utilizing multiple different genes with reasonable heterogeneity will be used to select most suitable candidate strains for whole genome sequencing. In vivo cultivation of clinical isolates is anticipated to create new laboratory strains. The results will significantly contribute to basic research in treponematoses and to our understanding of *Treponema* evolution, pathogenicity, epidemiology, and ecology. Genome sequences of *Treponema* infection in rabbits and hares may help to clarify the evolution of syphilis and diseases caused by related subspecies (e.g., yaws).

## **New *Austropotamobius torrentium* haplotypes revealed after molecular identification and phylogenetic analysis in Western Macedonia, Greece**

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**Keywords:** *mtDNA, Austropotamobius, phylogeography*

The Astacidae family is the only freshwater crayfish family present in Europe and is represented by 5 species that belong to 2 genera. The phylogeography of the family is very heterogeneous and it is largely shaped by the geography of drainage basins. The result is the shaping of a different genetic pools that ask for accurate identification and taxonomy to plan conservation actions. The goal of the present study was the identification of individuals of the Astacidae family populating rivers of the Florina county, using molecular markers, and the analysis of phylogenetic and phylogeographic relations of these individuals. The analysis was based on the barcoding gene of Cytochrome c oxidase subunit I (COI). All the crayfish individuals were identified as *Austropotamobius torrentium*, a fact of particular importance since Northern Greece is the southern reach of the species distribution. The recovery of genetic data for the species populations from other European countries and Greece allowed the study of the phylogenetic and phylogeographic relations and the revelation that the populations of Florina are a unique genetic pool of haplotypes that have not been recorded in any other population to date. The examined populations add to the genetic richness of the populations of the southern Balkans, which were probably a refugia shelter during the last glacial period and continue to be a genetic trust that requires careful management.



## Genetic variability of *Rhinolophus mehelyi* population at the northern margin of the species distribution range

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**Keywords:** bat conservation, founder effect, haplotype diversity

*Rhinolophus mehelyi* is a medium sized horseshoe bat, with a primarily Mediterranean distribution and vulnerable status all over Europe. The species has a highly fragmented range in Europe, with large declines noted in the XXth Century, especially in Western Europe. In Romania the species was known to occur in extreme SE Romania, but local populations, being affected by agriculture and intense habitat modifications, faced a dramatic decrease. Today's population numbers around 150 bats. Romania lays on the northern limit of the species range, with the two northernmost colonies known in Europe being in SE Romania (Tulcea) and SW Romania (Valea Cernei). In order to assess the genetic diversity of these bat populations at their range limits, we collected 128 skin biopsy punches from 3 locations from Romania and 5 locations from Bulgaria (European population strongholds). Two mitochondrial markers, the second hyper variable (HVII) domain of the mitochondrial D-loop region and the 12S region, as well as the nuclear recombination activating gene II (RAG2) were amplified by PCR. We calculated haplotype diversity ( $h$ ), nucleotide diversity ( $\pi$ ) for all samples, as well as the number of polymorphic sites ( $S$ ), and average number of pairwise differences ( $k$ ). Our intention is to evaluate the genetic variability of known Romanian *R. mehelyi* populations, and to compare it to a large core population from Bulgaria. Our intention is to test whether these two populations constitute remnants of a former more widely distributed (but unknown) population, or if Romanian populations are the result of colonisation events of a (formerly) expanding population. Our main hypothesis is that populations which are the results of a recent colonisation event will have overlapping, but greatly reduced haplotype spectra (genetic bottleneck), while old remnants should retain similar haplotype diversity in comparison to individuals belonging to the core population. The northernmost European population (Tulcea) has the lowest values for  $h$  and  $S$ , with diversity values increasing from north to south. We suggest that the Tulcea population is the result of a founder effect caused by the recent colonisation of the area.

## Yellow ear: etiology and incidence of pinna injuries in bats in Belgium

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**Keywords:** ear lesions, pinna, bats

The emergence of white nose syndrome has emphasized the need for more knowledge about diseases in bats. During the winter of 2012 we discovered 3 bats with ear lesions. Below, we illustrate our findings. The observed cases prompted us to screen for pinna injuries in bats. Between 29/01/2012 and 13/02/2012, a cold wave hit Belgium. This condition enabled the frost to penetrate in the caves much deeper than usual. Just after the cold wave, in 3 different caves of the area of Rochefort, we observed 3 bats (1 *Myotis bechsteinii* and 2 *Myotis nattereri*) close to the entrances, with external otitis. A thick purulent yellow ear discharge was visible at the tip of the pinnae and was sampled for bacteriological examination. Light microscopy of a Gram-stained smear made from the pus allowed to detect leucocytes and bacteria. The P.A.S. staining method excluded mycosis. The bacteriological culture identified two Gram-negative bacteria, *Serratia liquefaciens* and *Acinetobacter lwoffii*, and a Gram-positive bacterium *Kocuria rosea*. The bacteria we found are widespread in the environment but are not able to destroy the epidermis. In humans, similar bacteria are found in external otitis but additional etiologic factors are needed for an infection to start. Destruction of the cutaneous barrier, in association with moisture, is a common cause of external otitis. In our case, we assume that a frostbite lesion was secondarily infected by bacteria present in the moist environment of the cave. Masing (1984) reported that in Estonia, 3.8 % (n=52) of bats hibernating in natural caves had pinnae partially amputated, versus 0.4% (n=260) of bats hibernating in mines. He assumed this difference is due to the fact that mines are deeper than most of the caves, and better protected from the frost. At the entrance of a cave close to where the described cases were found, capture sessions with mist nets are organized every fall, for biological research purposes. This site is a known swarming site. From 2012 to 2017, the pinnae of the captured bats (n = 1056) were screened for lesions by visual inspection. Overall, 6% of bats showed pinna lesions (males 8%, females 2%). Only *Myotis* species were affected (*M. emarginatus* 11 %, *M. bechsteinii* 10%, *M. daubentonii* 2%, *M. nattereri* 2%). Pinna injuries in bats are common but are not well described in the literature. Frostbite in combination with bacterial infection is probably a frequent cause of such lesions, but other etiologies are highly probable, particularly for species like *M. emarginatus* which are known to hibernate far from the cave entrance. We have no explanation for the apparent sex ratio difference in the occurrence of these injuries.

## **Bat bites are only skin deep, but dog bites go clean to the bone: implications of comparative pathogenesis in reservoir hosts for human rabies**

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**Keywords:** *lyssavirus, zoonosis, bat*

Rabies, caused by infection with a lyssavirus, is an invariably fatal disease of all mammals. Lyssaviruses can be transmitted to people by bites of infected carnivores and bats. Bat-acquired rabies is becoming more common, and its diagnosis may be missed because the clinical presentation differs from that of dog-acquired rabies. Our aim was to compare rabies pathogenesis between carnivores and bats to identify underlying mechanisms for the differences in clinical presentation of human rabies. We performed a literature review for publications on lyssavirus infection in bats using the major search engines, with relevant search terms. Literature on lyssavirus infections in carnivores and people was identified through searches in handbooks and literature reviews. Our results showed three main differences in rabies pathogenesis between bats and carnivores. First, bat lyssavirus infections start in the skin, while carnivore lyssavirus infections likely start in the skin or muscle. Second, bat lyssavirus excretion probably occurs not only via salivary gland but also via tongue, while for carnivore lyssaviruses, excretion via the tongue has not been examined. Third, some bats lack lymphocytic encephalitis at the end stage of rabies, in contrast to carnivores, where lymphocytic encephalitis is reported in all end-stage cases. The preferential entry into sensory nerves of the skin in bat-acquired rabies versus motor nerves of the muscle in dog-acquired rabies, influences the pattern of spread to the central nervous system. This difference in pattern of virus spread may provide a mechanistic explanation for many differences in clinical presentation of human rabies. A better understanding of these differences should improve the recording of a patient's history, enable drawing up of a more sophisticated list of clinical characteristics, and therefore obtain an earlier diagnosis of rabies after contact with a bat or carnivore that has rabies.

## Could the thermal camera be an useful tool to help protecting the Seychelles sheath-tailed bat (*Coleura seychellensis*)? A pilot study.

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**Keywords:** *Seychelles sheath-tailed bat, thermal camera, census*

The Seychelles sheath-tailed bat *Coleura seychellensis* is listed as critically endangered on the Red List of the IUCN. This bat species was described in 1868. This species has been recorded from coastal boulder field caves with stable temperatures, horizontal ceilings and access into native palm woodland or marsh habitat. They need clear cave flyways not obscured by vegetation. The last census was done in 2009 restricted the presence of the sheath-tailed bats to 4 sites on 2 islands, Mahé and Silhouette. Abandoned roosts have been recorded from all four islands in the species historical distribution. Records from the last century and popular reports indicate that a strong decline in numbers and probably range has occurred. The surviving population is estimated to be as low as 50 to 100 individuals in total. Conservation actions are required based on research into the biology of the Seychelles sheath-tailed bat including basic information on feeding ecology and diet, habitat preference, movements, roosting habits and breeding ecology. This was a pilot study in which thermal camera was used to run a census of the Seychelles sheath-tailed bat in one site of the Mahé Island. The presence and the behavior of an animal can be detected over long distances thank the heat emitted from the animal and this is not an invasive technique that does not require artificial illumination. The regular use of thermal camera could improve the knowledge of the Seychelles sheath-tailed bat and help to protect the species by obtaining information on feeding areas and roosting caves.

## A case of swinepox in a wild boar (*Sus scrofa*) in Belgium

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**Keywords:** wildboar - dermatitis - poxvirus

A male wild boar, less than one year old and shot because of abnormal coat, showed multiple dark brown to black skin lesions up to cherry size spread over the whole body, including perineum and the external genital tract. On basis of necropsy, the boar was heavily infested by respiratory and digestive helminths, *Metastrongylus* spp. and *Ascaris suum*, respectively, but no other inside lesion was seen. From papules and crusts, histopathological examination, bacterial investigation and multi-sequencing were performed. Histologically, a severe exophytic dermatitis with crusts and bacterial foci was observed. For bacteriology, the skin swab revealed the involvement of *Staphylococcus aureus* as secondary infection. No parasite was seen based on cutaneous scrapings and trichograms. Finally, the skin-lesion involved agent was identified as swinepox virus by sequencing. Swinepox virus (SWPV), which replicates only in swine, belongs to the *Suipoxvirus* genus of the *Poxviridae* family. It's the etiologic agent of a skin disease of pigs, characterized by generalized pustular lesions and associated with high rates of illness (>80%) but low death rate (<5%). In domestic pigs, it occurs mainly with poor management and housing conditions. Swinepox virus outbreaks are often reported in farmed pigs but, at this day, only one case of swine pox in a wild boar (*Sus scrofa*) has been described (Austria, 2004). In the wild, infestation with *Haematopinus suis* could promote the infection.

## First Report of Abortion of a Roe Deer Related to *Brucella* spp in Greece

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**Keywords:** Abortion, *Brucella* spp, Roe Deer

Brucellosis is a zoonosis that remains endemic in mainland Greece. In wild ruminants *Brucella* spp infection follows a course similar to that in the cattle. Interactions between domestic and wild ungulates represent a major problem in epidemiology and the role of Roe Deer in some diseases of concern in livestock is yet unknown. A female Roe Deer (*Capreolus Capreolus*) approximately 2 years old was admitted to ANIMA -Wildlife Rescue and Rehabilitation Center from mountainous Nappaktia Greece, on 21.04.2013 with severe traumatic injuries. Supportive treatment immediately initiated with fluids (Lactated Ringer's, iv), steroids (Dexamethasone: 5 mg/kg, im, once) as well as antibiotics (Trimethoprim sulfadiazine: 20mg/kg, im, SID) that continued for 7 days. Thoracic x-rays did not reveal anything abnormal. One week later the deer was transferred to a protected recovery area in the National Park of Parnitha, since it recovered from the injuries. Abortion of 2 near- to-, full term fetuses (~2kg) with retention of embryonic membranes was noticed 3 weeks later. After sedation (Xylazine HCL:1.1mg/kg), the embryonic membranes were removed, and blood samples were taken for hematological, biochemical and serological examinations while the animal was treated with intrauterine antibiotics. Hematology and biochemistry were normal. In addition the blood serum was tested in the accredited National Laboratory of Athens (NLA), where the serological tests are carried out according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of OIE. The results were as follows: a) Rose Bengal Test (RBT): positive (visible agglutination, double-checked). b) Complement Fixation Test (CFT): Serum gave titer of 106,4 ICFTU/ml, which is considered to be positive (double-checked). Even though Rose Bengal is economical, simple and rapid screening test, confirmation is achieved by detection of IgG with CFT. The simultaneous use of both is recommended to obtain a maximal sensitivity for *B. abortus*, *B. melitensis*, and *B. ovis* infections. In conclusion, the seropositive results for *Brucella* spp in combination with compatible clinical signs suggested exposure of the Roe Deer to *Brucella* spp. It is believed that wild ruminants are occasional victims of Brucellosis "spill-over" from livestock, rather than true reservoirs. Roe Deer is a selective browser and less gregarious than other deer species but the pasture sharing and contact with feral livestock is common in mountainous Greece. This is the first confirmed case of Brucellosis infection in a Roe Deer in Greece. A more extensive survey on the prevalence of Brucellosis in wild ruminants of Greece should be carried out in order to assess the risk factor of Brucellosis in wild ruminants.

## **Mycoplasma conjunctivae occurrence in vectors and anatomic locations related to transmission and persistence**

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**Keywords:** *Infectious Keratoconjunctivitis, wildlife-livestock interface, mycoplasma*

*Mycoplasma conjunctivae* is an obligate microparasite that does not long resist environmental conditions and causes Infectious Keratoconjunctivitis (IKC) in Caprinae. This mycoplasma has a tropism to ocular structures where causes inflammation and tissue damage, leading to blindness and eye perforation in the worst cases. Despite it is considered strictly an ocular disease and direct contact the main way of IKC transmission, alternative routes and locations other than the eyes have not been thoroughly studied with sensitive molecular methods. With the aim to assess alternative locations of potential importance for transmission and within-host persistence, we studied the occurrence of *M. conjunctivae* by qPCR in nose cavity, external ear canal and the reproductive tract in relation to the eyes detection, and in flying insects captured in the vicinity of different IKC scenarios. A total of 205 wild (Pyrenean chamois *Rupicapra pyrenaica*=155; Iberian ibex *Capra pyrenaica*=46, mouflon *Ovis aries musimon*=4) and domestic (sheep *Ovis aries*=98) hosts and 472 flying insects were studied (associated with three *M. conjunctivae*-endemic sheep flocks and one IKC outbreak in a captive Iberian ibex population). We detected *M. conjunctivae* in ear 7.2 % (CI 95% 4.7-11.0) and nose swabs 9.5 % (CI 95% 6.4-13.9) of the ruminants without statistically difference of the prevalence among the species sampled. Its detection in nasal cavity was mostly associated with ocular infection 95.6% (CI 95% 79.0-99.8). However, only 52.6% (CI 95% 31.7-72.7) of the animals positive in ear swabs were also positive in eye swabs. Among the eye-positive ruminants, 61.1% (CI 95% 44.8-75.2) were positive in nose swabs and 27.8% (CI 95% 15.8-44.0) in ear swabs. Ct values of the qPCR (inversely related with target DNA) were significantly higher in the ear than the nose and eyes ( $p<0.01$ ) and higher in the nose than the eyes ( $p<0.01$ ). Among the flying insects captured, *M. conjunctivae* was detected in 7.1 % (CI 95% 2.0-22.6) of *Musca* spp. associated with the IKC outbreak and 1.7% (CI 95% 0.6-4.8) of *Musca* spp. associated with one of the *M. conjunctivae*-endemic sheep flocks. These results suggests that *M. conjunctivae* transmission may occur by direct contact from eye or nasal secretions and/or indirectly through flies, particularly from clinically affected individuals during IKC outbreaks. Being wild Caprinae spatially structured populations, flies may have an important role for transmission between groups and/or for cross-species spillover. The detection of *M. conjunctivae* only in external ear canals in some animals suggest that this location may be relevant for within-host persistence, as described in other mycoplasma infections. However its epidemiological relevance or the potential clinical consequences remains to be elucidated.

## Pasteurellaceae occurrence in relation to bronchopneumonia in a Pyrenean chamois population

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**Keywords:** *Pasteurella multocida*, bronchopneumonia, chamois.

Bronchopneumonia can cause mass die-offs in wild mountain ungulates. Epizootic outbreaks of bronchopneumonia are sporadically reported in Pyrenean chamois (*Rupicapra pyrenaica*), yet lesions of pneumonia are a relatively common finding in post-mortem examination of apparently healthy hunted chamois and the cause of death in sporadic cases. Coughing and respiratory distress is observed regularly in Pyrenean chamois populations, and the disease is suspected to be endemic and an important factor underlying the recruitment variability among social groups. We investigated bronchopneumonia to better understand this syndrome in relation to Pasteurellaceae occurrence and individual traits in a Pyrenean chamois population of the Eastern Pyrenees (Freser-Setcases National Game Reserve), Northeastern Spain. A total of 59 chamois were sampled during the regular hunting season in early spring and autumn 2015 and early spring 2016. Post-mortem examinations were performed in the field and sterile Amies swabs were collected from three different locations of the lungs, craneo-ventral area (preferentially right apical lobe), dorso-medial area (medial lobes) and dorso-caudal area (caudal lobes). The incisions were performed with sterile scalpel blades for each sample and from transitional areas if lesions were present. To determine the extension of the lesions, the lungs were collected and examined in the laboratory always by the same person. At the laboratory, the swabs were cultured in chocolate agar within 24h post collection and incubated for 24-48h at 37°C, 5% CO<sub>2</sub>. Strains compatible with Pasteurellaceae were isolated and identified by sequencing the 16S rRNA. Many of the chamois, 35/59 (59.3%, CI 95% 46.6-70.9) had macroscopic lesions of chronic bronchopneumonia consisting in firm and tan to dark red areas in the anteroventral lung lobes, extension ranging from 5% to 100% of the cranial and medial lobes, and in 8/35 lungs the lesions extended into 5-10% of the caudal lobes. Pasteurellaceae were isolated in 37.3% (22/59) of the lungs, being *Pasteurella multocida* the most frequently isolated (12/22), followed by *Mannheimia haemolytica* (6/22) and *Bibersteinia trehalosi* (6/22). *P. multocida* coinfections with *M. haemolytica* and *B. trehalosi* were detected in two chamois. Pasteurellas were primarily isolated in the craneo-ventral and dorso-medial areas of the lungs but also in the dorso-caudal areas. The isolation of Pasteurellaceae was not consistent but correlated with pneumonic lesions ( $X^2=4.6$ ,  $df=1$ ,  $p=0.03$ ) and were also isolated from lungs without evidence of disease. The presence of pneumonia was not related to sex, season or the age of chamois, suggesting an endemic disease occurrence. The high numbers of chamois with lesions of bronchopneumonia indicate the importance of this disease in this species. Pasteurellaceae, mainly *Pasteurella multocida*, apparently have an important role in the pathogenesis. The inconsistency of its presence in relation to the lesions suggests that not all correspond with active infection processes and that other factors and/or infectious agents apart from Pasteurellaceae in the lungs can trigger the disease. Further studies on other etiological agents and factors, including quantified molecular detection and subtyping of Pasteurellaceae strains, would shed more light on the bronchopneumonia complex aetiology and pathogenesis in chamois.



## Epidemiological and molecular surveillance of *Mycobacterium tuberculosis* Complex in wild ungulates from southern Spain, 2011-2018

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**Keywords:** *Mycobacterium tuberculosis* complex, Spoligotype, Wild ungulates, Spain.

During the last decade, despite the efforts involved in the eradication, the prevalence of tuberculosis (TB) in cattle has remained stable or even increased in the south of Spain. The increase in wild ungulate populations, which are considered natural hosts for TB in south-central Spain, may influence the national eradication program. The aims of this study were: to determine the seroprevalence and spoligotypes of *Mycobacterium tuberculosis* Complex (MTC), and to provide information on associated risk factors. Blood samples from 1,190 wild boar, 1,214 red deer (*Cervus elaphus*), 345 fallow deer (*Dama dama*) and 235 mouflon (*Ovis musimon*) were collected in 59 hunting areas in southern Spain between 2011 and 2018. Additionally, 146 TB-like lesions (TBLL) were obtained from 120 wild boars, 21 red deer and 5 fallow deer, during the post-mortem examination in 19 of these hunting areas. By species, the overall TB seroprevalence was 51.6%  $\pm$  2.84 (614/1190) in wild boar, 9.6%  $\pm$  1.65 (116/1214) in red deer, 6.8%  $\pm$  2.96 (19/279) in fallow deer and 1.3%  $\pm$  1.44 (3/235) in mouflon. A total of 116 (79.5%) of the 146 TBLL showed positive results to mycobacteria culture. Mycobacteria were isolated in 18 out of the 19 (94.7%) sampled hunting areas, and seven different spoligotypes of *Mycobacterium bovis*, including SB0295, SB0121, SB0134, SB1190, SB0265, SB0340 and SB2461 were identified. Significantly higher seroprevalence to MTC was detected in wild boar compared to the other species analyzed ( $P > 0.001$ ). A significantly higher seropositivity was found in wild boar ( $P < 0.001$ ), red deer ( $P = 0.05$ ) and mouflon ( $P = 0.043$ ) from fenced hunting areas compared to those sampled in open areas. Prevalence of MTC antibodies significantly increased in adult compared to young animals in wild boar, red deer and fallow deer ( $P < 0.05$ ). The seroprevalence obtained in wild ungulates, particularly in wild boar, and the presence of shared spoligotypes between wildlife and livestock, suggest that these species could be interfering in the TB eradication program in Spain.

## Types and diversity of Persian leopards' lesions resulting from the illegal use of snares and gin traps

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**Keywords:** *Gin trap, Persian leopard, snare*

The present report documents six cases of Persian leopards (*P. p. saxicolor*, recently revised as *P. p. tulliana*) in the course of the last 6 years being caught accidentally in snares or gin traps that were set by local people against wild boars (*Sus scrofa*) or less frequently jungle cat (*Felis chaus*), which three of them are being described in this abstract. It details the type and diversity of lesions resulting from these trapping methods, and informs a broader audience about the newly reported threat posed by snares and gin traps to leopards in Iran. Three of these leopards died as result of severe injuries, two of them were rescued and survived albeit disabled and one animal could be released to the wild. These cases reveal the growing problem of habitat disturbance and loss for leopard and their natural prey in the Alborz mountain range and Caspian lowlands of Iran. Case number one was found near Gorgan, Golestan Province, trapped by a leg in a wire snare deployed against wild boar. Clinical investigations revealed a deep incision located immediately above the radio-carpal joint (wrist) of the left foreleg, with damaged underlying soft tissues and a swollen post-traumatic foot with greyish mottled skin. Ultrasound and radiographic examinations of the left forelimb confirmed severe ante-brachial muscle, tendons, and radial nerve injuries associated to a wet gangrene of the distal end of the foot. The presence of a potentially life-threatening gangrenous process commanded a trans-humeral amputation, carried out two days later. Following a prolonged antibiotic treatment, the animal recovered successfully from the operation and was transferred to the Tehran Zoo. Case number two reported at Gilan Province near the Siahkal County, close to Deylaman Prohibited Hunting Area. The animal was found dead, strangled by a wire snare set by local farmers. Case number three in mid-November 2013 local people in Mazandaran Province spotted a lame leopard with a metal gin trap hanging from the distal end of the right forelimb. Then on January 3rd, 2014 local farmers found near Tonekabon County, Mazandaran Province, less than 5 km from the location of the first observation, a leopard with a recently amputated right foot. The animal was in medium body condition with signs of mild dehydration, and had a partially healed amputation of the right foot at carpal joint level with no signs of local infection or necrotic process. Radiographs of the right forelimb, confirmed the amputation of the foot with distortion of carpal bones, and active laminar periosteal reactions in several regions of the distal part of the affected limb, possibly as a reaction to repeated trauma or infectious processes. A computerized-tomography (CT) scan, confirmed the presence of a metallic fragment in the spinal canal of L6 which was likely responsible of the paralysis of hind legs. The animal showed no deep pain sensation and no proprioception in hind limbs. Due to the bad prognosis of the vertebral injuries, the presence of multiple metallic foreign bodies and assumed suffering, the animal was euthanized.

## Severe conjunctivitis associated with *Chlamydia felis* infection in a free-ranging Eurasian lynx (*Lynx lynx*)

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**Keywords:** *Conjunctivitis, Eurasian lynx, Chlamydia felis*

*Chlamydia felis* is one of the most common specific cause for conjunctivitis in the domestic cat (*Felis catus*). Clinical signs typically include conjunctivitis with marked hyperaemia, chemosis, blepharospasm and ocular discharge. It can also be associated with fever, infection of the upper respiratory tract, pneumonia and reproductive disorders. However, in free-ranging wild felids information about the occurrence, epidemiology and health impact of *Chlamydia* spp. infections are scarce. In March 2017 a female adult Eurasian lynx (*Lynx lynx*) was captured in the Jura Mountains of Switzerland in the context of a translocation programme. It presented with a marked unilateral conjunctivitis with severe hyperaemia of the nictitating membrane, chemosis and muco-purulent ocular discharge. No injury of the ocular structures was observed. The animal was in a good body condition and the clinical examination revealed no further abnormalities. Conjunctival, oropharyngeal and rectal swabs as well as blood samples were collected for laboratory tests and the animal was brought to a quarantine station. *C. felis* DNA was detected in both eyes while the lynx was negative for all other agents tested, including feline herpesvirus-1, feline calicivirus, *Mycoplasma felis*, feline leukaemia virus, feline immunodeficiency virus, parvovirus and canine distemper virus. The lynx was subsequently treated with oxytetracycline and ketoprofen. Following treatment, the ocular changes progressively resolved. At clinical examination two weeks after capture, both eyes were clinically inconspicuous and the conjunctival swabs negative for *C. felis*. The animal was considered healthy and released into the wild. It appeared on a camera-trap picture three months later in good body condition and with roughly unsuspecting eyes. To our knowledge, this is the first description of a *C. felis* infection with associated conjunctivitis in a free-ranging felid. The observed ocular signs and the rapid clinical improvement under treatment are in agreement with descriptions in the domestic cat. Considering the apparently isolated occurrence of the disease in lynx, a domestic or wild cat (*Felis silvestris*) may have acted as a source of infection, as the lynx shares its habitat with both domestic and wild cats. However, to date there are no data on the occurrence of subclinical *C. felis* infections in lynx. This case highlights the benefit of a quarantine period to treat and prevent the spread of infectious diseases in the framework of translocation programmes.

## European College of Zoological Medicine Residency in Wildlife Population Health

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**Keywords:** *Training programme, veterinary specialisation, wildlife health*

With increased understanding of the impact of disease on wildlife populations, there is a growing need to train wildlife health professionals. Whilst some post-graduate courses relating to wildlife health and conservation medicine exist, there were no formal residency programmes available in this field until recently. The aim of this poster is to increase awareness that such specialist training programmes now exist in Europe. The European Board of Veterinary Specialisation (EBVS, established in the early 1990s: <https://ebvs.eu/>) is the umbrella organisation for veterinary specialties within Europe and includes 27 veterinary specialist colleges. The European College of Zoological Medicine (ECZM, established in 2009: <https://www.eczm.eu/>) is one of these colleges and it is divided into five subspecialties: three of these are taxon-based (Avian, Herpetology, and Small Mammal) whilst the remaining two focus on the disciplines Zoo Health Management, and on Wildlife Population Health (WPH). The WPH specialty was formed in 2009 and currently has 58 Veterinary Specialists in 22 countries. A major goal of the ECZM is to build an active community of Veterinary Specialists in the field by offering 3-year residency programmes to train future Diplomates. The WPH residency syllabus encompasses five broad subjects comprising: Wildlife Population and Ecosystem Health; Wildlife Pathology and Disease Investigation; Wildlife Disease Surveillance and Preventive Medicine; Wildlife Medicine; and Research Studies. In contrast to the four other ECZM subspecialties which are clinically-orientated, WPH focuses on research and epidemiology in order to understand how diseases affect wildlife populations. To be eligible to commence a WPH residency, a candidate will need to have completed a minimum of a 12-month clinical internship or been working in veterinary practice for two years. If a candidate completes a WPH residency and fulfils the publication requirements (two first-author peer-reviewed papers), they qualify to take the written specialist board examination. If the board exam is passed, the successful candidate obtains a diploma, becomes a Diplomate of the ECZM and is awarded the title “European Veterinary Specialist in Wildlife Population Health”. As of March 2018, there are eight active WPH residents in Croatia, Germany, Switzerland, and the United Kingdom. The first WPH residency in the UK, run jointly by the Royal Veterinary College (RVC) and Zoological Society of London (ZSL), started in 2015 and is a 3-year full-time postgraduate programme. It has a built-in master’s degree (Master of Veterinary Medicine) awarded by the RVC which allows the resident to complete all the modules of the MSc Wild Animal Health (run jointly by RVC and ZSL since 1994) as well as other MSc modules relevant to wildlife research at RVC, for example on Veterinary Epidemiology. This programme will have its second resident starting in 2018. In conclusion, it is hoped that the training provided through ECZM WPH residency programmes will help to build a growing network of wildlife health professionals in the future.

## Fatal co-occurrence of *Mycoplasma ovipneumoniae*, *Mycoplasma agalactiae* and Pasteurellaceae in an alpine chamois

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**Keywords:** mixed infections, *Rupicapra r. rupicapra*, diagnostic process

Co-infections, or alternatively called mixed infections, are the rule in natural conditions. Furthermore, wildlife has to face the pathological effects known to be related to multiple and concomitant infections. Focusing on wild ungulates, the co-occurrence of multiple strain infection can indeed lead to severe disease outbreaks and the diagnosis of all etiological agents involved is necessary for the control strategies of the disease. Among strains of main interest, infections with multiple *Mycoplasma* species and/or in association with Pasteurellaceae can occur severely. Although for the latter a precise microbiological diagnosis can be obtained, for *Mycoplasmas* the small number of biochemical and physiological properties available together with the serological cross-reactions between subspecies or types complicate the identification and the detection of co-infections. Conversely, molecular approaches can overcome these limitations providing useful tools for rapid and reliable identification allowing the detection of multiple infections. The need is to apply a diagnostic process that combines techniques that allow the typing of all the etiological agents involved in the disease. Here we propose the case of the co-occurrence of two *Mycoplasma* species and Pasteurellaceae detected in an old (>6 years old) female chamois (*Rupicapra r. rupicapra*) culled during the 2017 hunting season. The animal was culled since its poor body conditions and the hypertrophic udder. Macroscopically, the udder was enlarged and lungs showed bronco-pneumonia, multiple abscesses were recorded in both organs. Liver was congest and kidneys had an altered parenchyma. All the organs were cultured on blood agar and Gassner media; PPLO agar and broth were used for bacteriological exams of lungs and udder. DNA was extracted from both organs and the cultured *Mycoplasma* spp. colony; four real-time PCR protocols were designed to differentiate *Mycoplasma* species that likely infect chamois: *M. ovipneumoniae*, *M. agalactiae*, *M. bovis* and *Mycoplasma mycoides* cluster. Bacteriological analyses showed *Bibersteinia trehalosi* in lung, *Mannheimia haemolytica* in udder and *Mycoplasma* spp. in both organs. PCRs detected *M. ovipneumoniae* and *M. agalactiae* in the lung while the latter was recorded even in the udder. Our diagnostic process showed the fatal co-occurrence of bacterial and multiple *Mycoplasma* infections in chamois giving evidence of the severity of the disease. The use of these series of PCRs allowed the identification and differentiation of two *Mycoplasma* species and lead to exclude other *Mycoplasmas* that can affect chamois. *M. ovipneumoniae* and *M. agalactiae* are widespread in wild and domestic ruminants and are responsible of both subclinical infections and severe disease. Particularly, *M. agalactiae* infection can affect individuals causing agalactia in lactating females, conjunctivitis, pneumonia and arthritis. Moreover, the pathogenic role of Pasteurellaceae on these animal species is documented. As during summer pastures chamois can have a spatial overlap with flocks, sheep and goats may have a role in the spread of these infections crossing the wild-domestic interface. Further analyses should be performed to compare strains of *Mycoplasmas* and Pasteurellaceae from wild and domestic ruminants to assess if an interspecies transmission really occurs and to study the capacity of these changing microorganisms to adapt to different species.

## How specific and sensitive is the visual diagnosis of Sarcoptic mange in free-ranging Iberian ibexes?

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**Keywords:** Diseases management, *Capra pyrenaica*, *Sarcoptes scabiei*

Sarcoptidosis is a parasitic disease caused by *Sarcoptes scabiei* affecting wild mammals worldwide including the Iberian ibex (*Capra pyrenaica*). Selective culling of the scabietic ibexes is one of the disease management measures applied in Iberian ibex populations either to prevent the parasite spread or for animal welfare issues in severe infestations. Despite visual identification of compatible lesions in field conditions is assumed to be accurate enough to decide which ibex must be culled, false negative and positive cases occur, especially in initial stages of the illness and in disorders causing similar symptoms, respectively. The aim of this work is to determine the sensitivity and the specificity of this methodology by analysing available data of 403 Iberian ibexes hunter-harvested in the Espacio Natural de Sierra Nevada between 2002 and 2015. Before shooting, spotted ibexes were classified as positive or negative to Sarcoptic scabiei by visual inspection with 10 X 42 binoculars. Next, microscopic inspection of the hydroxide potassium digestion of five 2.5x2.5cm skin pieces (6.25 cm<sup>2</sup> each) was performed in the laboratory to confirm or discard mite presence. Using General Linear Models (GLM), we explored whether the observed variability in the rate of false negative and false positive cases was explained by the effects of sex, age or the period of the year (Wet period included winter and spring while Dry refer to summer and fall). Then, sensitivity and specificity were calculated. Statistical analyses were made using the R software version 3.2.2. According to our model selection, specificity of the visual diagnosis was explained by the additive effects of sex and the period of the year. For the sensitivity, however, the model including the additive effects of age and the period of the year was the best. The visual diagnosis of sarcoptic mange resulted considerable less specific (false positives) in females (16.13%) and during the wet period (43.53%). Sensitivity (false negatives), besides, was lower in juveniles (80.56%) and during the dry period (75.36%). Therefore, the risk to shot a healthy ibex increases for females and during wet period. Otherwise, it is easier to pass up infected animals in dry period or if they are juveniles. Concluding, this method has been seen to be more sensitive than specific. Thus, it is important to check out the use of this visual method for culling purposes as healing animals may also be alopecic and shooting them may be counter-productive for population resilience.

## **Novel Salmonella variant associated with mortality in great spotted woodpeckers (*Dendrocopos major*) from Great Britain.**

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**Keywords:** *Salmonella*, woodpecker, disease

Garden Wildlife Health (GWH) is a citizen science project that conducts scanning disease surveillance of garden birds, among other species, across Great Britain (GB). Whilst Passeriformes, principally Fringillidae species, constitute the majority of this convenience sample, members of the Picidae family (Piciformes) are comparatively under-represented, comprising only 0.1% (n=7) of all avian cases examined post-mortem, 2013 – 2017 inclusive. Of these seven birds (from seven incidents), all were great spotted woodpecker (*Dendrocopos major*): the most common cause of death was trauma (n=4), with infectious disease (n=2) and euthanasia (n=1) also recorded. As very little is known about the conditions affecting free-living Piciformes, the objective of this study was to further investigate the two cases of infectious disease, exploring the aetiology, presentation and pathogen characterisation. Both birds, hereafter referred to as woodpecker 1 (WP1) and woodpecker 2 (WP2), were found dead in the same county in South East England, approximately 10 miles apart, in December 2013 and June 2016, respectively. Both were adults (WP1=male, WP2=female) in poor body condition, based on superficial pectoral muscle (SPM) mass and absence of fat deposits. Macroscopic abnormalities included skin lesions (WP1 and 2), and discolouration of the liver and lung (WP2). In each bird, a *Salmonella* sp. was isolated from the small intestinal contents, liver and lung, identified by colonial and Gram's stain morphology. The most significant histopathological findings were dermatitis (WP1 and 2), necrotising myositis (WP1) and likely meningitis (WP2). Gram negative rod-shaped bacterial aggregates were observed in multiple tissues. Immunohistochemistry targeting the *Salmonella* common structural antigen demonstrated specific immunoreactivity in all tissues examined, co-localising *Salmonella* with inflammatory lesions in multiple sites in both birds, including skin, SPM and heart. The multi-locus sequence types (MLST), determined from whole genome sequence data, were identical for both of the isolates. This MLST was shown to be a single locus variant of the MLST for a strain of *S. Hessarek* (isolated from a human in GB). Single nucleotide polymorphism (SNP) analysis revealed that across the whole genome, the isolates from WP1 and WP2 differed by 2 SNPs, and each differed to *S. Hessarek* by 2690 SNPs. Biochemical analyses established that the WP isolates shared the same biochemical profile and serotyping showed they also had the same antigenic profile (4,5,12 : a : 1,5), but this differed from *S. Hessarek* (4,12,[27] : a : 1,5). These findings support tentative identification of both WP isolates as an 0:5 variant of *S. Hessarek*, pending more detailed characterisation underway at the Institute Pasteur. The vast majority of *Salmonella* serovars isolated from garden birds in GB are *S. Typhimurium*. *Salmonella* *Hessarek*, however, has now been reported from wild Passeriform multiple mortality events in GB, mainland Europe and the Middle East, with starlings (*Sturnus vulgaris*), song thrushes (*Turdus philomelos*) and blackbirds (*Turdus merula*) affected. Here, we present two further cases where *S. Hessarek*, or a variant thereof, has caused disseminated salmonellosis, contributing to the death of a wild bird, and the first report of potential *S. Hessarek*-associated mortality in a Piciform species.

## **Red kite (*Milvus milvus*) white blood cell changes during migration and winter rest in Huesca, Spain**

Paula Yagüez i López-Jurado

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**Keywords:** *Red kite, migratory stress, heterophil/lymphocyte ratio*

The red kites' (*Milvus milvus*) population is in decline in Europe. This medium sized raptor is migratory and many red kites from central and northern Europe winter on the Iberian Peninsula. Migration implies physiological effort and social stress, which could lead to increased susceptibility to certain pathogens. Studies have shown that the effects of migratory efforts vary considerably between species and birds populations. The estimated total white cell count (TWCC) and the heterophil/lymphocyte (H/L) ratios from thin blood smears are a low cost, widely accepted primary approach for the evaluation of the immune state of birds. We examined 129 red kites' blood smears from red kites captured for banding at the Binaced vulture restaurant (Huesca, Northern Spain) during autumn migration (the September to November), wintering (December and January); and during (spring migration) February and April in three different wintering seasons. Females had a significantly lower H/L ratio than males and adult red kites showed significantly higher TWCC and H/L ratio than juveniles in their first year of life. Red kites captured in the winter rest period (between December and January) showed significantly higher H/L ratios than those captured during migrations, both southbound and northbound. The significantly higher H/L ratio in wintering kites is also observed when segregating the results by sex and age. The global H/L ratio of the three different winters rests were higher in the 2014/2015 winter. No relation between body condition and TWCC or H/L ratio was evident. Our results may support the assumption of a negative effect of stress and physical effort during migration on cellular immunity in red kites. However, other factors such as pathogen exposure and stress during the capture and restraint could also have influenced the results.



## Herpesviruses in free living owls in Slovenia

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**Keywords:** free-living owls, herpesvirus, PCR

Herpesviruses (HV`s) affects a wide range birds of prey and were frequently identified in falcons, owls, eagles and buzzards (1, 2, 3, 4, 5). The aim of our study was to investigate HV infections in wild owls in Slovenia. Altogether 55 wild dead owls were collected at various locations in Slovenia between 1995 and 2015. Collected owls belonged to seven different species: Tawny Owl (*Strix aluco*; N=19 ind.), Long-eared Owl (*Asio otus*, N=15 ind.), Ural Owl (*Strix uralensis*, N=11 ind.), Western Barn Owl (*Tyto alba*, N=3 ind.), Eurasian Scops Owl (*Otus scops*, N=3 ind.), Eurasian Eagle Owl (*Bubo bubo*, N=3 ind.), and Short-eared Owl (*Asio flammeus*, N=1 ind.). For the detection of HVs, a nested PCR that target a region of the HV DNA polymerase gene was used (6). HV was detected in organs of 8 out of 55 wild owls (14.5 %) in three species, Eurasian Eagle Owl (2/3), Ural Owl (4/11) and Long-eared Owl (2/15). Phylogenetic analysis of the partial DNA polymerase gene nucleotide (nt) sequences showed that identified HVs belonged to the three distinct highly supported genetic groups related to the avian and mammal alphaherpesviruses. One strain was identical to the columbid herpesvirus 1 (CoHV1) while the other strain had an nt sequence very similar to the gallid herpesvirus 2 (GaHV2). Other six strains showed a more distant relationship to other alphaherpesviruses of birds and mammals. Our research into the HVs in wild owls showed that owls could be infected with HVs that are different from CoHV1. Among these viruses are known HVs, such as GaHV2 or Marek's disease virus, as well as novel HVs.

## New tool for improving the post-mortem diagnosis of diseases in wild boar: the use of serum biochemistry

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**Keywords:** Cerebellar sinus, post-mortem interval, serum

**Background.** The diagnosis of peracute diseases through gross and histologic examination can be challenging. In such cases, biochemical analyses could provide complementary information to assist post-mortem diagnosis. Arena-Montes et al (2013) proposed the blood lake present in the cerebellar sinus to sample blood post-mortem. However, progressive changes of biochemical parameters after the death could hamper the interpretation of biochemical results. The aim of this study was to evaluate the effect of time on common biochemical parameters from blood collected after death from the blood lake of wild boars (*Sus scrofa*). **Methods.** The blood of 23 wild boars killed during hunting in winter conditions was sampled once through the occipital foramen in the cerebellar sinus. Body condition, age and sex of boars were recorded. Groups of three individuals were sampled immediately after death, and hourly from 2 to 8 hours (T8h, n=2) after death. Samples of whole blood were promptly sent to the laboratory. They were centrifuged, degree of haemolysis was noted, and biochemical parameters were measured with a wet chemistry analyser. Data were analysed using principal component analysis and linear regression modelling. **Results.** Albumin, ALT, potassium, fructosamine, creatinine, globulins, and total proteins tended to increase with time whereas sodium and chloride concentration decreased with time. A segmented regression analysis indicated a stability of blood quality for two hours after death, followed by a linear decrease of its quality. **Conclusion.** Further studies are planned to collect a larger sample, to allow confirmation of these results and identify reference intervals.

## Towards protocol building for biodiversity mapping of terrestrial gastropods

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**Keywords:** *Gastropods, Biodiversity, GIS*

Among gastropods, terrestrial snails play an important role in the fauna of Greece. Greece is one of the richest countries in Europe in terms of terrestrial snails, which is due to its diverse geomorphology. They live in all the ecosystems which are found on mainland and islands in Greece, from sea level to the peak of Mt. Olympus. The main danger for terrestrial snails are the fragmentation and destruction of their habitats. Since, almost half of the species found in Greece, have a very limited spread, any destruction of their habitat could have a direct impact on their population status. According to the “Red Book of Endangered Animals in Greece”, out of 680 species, only for 372 species there exists available information about their population. From those, 211 are of lesser concern, as they are relatively common or widespread, sixty three are vulnerable, twenty eight are endangered and seventy are critically endangered. Therefore, there is a need to sample and map the species distribution based on a specific protocol. In this work, a first attempt to set up such a protocol is attempted. This protocol, up till now, includes four stages: preprocessing, sampling, sorting - identification at species level and finally input the data into a data base. A basic tool for the data analysis is the Geographic Information System (GIS). GIS allow the sampling strategy for selection of habitat, recording, visualization and processing of ecological data. Firstly, with the use of GIS, land uses can be selected, which are evaluated as being available for sampling sites (e.g. based on CORINE), for the recording of gastropods. Sampling could be carried out in the selected areas, where habitat data (geographic location, land use, altitude, etc.) is recorded and individual samples taken. Once the gastropods are identified in the Lab, all collected data is imported into a GIS database for further spatial analysis.

## **Nest mite communities of migratory and sedentary white storks (*Ciconia ciconia*) and migratory black storks (*Ciconia nigra*) at the end of the migratory period**

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**Keywords:** *Nest mites, storks, migration*

At least 2500 species of mites from 40 families are associated with birds. These species can be divided into those that inhabit the nest and those that reside primarily on the body of the host. Black stork (*Ciconia nigra*) and white stork (*Ciconia ciconia*) nests create a specific niche for mites, which is characterized by host phenology. We hypothesize that storks use migration as an escape method to reduce parasite burden including those of parasitic mites. Here we characterize the composition of mite communities in nests of white and black storks with the combined methodology of molecular and morphological identification and determined the dominant species in relation to the characteristics of this type of microhabitat. We compared the mite community composition of nests between white stork and black stork and, between white stork nests belonging to migratory white storks and that are empty outside the breeding season and those from storks that have become resident and that are occupied by storks throughout the year. A total of 12 white stork and 5 black stork nest material samples were collected in the province of Ciudad Real during the post-migration period. In total 1619 mites belonging to 3 orders (Mesostigmata, Trombidiformes (Prostigmata suborder) and Sarcoptiformes (Oribatidae suborder and Astigmata cohort) were extracted and classified in 35 species/morphology groups. The most abundant species found in the nest material belonged to the Astigmata cohort (63%) and Prostigmata suborder (19%). However, the Mesostigmata mites had a greater diversity of species (22 species). Black stork nest mite communities differed from those of (migratory) white storks although the differences were not significant, possibly because both species share the same habitat type and phenological behaviour (migration). Some parasitic mites such as *Dermanyssus gallinae* were also found. The abundance and richness of mite species were significantly higher in the nests of resident white storks than in the nests of migrating white storks. This could be related to the year round presence of the host in the nest of resident storks. Also, as the nests of resident storks were located in an anthropized habitat (landfill) and those of migratory storks in a less human influenced landscape the habitat could have an impact on the mite communities in the nest.

## Serological survey of sarcoptic mange in Mediterranean Iberian ibex (*Capra pyrenaica*) populations

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**Keywords:** *Sarcoptes scabiei*, ELISA, prevalence

Sarcoptic mange is a mite parasitosis caused by *Sarcoptes scabiei* described in a wide range of species worldwide. Sarcoptic mange has affected most Iberian ibex (*Capra pyrenaica*) Mediterranean populations since 1987, some of them with devastating consequences. However, both demographic effects and management have differed amongst populations and outbreaks. In wild ibexes, sarcoptic mange is usually diagnosed through skin scraping or potassium hydroxide (KOH) digestion to detect *S. scabiei* under scrutiny. Otherwise, visual diagnosis of compatible lesions is also functionally accepted in field conditions when the disease has already been locally confirmed by means of the aforementioned gold standard techniques. Enzyme-linked immunosorbent assays (ELISA) have also been successfully used to detect IgG against *S. scabiei* in different species, including wildlife; therefore it may be a useful tool for the surveillance of sarcoptic mange in ibex populations. The objective of this study is to perform a retrospective analysis of seroprevalence against *S. scabiei* in the Mediterranean Iberian ibex populations, in order to understand the differences in outbreaks evolution among them. Therefore, our aims are (1) to utilize a recently set-up ELISA (91.7% of sensitivity and 97.4% of specificity) to determine the prevalences across different ibex populations along the Mediterranean arch of the Iberian Peninsula and (2) to investigate the relationships between seroprevalence and the differential traits (demographic effects and management) of every affected population and outbreak. Between 2004 and 2018, 995 serum samples were collected from either selectively hunted, captured for management purposes or found dead ibexes from Sierra Nevada (n=484), Murcia (n=91), Sierra de Cazorla (n=42), Ports de Tortosa i Beseit (n=345) and Muela de Cortes (n=33). We used a validated ELISA using *S. scabiei* var. *canis* IgG serologic test (AFOSA GmbH, Germany) and an avidin-biotin detection system with a biotinylated secondary antibody (Pierce® Biotinylated Recombinant Protein G, Thermo Fisher Scientific Inc., Rockford, USA) that provides a precise and accurate detection of goat primary antibodies. Statistical analyses were performed using R software version 3.2.2 to assess the effect of individual and population traits. The preliminary results show a 22% mean seropositivity against *S. scabiei*, interval ranging from 0 to 54.5% depending on population and the year studied. This is an on-going study. Further detailed results analyzing the causes for the differences found among years and populations will be presented and discussed.

## **Trichomonas gallinae genotype B in hand-reared wild Mississippi kites (*Ictinia mississippiensis*)**

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**Keywords:** *Trichomoniasis, captive raised, white plaques, oral lesions*

A total of 6 nestling Mississippi kites (*Ictinia mississippiensis*) were presented to the Oklahoma State University Center for Veterinary Health Sciences (Stillwater, OK) after being found on the ground. The animals were hand-reared as following the service protocol and were developing as expected, however, after 1 week, one animal developed white plaques in the mouth. Over the next 5 days, all animals developed similar lesions. Main differentials included *Trichomonas* sp., *Capillaria* sp., *Candida* sp., poxvirus, or bacterial infection. Repeated wet mounts were performed in order to assess the presence of *Trichomonas* sp. but yielded negative results. Cytology was suggestive of fungal hyphae however, fungal culture was negative. Due to poor performance and growth, one animal was euthanized. *Trichomonas* sp. was identified on necropsy and all remaining kites were treated with metronidazole (50 mg/kg PO q24h for 5 days). Samples from the dead animal were submitted for PCR testing which yield a final identification of *Trichomonas gallinae* with 100% coverage and 99% identity with genotype B. All remaining animals improved and were later released back into the wild. To author's knowledge, this is the first report of *Trichomoniasis* in Mississippi kites, a primarily insectivore bird of prey. Although the source of parasite remains unknown, it is suspected that one of the animals was carrying it and this was spread to the remaining birds by means of hand feeding.

## Complete Blood Counts and Hematologic Values in Bobwhite Quail (*Colinus virginianus*)

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**Keywords:** bobwhite quail, hematology, estimate, white cell count

Northern bobwhite quail (*Colinus virginianus*) have experienced decline throughout much of their range. They are commonly captive-bred and raised for release to support wild populations. There is limited hematologic data for this species. Complete blood cell counts in eighteen 49-day-old captive raised quail were performed. The data can be used to contribute towards the creation of age-specific reference intervals for the species. This age is important as it is when animals may be medically assessed prior to release in to the wild. Red and white blood cell counts, cell differentials, packed cell volumes, and total solids were determined for each animal. White blood cell counts were obtained by estimates from blood smears using both 400x magnification and 1000x magnification, as well as an indirect method using a Phloxine B stain. White blood cell counts using 1000x magnification were found to underestimate values when compared to the other two methods.

## Investigation of temporal shedding of bacterial fecal pathogens in captive monkeys

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**Keywords:** *Shigella, monkeys,*

The illegal wildlife trade in Malawi is a concern for primate welfare and ecological health. Confiscation, rehabilitation, and reintroduction of primates is an effective strategy for improving both wildlife health and primate welfare. Rehabilitated animals are screened for certain diseases prior to reintroduction into the wild, though there is little known about infectious organisms present in wild primates in Malawi and their significance in wild animal health. Bacterial fecal pathogens, including *Shigella* spp and *Salmonella* spp are examples of organisms capable of causing clinical disease in humans and nonhuman primates; however, these organisms have been cultured from captive primates at a rehabilitation facility with and without animals showing clinical signs of disease. The primary objective of our study was to attempt to identify temporal shedding patterns of *Salmonella* spp and *Shigella* spp as well as investigate association with concurrent gastrointestinal parasite infection. Two methods were used to sample vervet monkeys (*Chlorocebus pygerythrus*) and blue monkeys (*Cercopithecus mitis*) housed at the rehabilitation facility: (1) sampling for three consecutive days at 14-day intervals and (2) sampling at 7-11 day intervals. Fecal pathogen cultures and biochemical identification tests were completed by a local laboratory. Fecal swabs were streaked directly onto xylose lysine deoxycholate (XLD) and MacConkey agar plates. Each culture was evaluated by disc diffusion antibiotic sensitivity testing. Gastrointestinal parasites were identified by fecal flotation, direct fecal smears, and light microscopy. Behavioral observations of characteristic indicators of stress in primates (scratching, pacing, self-grooming, and yawning) were conducted to approximate physiological stress. Over 2 months, 17 animals (15 vervets and 2 blue monkeys) were each sampled between 4 and 9 times, for a total of 103 cultures; 98 parasite exams were also performed during this time period. 11 individuals cultured positive for either *Shigella* spp (10) or *Salmonella* spp (1) at least once during the study period. 9/11 only had one positive fecal culture while 3/11 cultured positive more than one time; positive cultures accounted for 16/103 total cultures. During the sampling period only one *Shigella* spp positive monkey displayed concurrent clinical signs of shigellosis, which included frequent low-volume stools with blood and mucus. Gastrointestinal parasites detected included *Balantidium coli*, hookworms, *Strongyloides* sp. and *Trichuris* sp. There was no significant correlation between culture positivity for a fecal pathogen and concurrent parasitic infection. At the conclusion of 100 hours of behavioral observations, a stress score was calculated as the average number of characteristic stressful behaviors displayed by an individual primate per hour. There was no significant difference between the stress score for primates culturing positive for fecal pathogens and those who cultured negative. This study did not reveal a clear temporal pattern for shedding fecal pathogens and confirmed that primates could shed *Shigella* spp without exhibiting clinical signs. More information on the ecology and significance of shedding of fecal pathogens is needed to determine the significance of a fecal pathogen positive culture in a non-clinical animal and the potential impact on animal health.



## **Outbreak of Canine Distemper in Red foxes (*Vulpes vulpes*) in Austria**

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**Keywords:** Austria, Canine Distemper Virus, Red foxes, *Vulpes vulpes*

In March 2018 a sudden increase of diseased foxes (*Vulpes vulpes*) was noticed by hunters in Lower Austria. The foxes showed symptoms such as staggering walk, apathy and loss of shyness. Within one month 27 foxes (including three from Vienna) were submitted for necropsy, and many more deaths were reported. Macroscopically the foxes showed varying pathological lesions, ranging from pyometra to lung lesions, as well as parasitic enteritis, this in combination with a partly high degree of autolysis, made a clear-cut diagnosis of CDV at first glance impossible. A first histological screening showed the pathognomonic eosinophilic intra-cytoplasmic viral inclusions in the epithelium of bronchi, as well as a multifocal demyelination of white matter in the brain. To secure the diagnosis tissue samples (lung, spleen and brain) were analyzed using PCR. 16 out of 19 foxes submitted had a positive result for canine distemper virus. The sequence of the virus analysis is ongoing. Canine distemper has been circulating to a low degree in Lower Austria and Vienna in the last years, mainly affecting badgers (*Meles meles*). This current outbreak of the disease in foxes might be due to the large population of foxes, and the increase in stress due to the mating and birth season. It again highlights the importance of monitoring wildlife diseases especially in the light of the one health perspective. As well as it again highlights the importance of a good communication between local people and experts, to make sure such outbreaks do not go unnoticed.

## **Bifidobacterial occurrence in in cotton-top tamarin (*Saguinus oedipus*) and emperor tamarin (*Saguinus imperator*)**

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**Keywords:** cotton-top tamarin, emperor tamarin, bifidobacteria

Unlike in humans, the diversity of bifidobacteria in the gut of non-human primates is poorly understood. In the present work, for the first time, the bifidobacterial population in cotton-top tamarin (*Saguinus oedipus*) and in emperor tamarin (*Saguinus imperator*) has been studied. The main objective of this study was the isolation and the identification of bifidobacterial strains from one adult subject of the cotton-top tamarin and from one adult subject of the emperor tamarin. The analysis of their bifidobacterial communities by molecular methods was assessed by culture dependent and culture independent techniques. Viable bifidobacteria ranging from 8 to 9.0 log<sub>10</sub>/g of faeces were found in both tamarins and sixty-three fructose-6-phosphate phosphoketolase positive strains were isolated and characterized. Rep PCR analysis with primer BOXA1R clustered these isolates in fifteen different groups. The 16S rRNA gene sequence analysis confirmed the presence of 15 different Clusters (nine Clusters for each tamarin) and six out of them were species previously described in other Callitrichidae (common marmoset): *Bifidobacterium callithricos* (Cluster V), *Bifidobacterium stellenboshense* (Cluster VIII), *Bifidobacterium myosotis* (Cluster X), *Bifidobacterium biavatii* (Cluster XII), *Bifidobacterium tissieri* (Cluster XIII) and *Bifidobacterium catulorum* (Cluster XIV). The remaining nine Clusters represent new species. Recently 4 clusters have been described as the novel species *Bifidobacterium aerophilum* (Cluster I), *Bifidobacterium avesanii* (Cluster II), *Bifidobacterium ramosum* (Cluster III) and *Bifidobacterium callitrichidarum* (Cluster XI). Clusters IV, VI, VII and IX are new species under description and Cluster XV is a new species under study. These new species show phenotypic similar features such as microaerophilic growth. Based on our results, bifidobacteria could be considered natural inhabitants of the gut of the cotton-top tamarin and emperor tamarin. An interesting finding is the high number of bifidobacterial clusters found in every single tamarin showing a high intra/inter-subjects diversity and similarity: this is in contrast with the few numbers of species (at maximum 4) usually found per human host. Interestingly, comparing data on studies of bifidobacterial distribution in New World Monkeys (common marmoset and tamarins), it can be observed that some bifidobacterial species were exclusively present in Common marmosets or tamarins, whereas other species were widely shared across all these Callitrichidae. Consequently, such complex and heterogeneous bifidobacterial communities seem to be composed of some bifidobacterial species more intimately close to their host (probably being genetically adapted to the host) and a core of species potentially shared across different host species, which reflect the co-evolution of these beneficial microorganisms with their host.

## **Canine Distemper: an actual old issue for wildlife conservation**

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**Keywords:** *distemper, conservation*

Canine distemper is a worldwide infectious disease caused by a Morbillivirus, related to Paramyxoviridae, along with rinderpest virus, peste-des-petits-ruminants virus, phocid distemper virus, measles virus and cetacean Morbilliviruses. Canine distemper virus (CDV) is mainly known because it's the causative agent of a severe systemic disease characterized by a variety of symptoms in dogs. CDV is an enveloped, negative-sense RNA virus, that infects different cell types of various organs and tissues. CDV infection is characterized by a systemic and/or nervous clinical course and viral persistence in selected organs including the central nervous system (CNS) and lymphoid tissue. Symptoms include fever, respiratory and enteric signs, and neurologic disorders. Domesticated dogs, unvaccinated or improperly vaccinated populations, are the main reservoir of CDV, which is a multihost pathogen. In fact, this virus occurs in all members of the Canidae and Mustelidae families, some members of the Procyonidae, Hyaenidae, Ursidae and Viverridae families. Also has been reported in large felids and marine mammals. More recently, CDV was reported in non-human primates with high mortality rates. CDV is highly contagious and has been responsible for severe population declines in both terrestrial and aquatic wildlife. In the last years, outbreaks have been documented in a wide range of wildlife species. In this study we analyzed samples from wolves, foxes and dogs from central Italy. All samples were collected from dead animals which were sent to the Diagnostic Units of IZSUM and subjected to necropsy. We also had the possibilities to conduct analysis on infected and symptomatic live animals, hosted in a Wildlife Rescue Centre. Our study shows a wide CDV circulation involving different ecotypes and species in the investigated area, confirming the fact that the spread and incidences of CDV epidemics in wildlife are worldwide increasing. An adult male infected wolf (*Canis lupus*) was also monitored during the hospitalization and showed a long period of viral shedding after the regression of the neurological symptoms, before the reintroduction in nature. The aim of this study is to review a well-known disease in domestic dog but with a lack of knowledge of pathogenesis and dynamics in wildlife, and to pinpoint the virus epidemics in central Italy and investigate the CDV issues for fauna conservation

## **Immunohistochemical investigation of the cross-reactivity of selected cell markers in formalin-fixed, paraffin-embedded lymphoid tissues of Franciscana (*Pontoporia blainvillei*)**

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**Keywords:** *Cetacean, Immunopathology, Lymphoid system*

A considerable amount of knowledge on natural and anthropogenic pathologic conditions affecting different cetacean species has been gained over the last decades. Nonetheless, the immunopathological bases for most of these processes have been poorly documented or remain unknown. Comparative immunopathological investigations in these species are precluded by the limited number of specific antibodies, most of which are not commercially available, and the reduced spectrum of validated and/or cross-reactive ones. To partially fill in this gap of knowledge, a set of commercially available primary antibodies were tested for cross-reactivity against leukocytes and cytokines in formalin-fixed, paraffin-embedded (FFPE) lymphoid tissues (lymph nodes, spleen and thymus) of three bycaught, apparently healthy and fresh Franciscanas (*Pontoporia blainvillei*) using immunohistochemistry. On the basis of similar region specificity within the lymphoid organs, cellular morphology and staining pattern with human control tissues, 13/19 primary antibodies (caspase 3, CD3, CD57, CD68, FoxP3, HLA-DR $\alpha$ , IFN $\gamma$ , IgG, IL4, IL10, Lysozyme, TGF $\beta$  and PAX-5) exhibited satisfactory cross-reactivity. Our results expand the spectrum of suitable cross-reactive primary antibodies in FFPE cetacean tissues. Further comparative immunopathological studies focused on infectious diseases and ecotoxicology may benefit from establishment of baseline expression of immunologically relevant molecules in various cetaceans species. Acknowledgement: FAPESP and CNPq

## Can Irish seals be One Health sentinels? A pilot study

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**Keywords:** One Health, Antimicrobial Resistance, Influenza A, Seals

Vale, A. (1,3), Shubin, L. (2), Byrne, BA. (2), Leonard, F. (1) and Barry, G. (1) (1) School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland (2) School of Veterinary Medicine, University of California, Davis, United States of America (3) School of Science, Institute of Technology, Sligo, Ireland WHO, OIE and FAO recognize the importance of antimicrobial resistant (AMR) bacteria and influenza viruses in animal and human health worldwide. Harbour and grey seals are native marine mammals that can be frequently seen on Irish shores. These protected species have an important role as top predators in the marine food chain and their conservation is vital. Furthermore, interactions between seals and other animals are frequently reported, which may facilitate inter-species disease dissemination. Our study addresses the need to gather information on seal health and inform on the potential of using seals as One Health sentinels for AMR bacteria and Influenza viruses. Nasal and faecal swab samples were collected from 23 harbour seals and 2 grey seals in rehabilitation at Seal Rescue Ireland in the Summer of 2017. Viral RNA was isolated from nasal swabs for Influenza A detection by reverse transcription (RT) PCR and faecal samples were screened for the presence of  $\beta$ -lactamase-producing *E. coli* using selective chromogenic medium (TBX) supplemented with cefotaxime. *E. coli* antimicrobial susceptibility profiles were determined by VITEK2 (Biomérieux®) and multiplex PCRs for  $\beta$ -lactamase genes detection were performed on appropriate isolates. Nineteen other cefotaxime-resistant bacteria isolated from faeces were identified by 16s rRNA PCR and the presence of  $\beta$ -lactamase coding genes investigated. All 25 nasal swabs tested negative for Influenza A virus. Seventy-three per cent of *E. coli* investigated were multidrug resistant (MDR). AMR genes blaTEM-1 and blaOXA-1 were detected in *E. coli* sampled from 4 (16%) and 9 (36%) seals, respectively. Other cefotaxime-resistant bacteria were identified as the Enterobacteriaceae: *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Enterobacter ludwigii* and *Enterobacter cloacae* and none of these bacteria carried  $\beta$ -lactamase genes. No extended-spectrum beta-lactamase (ESBL) genes were detected. This is the first pilot study investigating the presence of AMR genes and Influenza A in Irish seals. MDR bacteria and AMR genes blaTEM-1 and blaOXA-1 were detected in *E. coli* isolated from young wild seals with no previous antibiotic treatment. ESBL genes and Influenza A were not detected in the population investigated. Our findings suggest the circulation of AMR bacteria in the ecosystem and illustrate the potential role of seals as sentinels for the presence of AMR bacteria in the environment.

## Guiana dolphin mass-mortality linked to cetacean morbillivirus, Brazil

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**Keywords:** *Paramyxoviridae, Emerging viruses, Marine Mammals*

Cetacean morbillivirus (CeMV) is a highly infectious pathogen, first detected in the northern hemisphere in 1988–1990, and responsible for numerous cetacean mass die-offs worldwide. Currently, there are three well characterized strains: the porpoise morbillivirus, the dolphin morbillivirus, and the pilot whale morbillivirus, and three less known strains, including the novel Guiana dolphin strain (GD)-CeMV, recently identified in a single specimen from Brazil. To date, no epizootics linked to CeMV have been detected in the South Atlantic. Between November and December 2017, an unusual mortality event (UME) involving 56 GDs began in Ilha Grande Bay, Rio de Janeiro, Brazil. Necropsies were performed on 20/56 (37.7%) animals. Tissue samples of major organs were collected and fixed in 10% neutral buffered formalin for light microscopy examination or frozen (-80°C) for PCR analysis. Primers targeting highly conserved fragments of the phosphoprotein (P) gene and RNA-dependent RNA polymerase protein coded by the L gene (primers RES-MOR-HEN) were used. CeMV genome was detected in 15/20 (75%) animals for the P gene and 6/6 (100%) animals for the L gene, including one animal which had histomorphological evidences of viral replication (intranuclear and intracytoplasmic eosinophilic inclusion bodies, INCIBs). Amplified products were sequenced and compared with sequences of CeMV by using BLASTN. Identical sequences were obtained for the L gene and two sequences with variation in one nucleotide position were obtained for the P gene. Sequencing of a 405bp amplified fragment of the CeMV P gene revealed 99-100% identity to the GD-CeMV strain and 78-82% identity with other CeMV strains. A 443bp amplified fragment of the CeMV L gene revealed 74-75% identity to CeMV and other morbillivirus species. Partial P and L gene sequencing and analysis using MEGA7 (<http://megasoftware.net/>) corroborate that the GD-CeMV strain differed from other morbilliviruses and represents a distinct lineage. Microscopically, one specimen presented lesions consistent with CeMV infection, including marked, multifocal, subacute bronchointerstitial pneumonia with type II pneumocyte hyperplasia, syncytia and scattered intraepithelial INCIBs; mild to moderate, multifocal histiocytic and lymphoplasmacytic mastitis with necrosis and epithelial INCIBs; and multicentric lymphoid depletion. Furthermore, moderate to severe parasitosis by *H. brasiliensis* likely accounted for severe comorbidity in most cases. Intense viral replication in the mammary acinar epithelium in a lactating female may imply a vertical transmission route, besides the horizontal aerogenous and direct contact routes. Herein, we provide compelling molecular and pathologic evidences that associate GD-CeMV infection to the ongoing Guiana dolphin mass die-off in Rio de Janeiro, Brazil. While the factors underlying the die-off are being investigated, our results indicate GD-CeMV likely is playing a major contributory role. The data hereby reported increase the body of knowledge on health and disease aspects of this endangered species. By the time of submission, the UME had claimed more than 200 Guiana dolphins in Rio de Janeiro state, and appeared to be extending southwards. The environmental consequences and conservation impacts coupled with the anthropogenic threats are expected to be dramatic. **Acknowledgement:** FAPESP, CNPQ, FAPERJ, UERJ and PETROBRAS

## **Fatal case of disseminated phaeohyphomycosis in a free-living common toad (*Bufo bufo*) in the United Kingdom caused by *Exophiala* sp.**

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**Keywords:** *amphibian disease, black yeast, fungal infection*

In recent decades there has been increased awareness of the importance of multiple infectious disease agents causing wild amphibian mortalities, population declines and even species extinctions. *Batrachochytrium dendrobatidis*, *B. salamandrivorans* and *Ranavirus* are particularly well recognised in this regard. As a consequence, increased scanning surveillance efforts are being targeted at wild amphibian populations, with the objective to better understand the endemic conditions affecting them, thereby facilitating rapid detection of novel or emerging pathogens. Garden Wildlife Health (GWH) conducts disease surveillance of wild amphibians in Great Britain throughout the calendar year. A single adult female common toad (*Bufo bufo*), found dead in a garden pond in Ayrshire, Scotland, was reported in September 2016, and a subsequent post-mortem examination was performed by GWH at the Institute of Zoology, ZSL. The animal was in normal body condition based on assessment of muscle mass and coelomic fat bodies. Macroscopic examination revealed multiple abnormalities, including numerous dark red papular skin lesions (circa 1-2 mm diameter) over the back and dorsal aspects of the limbs, hepatomegaly, and a large undifferentiated soft tissue mass in the coelomic cavity of 2.7g and approximately 3.5 x 2.3 x 1.8 cm in size. Histopathological examination revealed severe hepatitis and coelomitis with intralesional pigmented fungal hyphae consistent with phaeohyphomycosis. Direct microbiological examination of the liver isolated the fungus *Purpureocillium lilacinum*, an environmental saprophyte and potential contaminant. Polymerase chain reaction targeting the internal transcribed spacer region and large ribosomal subunit rDNA was conducted on DNA extracted from a sample of affected liver. The sequence data obtained (ITS sequence 234 bp) identified a black yeast of the genus *Exophiala* sp. (Chaetothyriales), with closest identity (95%) to *E. cancerae*; however, the possibility of an infection or co-infection with the closely related *E. salmonis* could not be excluded. Various waterborne species belonging to the black yeast genus *Exophiala* can cause cutaneous or disseminated infections in ectothermic aquatic animals. Whilst mixed fungal infection could not be excluded, based on the microscopic appearance of the pigmented fungal elements and the known disease manifestations caused by *Exophiala* sp., this agent was considered to be the primary pathogen in this common toad. To the authors' knowledge, this is the first description of *Exophiala* sp. in a common toad and the first report of phaeohyphomycosis affecting a free-living amphibian in Great Britain. To date, the only other report of an *Exophiala* sp. infection in Bufonidae involved *E. cancerae* in the liver of a green toad (*Pseudepidalea viridis*) in Israel. Whilst we found no evidence of significant concurrent infection or generalised debility in this common toad, it has been postulated that immunosuppressed or stressed amphibians are most susceptible to phaeohyphomycosis, and that traumatic injury to the skin may predispose to infection with these environmental fungi. Phaeohyphomycosis appears to most likely be a sporadic cause of mortality in individual wild amphibians but this finding adds to our knowledge of the growing list of infectious diseases affecting these species in Great Britain.

## Status of Marine Mammals' and Reptiles Strandings in the Greek Coasts between 2014-2015.

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**Keywords:** *strandings, marine mammals, marine reptiles, morbidity and mortality*

The Mediterranean Sea, including Black Sea, hosts a diverse fauna, with a total of 27 different species of marine mammals and reptiles known to exist or to have existed in the region, whereas the Greek Seas represent one of the most important marine areas, hosting a large number of different marine species. A variety of types of marine species strandings have been identified ranging from alive or dead, single animals to the strandings of mixed marine species and sporadically mass strandings. Natural factors, such as diseases, navigational errors, abnormalities in the Earth's magnetic field, oceanographical or climatic events, escaping predators, pursuing prey and anthropogenic causes, such as boat collisions, entanglement in fishing gear, intentional harassment, navy or seismic sonar testing and pollution are the main reported causes of these strandings. The stranding events provide useful information for determining marine species' biology, physiology, occurrence, distribution, abundance as well as morbidity and mortality causes. Marine species' stranding data were obtained mainly through the National Stranding Network Marine Species' Database established by the Hellenic Centre for Marine Research in collaboration with the School of Veterinary Medicine of Aristotle University of Thessaloniki (AUTH). The stranding database includes information concerning the species identification (7 cetaceans, monk seals and 3 marine turtles) geographical area of findings, body characteristics and measurements, causes of morbidity, and mortality from the Hellenic Coast Guard's official notices of the first inspection of the animal, the autopsy/necropsy report of the Veterinary Authorities or from ARION's Veterinary Network. This is a study for data analysis of marine mammals and reptiles stranded during the period 2014-2015, like Cetaceans (striped dolphin - *Stenella coeruleoalba*, bottlenose dolphin - *Tursiops truncatus*, Risso's dolphin - *Grampus griseus*, Cuvier's beaked whales - *Ziphius cavirostris*, shortbeaked common dolphin - *Delphinus delphis*, sperm whale - *Physeter macrocephalus*, harbour porpoise - *Phocoena phocoena*), Pinnipeds (monk seals - *Monachus monachus*) as well as marine Reptiles (leatherback turtle - *Dermochelys coriacea*, green turtle - *Chelonia mydas*, loggerhead turtle - *Caretta caretta*) found along the Hellenic coastline - live, sick, injured, orphaned or dead. The geographic distribution of stranded animals, biological data, as well as, some morbidity and mortality causes are analyzed in the present study. The majority of dead or injured *C. caretta* stranded in the Ionian Sea, those of *M. monachus* in S. and C. Aegean, while the majority of *S. coeruleoalba* and *T. truncatus* stranded in the N. and C. Aegean Sea. Approximately in 65-70% of the stranding events the causes could not be identified since necropsy was not performed, almost 30-35% were related to human activities (fishery activity, boat collision, Navy or seismic sonar testing, deliberate killing) or infectious factors, while 5-10% were alive (injured, sick and orphan). In comparison to the strandings reported in previous years (2010-2013) the number of *C. caretta*, *C. mydas* and *M. monachus* presented a relative increased number of events/year. The total number of strandings species were 213 and 437 cetaceans, 45 and 62 monk seals and 1251 and 2117 turtles in 2014-2015 and 2010-2013, respectively.



## Causes of Stranding of Loggerhead Turtles (*Caretta caretta*) in Northern Greece (2010-2018): A Retrospective Study

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**Keywords:** stranding, *Caretta caretta*, rehabilitation

The endangered Loggerhead Sea Turtle (*Caretta caretta*) nests mainly in the Eastern Basin of the Mediterranean Sea, and Greece hosts about 60% of the nesting areas. In this retrospective study, 76 cases of stranded Loggerhead Sea Turtles are presented. From 2010 to 2018, a total of 76 stranded Loggerhead Sea Turtles were submitted to the Exotic and Wildlife Clinic of the School of Veterinary Medicine, AUTH, by the Hellenic Marine Stranding Network. All animals came from the North Aegean area and the strandings occurred mainly during the winter season (32/76). Most of the animals were females (61/76), and according to the size and weight, they were estimated as adults (50/76) and juveniles (26/76). The main causes of admission were injuries (40/76), depression (19/76) and ingestion of fish hooks (17/76). After a detailed clinical examination, radiographs were performed and blood samples were taken for hematological and biochemical examinations, as well as fecal samples for parasitological examinations. Supportive treatment with fluids and electrolytes was immediately initiated. Anorectic animals were forced-fed with special food enriched with vitamins and minerals. Traumatic injuries, which were the most common cause of admission (40/76), included carapace (3/40) or plastron (1/40) fractures, limb fractures (2/40), skull and mandible fractures (27/40), or a combination of the above (7/40). Almost all injuries were related to propeller or fish spear impaction. According to the severity of the injuries, turtles were lethargic, anorectic, with locomotion and/or neurologic abnormalities. Supportive care, wound care, broad-spectrum antibiotics and analgetics were used at the first stage of treatment. Shell fracture repair was successfully performed in some of the animals. Bone fractures were stabilized by external/internal fixation and in severe cases amputation was performed. Some of the skull and mandible fractures were surgery managed as well. More than half of the turtles (23/40) recovered and were released into the wild, whereas the rest (17/40) died during the first days of rehabilitation. Seventeen sea turtles had ingested hooks with fishing lines. Endoscopic and/or surgical removal of esophageal and gastric fishhook was performed. Since those turtles were in good physical condition, and no other problems were observed, they were released to the sea after their recovery. Ten juvenile animals suffered from severe hypothermia and cold stunning accompanied by malnutrition during early winter. After symptomatic treatment and nutritional support, all except one fully recovered and returned back to the sea. Nine animals had clinical signs of respiratory problems: lethargy, anorexia, dyspnea, abnormal respiratory sounds and buoyancy issues. Radiographic findings revealed severe pneumonia. A combination of supportive treatment (fluids and electrolytes), nutritional support, and broad-spectrum antibiotics was administered. Six animals fully recovered and 3 died. In conclusion, human-related traumatic injuries were the main causes of Loggerhead Sea Turtle strandings. Our results (55 releases – 76% of all animals) demonstrate that immediate intervention in sea turtle stranding events, as well as the appropriate treatment is essential for high survival rate and successful rehabilitation and release back in the wild.

## **An unusual case of peripheral neuropathy in a captive Brown pelican (*Pelecanus occidentalis*)**

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**Keywords:** *Pelecanus occidentalis*, Pathology, Lymphocytic aggregates

Wild bird pathologies are widely studied, although little is known about certain diseases. Commonly, we use domestic animal diseases as a model to explain wildlife illnesses, but they are not often related. Furthermore, investigating studies of these diseases becomes harder, when these wild animals have been in close human contact, due to the possible interferences in the results. This report shows the case of a 6-year old, male Brown pelican (*Pelecanus occidentalis*) from the Fresno Chaffee Zoo (California, USA) that died after some days showing illness and ataxia episodes. The pelican had been on topic NSAIDS treatment two days prior to death. The necropsy revealed a subcutaneous hemorrhage on the right leg, as well as pale and mildly enlarged kidneys. In the histological examination, there was mild to severe lymphocytic perivascular infiltration of the peripheral nerves (sciatic, brachial plexus, vagus and splanchnic). In order to identify the specific population of lymphocytes involved in these lesions, immunohistochemistry was performed. Results confirmed that both, B and T lymphocytes populations were present, suggesting that these findings did not have a neoplastic origin. In birds, lymphocyte aggregations can have numerous origins. However, most common ones are those related to virus, although Avian Influenza and West Nile virus tests were negative in this animal. To the authors knowledge, there are no previous cases on this species reporting these findings. Better understanding of relations between wild animal diseases, and human actions, will allow us to achieve finer knowledge about how to handle and take care of wild animals welfare.

## **Distribution of the protected sea urchin *Centrostephanus longispinus* (Philippi, 1845) in the Dodecanese (south Aegean Sea, eastern Mediterranean)**

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**Keywords:** endangered species, sea urchins, Aegean Sea

The needle-spined sea urchin, *Centrostephanus longispinus* (Philippi, 1845) is a species protected by National Laws and European/International Conventions; it is referred in Annex IV of the Habitat Directive (CD 92/43/EEC), in Annex II of the Bern Convention, and in Annex II of the Barcelona Convention Protocol. The urchin lives in *Posidonia oceanica* beds, on detritic, and on various rocky bottoms, more often on coralligenous beds, where calcareous coralline algae thrive. It is considered as cryptic occupying crevices and overhangs. It is distributed throughout the Mediterranean, being rare in the western basin and inferred to be frequent to the warmer eastern one, due to its thermophilic character and despite the total lack of relevant studies. Although *C. longispinus* is considered as endangered or threatened, its population status has not been assessed, and the only existing information is limited in western Mediterranean populations (France, Italy and Spain). Considering all the above the present work aims at assessing the distribution of *C. longispinus* populations in the Dodecanese island complex, using a non-destructive method. Relative population abundance was estimated using the ACFOR scale, by diving along strip transects on pre- and coralligenous cliff bottoms (up to 50 m depth) at seven islands (Astypalaia, Agathonisi, Chalki, Kalymnos, Patmos, Symi, Tilos). *Centrostephanus longispinus* was observed in six out of the seven islands surveyed, as no specimens were observed in Symi stations. In shallow depths (< 20 m) the species occurred exclusively within crevices of steep cliffs covered by coralline algae enclaves, whereas in deeper it was also found on moderately inclined cliffs, and especially on their lower part that proximate to detritic sands and maerls. Its relative abundance ranged from PR (present, in Kalymnos, Patmos) to O (occasional, in Tilos), being R (rare) in most cases (Astypalaia, Agathonisi, Chalki). It seems, therefore, that the species is widely distributed in the study area, but its relative abundance is rather low, at least in the surveyed depth zone as the species is commonly reported as by-catch from trawl fisheries. Our results, although preliminary, indicate that the populations of *C. longispinus* on submarine cliffs are strongly related with the presence of coralligenous bottoms. In the Dodecanese, the environmental characteristics of water masses, i.e. oligotrophy and great transparency, allow the penetration of light in greater depths, thus, 'pushing' the coralligenous in deeper or shrinking its presence in crevices on steep rocks, and so does *C. longispinus*. However, more information is needed to explain *C. longispinus* distribution, assess its population status and set on priority actions as demanded by EU Laws and International Conventions.

## **Optimization of sampling techniques and molecular detection of Herpesviridae in Neotropical primates.**

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**Keywords:** *Herpesvirus, Neotropical primates, non-invasive saliva collection*

Neotropical primates in Peru are frequently trafficked and in prolonged contact with humans, domestic animals, and wildlife. This can facilitate disease emergence, transmission, and spillover events. Currently, the surveillance of infectious diseases in Neotropical primates is limited by the use of invasive testing, non-adequate sample storage under field conditions, low availability of diagnostic tests, and the cost of commercially available assays. Herpesviruses (HV) are agents of major concern for the reintroduction of primates that have been subjected to prolonged captivity, which exposes them to a diverse range of HV species that can be lethal for non-adapted primate species. In addition, the diversity of the Herpesviridae family is broad, and many limitations exist for the diagnosis of HV in non-human primates using available laboratory tests that are specifically designed for human HV or certain simian HV (e.g. HSV1, herpesvirus B). In order to improve diagnostic capabilities in these sites, we performed a cross-sectional study of HV identification in Neotropical primate species hosted at a rescue center for trafficked primates in the Peruvian Amazon. Our goals were to optimize a non-invasive method to collect saliva samples and to test the application of a consensus PCR for the diagnosis of HV field isolates. A total of 40 monkeys belonging to 8 different species were offered sterile ropes coated in various attractants, and behavior, chewing time, and volume of the solution eluted from samples was recorded. On a different date and under anesthesia, we performed oral swabbing and venipuncture of the same individuals to obtain saliva and whole blood samples. Molecular analysis was carried out to the Centro de Investigaciones Tecnológicas, Biomédicas y Medioambientales, in Lima, Peru. DNA isolation was performed and cytochrome b detection through PCR was applied as a mammalian internal control. A consensus PCR to detect and determine HV species was performed. Among different monkey species, overall percent of rope acceptance ranged from 71%-100%. Mean rope chewing time was 35 – 60 seconds, with a significant difference in time spent chewing the rope in *Alouatta seniculus* ( $p=0.033$ ) and *Ateles chamek* ( $p=0.044$ ) based on attractant used. There was no correlation between volume of eluted solution and chewing time or individual weight. The consensus HV PCR amplified a region of the dPol gene in 42.4% of the samples, and of the Terminase gene in 4.8% of the samples, detecting Human alphaherpesvirus 1, Saimirine gammaherpesvirus 2 and Ateline alphaherpesvirus 1. Herpesviral DNA was detected in 38.9% of blood samples and 74.4% of saliva samples using both molecular markers. Genetic sequencing to conduct phylogenetic analysis of HV species detected in this population of primates is underway. These data demonstrate that non-invasive saliva collection using chewed ropes is feasible and expands our knowledge of HV diversity at a species level in captive Neotropical primates in Peru. Our results can simplify respiratory pathogen surveillance at settings with limited resources and improve our capacity to screen rescued animals for opportune decisions in the prevention of disease.

## **Seroprevalence to *Brucella* spp. In wild boars of Campania region during the 2016-2017 hunting season**

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**Keywords:** *Brucella, wild boar, seroprevalence*

Brucellosis is an infectious disease caused by members of the *Brucella* genus, undergoing to specific eradication plans worldwide in domestic ruminants. However, Brucellosis is frequently reported among wildlife populations in Europe and particularly in the Eurasian wild boar which population is in a progressive expansion throughout Europe in the last decades, raising concerns regarding the control of diseases in this species. Thus, in the present study we carried out a cross-sectional survey in wild boars in Campania region to assess the sero-prevalence against *Brucella* and to provide information on associated risk factors using both Rose Bengal Test (RBT) and a competitive enzyme-linked immunosorbent assay (cELISA) on 513 sera from hunter-killed wild boar sampled between 2016 and 2017 in Campania region, southern Italy. Our results demonstrated an apparent prevalence of 11,31% (95% CI 2,61-20,00%). Prevalence was significantly affected by the region of sampling but not by age or by sex of the animals. The relative sensitivity of the RBT versus the cELISA was 8.6% while the relative specificity was 96%. Results of the present study indicates a moderate sero-prevalence in wild boar, and then a wide exposure risk to *Brucella* spp in Campania region. *Brucella* presence in agroecosystem may have important implications not only for biodiversity conservation practice but also, and principally, for animal and human health, finally could represent a growing risk of spillback to outdoor-farmed pig herds. However, further investigations are needed using bacterial isolation as well as PCR, to better characterize the responsible *Brucella* serotypes involved.

## **A Molecular Study of Seven Selected Pathogens in Cypriot Mouflons (*Ovis orientalis ophion*) Using Archived Blood Stored on Filter Papers**

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**Keywords:** *Mouflon, Anaplasma phagocytophilum, Babesia*

The Cypriot mouflon (*Ovis orientalis ophion*) may be infected with pathogens which are important for both human and animal health. This free-ranging ungulate shares areas with domestic ruminants at the borders of Pafos forest, which constitutes its habitat. Fifty mouflon blood samples archived on filter paper during the years 2010 – 2014 were tested by real-time polymerase chain reaction for seven selected pathogens, namely *Coxiella burnetii*, *Brucella* spp., *Anaplasma* spp., *Anaplasma phagocytophilum*, *Babesia* spp., *Leptospira* spp. and *Toxoplasma gondii*. One mouflon (2%) was positive for *C. burnetii*, two (4%) for *Anaplasma* spp., two (4%) for *A. phagocytophilum*, one (2%) for *Babesia* spp. and one (2%) for *Leptospira* spp. Sequencing analysis revealed that the *C. burnetii* identified was 100% homogenous (EU448144) with a sequence previously reported in mouflons from Cyprus. It also identified the *Anaplasma* spp. isolates as *A. ovis* which were 100% homogenous (FJ460442) with samples isolated from sheep and goats in Cyprus. *Anaplasma phagocytophilum* positive results were 100% homologous with previous isolates from goats and sheep in Cyprus. The *Babesia*-positive sample was identified as *Babesia canis canis* (KF499115). Apart from *Coxiella burnetii* and *Anaplasma ovis*, the other three pathogens are reported in Cypriot mouflons for the first time. The results suggest that Cypriot mouflons may support systemic infections with *C. burnetii*, *A. ovis*, *A. phagocytophilum*, *Babesia* spp. and *Leptospira* spp. Furthermore, the sequencing analysis results suggest the potential circulation of infection with *A. ovis* and *A. phagocytophilum* among domestic small ruminants and mouflons, through infestation with ticks. To the best of our knowledge, this is the first time *Babesia canis canis* has been reported in mouflons. Further studies should aim to identify the potential reservoir capacity of mouflons for the infectious agents identified above, and to investigate the epidemiological links between Cypriot livestock and wild mouflons.

## **Gastrointestinal parasites of ungulates in Greenland: Possible transmission between wild muskoxen (*Ovibos moschatus*) and free-roaming domestic sheep (*Ovis aries*)?**

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**Keywords:** Arctic ungulates, Gastrointestinal parasites, Greenland, Lungworms, Next-generation sequencing, *Ovibos moschatus*, *Ovis aries*

In Greenland, muskoxen (*Ovibos moschatus*) have been translocated from the north-eastern region to the west coast for hunting purposes and tourism. The first translocations in the 1960s were highly successful, and subsequent translocations have resulted in five distinct muskox populations distributed along the west coast of Greenland. The latest translocation, in 2014, introduced muskoxen into the sheep (*Ovis aries*) farming district in the southwestern part of Greenland. There, overlapping habitats of wild muskoxen and the free-roaming domestic sheep may enable interspecies transmission of parasites – such transmission from sheep to muskoxen has previously been suggested in other regions where the habitats overlap. We applied microscopy of faecal samples and a next-generation sequencing approach to investigate gastrointestinal parasites in Greenland muskoxen and sheep. We present selected preliminary results from these analyses. Baseline data on the prevalence and geographical distribution of parasites are highly relevant in order to outline and understand the risks of emergence of parasitic disease in animal populations in Greenland.

## **Aspiration of spruce twigs in wolves (*Canis lupus*)**

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**Keywords:** *wolf, pneumonia, aspired foreign body*

The Swedish wolf population is subject to extensive disease surveillance to increase the knowledge of wolf morbidity and mortality. We here present three cases of aspiration of spruce twigs in wild wolves. Two of the aspiration cases had resulted in suppurative bronchopneumonia and one case in fatal haemothorax. In all three cases, the cause was inhaled foreign material in the form of spruce twigs, between 6 and 12.5 cm in length. The three cases were recorded from a total of 147 necropsied wolves, together with 14 incomplete carcasses, comprising all the wild wolves found dead or euthanized in Sweden between the years 2011 and 2014. The aspiration of twigs presumably occurs when the wolf is running hard and breathing with open mouth, such as during a hunt in a dense spruce forest. The needles along a spruce twig act as barbs, so when a twig is inhaled it can only move in one direction, making it impossible for the wolf to cough it out. In other wildlife species, two reports of grass awn aspiration in stone martens (*Martes foina*) have been published, but no other reports of inhaled foreign bodies in the thorax. In domestic dogs there are multiple reports of aspired foreign bodies causing respiratory distress. Most frequently, grain ears and grass awns, and less commonly solid objects like twigs and stones, are found in the lesions. Only a few reports on inhaled twigs in other species exist, including a human and an equine case. Although a rare finding, inhaled twigs pose a potential risk for developing fatal lung lesions in wild wolves.



## **Parasitic Fauna of the American Mink, *Neovison vison*, in Valencian community, Western Spain.**

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**Keywords:** *American mink, Neovison vison, Parasitofauna, Spain*

The American mink (*Neovison vison*) is a mammal considered as an invasive species in European countries, so its presence becomes an important hazard for the conservation of other species in danger of extinction, such as the European mink (*Mustela lutreola*). The parasitofauna of the mink has been studied in several European countries, however, in Spain there are few studies reported. No research has yet been carried out at the Valencian Community (East Iberian Peninsula) where the species has been released from fur farms in the past decades, colonizing diverse rivers. In the present study, a complete ecto and endoparasitic study was carried out in a total of 75 American minks from three rivers of the study area. Parasitological techniques were used to study the presence of parasites on skin, contents of stomach and intestine; Baermann migration of lungs for nematode larvae detection, as well as serological analysis using latex agglutination for detection of *Toxoplasma gondii* were carried out. Global prevalence of parasitosis was 22.7%. The prevalence in locations within the animals was: ectoparasites 20.5%; stomach parasites 10%; intestinal parasites 5.6%. Regarding a broncopulmonar parasites, larval stages of nematodes was observed in a 4.2% of the lungs studied. Antibodies against *T. gondii* were detected in the 7.4% of the serums analyzed. The identification of parasites was: two species of ticks (*Ixodes ricinus* and *I. hexagonus*) and one species of flea (*Ceratophyllus* sp.). Six species of endoparasites (*Molineus patens*, *Physocephalus* sp., *Vigisospirura potekhina*, larval stages of broncopulmonary worms, and larvae (LI) of a bot fly of the family *Calliphoridae* where identified within the intestinal contents. This is the first study on parasite diversity in the American mink in the Valencian Community, however, more studies are required to know in depth the incidence and parasitic load of these mustelids, which can be a biological hazard to other endangered mustelids such as the otter (*Lutra lutra*) or the European mink.

## Diagnosis improvement of oropharyngeal avian trichomonosis: application to sampling Bonelli's eagle chickens in nests

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**Keywords:** *Trichomonas gallinae*, Bonelli's eagle, Diagnosis

Oropharyngeal trichomonosis in birds is caused mainly by the protozoan *Trichomonas gallinae* (*T. gallinae*). The disease affects the upper digestive tract of the birds causing granulomas that impede the normal breathing and swallowing of the food, and it could provoke the death by starvation. Diagnosis is made by culture of swabs in TYM médium, and visualization of motile trophozoites. Since 2012, diagnosis of oropharyngeal trichomonosis in nests of Bonelli's eagles (*Aquila fasciata*) has been carried out in the context of a Life Conservation program from the European Union by the wildlife recovery center GREFA (Madrid, Spain) and by the local Government of the Valencian Community. Sampling was done in several regions of Spain, but some of the nest are difficult to access and the arrival of the culture to the laboratory is delayed, causing false negative results. For that reason, three alternative methods have been tested in order to improve the detection of the infection: culture in TYM media, PCR using oropharyngeal swabs and PCR using the sediment of the culture tubes. The diagnostic methods were applied to three different populations: a controlled population of wood pigeon (*Columba palumbus*) located at the wildlife recovery center of GREFA (Madrid, Spain) near the diagnostic laboratory (n=35), a population of Bonelli's eagle located in different nests at less than three hours far from the diagnostic laboratory (n=11), and a population of Bonelli's eagle dispersed in a wide geographic area and located at more than 8 hours by road from the laboratory (n=37). The sampling procedure was as follows: one swab was used for culture in TYM media, while a second swab was preserved at -20°C for DNA extraction. After the observation of the cultures at the microscope for 5 days, 1 ml was obtained and centrifuged at 3000 rpm for 5 min. and the sediment was employed for DNA extraction. A PCR that amplify the ITS fragment of *T. gallinae* was applied to both samples of DNA, the one obtained from the swab (Swab-PCR) and the one obtained from the culture sediment (Sediment-PCR). In the wood pigeon population (n=35) near the diagnostic laboratory, 68.6% of the samples were positive to the culture in TYM, while the Swab-PCR revealed 88.6% of the samples positives and the Sediment-PCR displayed 91.4% of samples positives. Results of the samples obtained from the Bonelli's eagle nests (n=11) located closed to a diagnostic laboratory show similar results when culture and Sediment-PCR were compared (5 positives / 11) while Swab-PCR showed a lower sensitivity (3 positives / 11). Finally, 8% prevalence was obtained when culture was employed in nests located far away from the diagnostic laboratory, while Swab-PCR displayed 45.9% of samples positives and Sediment-PCR revealed 75.7% of positives. In conclusion, Sediment-PCR was the most sensitive method to detect avian oropharyngeal trichomonosis. Cultures in TYM medium are useful to obtain fresh isolates, but the distance from the nest is an inconvenience. In that situations, Swab-PCR is a good alternative.

## The role of the invador California Kingsnake (*Lampropeltis californiae*) in the life cycle of local parasites in Gran Canaria, Spain

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**Keywords:** *Lampropeltis*, *Helminths*, *Invasive species*

California kingsnake (*Lampropeltis californiae*), was introduced in Gran Canaria twenty years ago and nowadays its impact represents a huge ecological problem due to the predation on endemic fauna. The origin of this invasion is presumed to be by accidental escapes or releases of captive-bred specimens. Three different populations of California kingsnake can be found: the main one at the east, other population at north-west, and the last one at the south of the island. Since 2007, an eradication program has been carried out to control the populations and study their biology. The main objective of this study was to contribute to the biology and epidemiology of these helminths found in California Kingsnakes in Gran Canaria and their relationship with the island's native species. From a total of 106 snakes collected, 64 were analysed by trichinostomy. They were positive for larvae species identified as Nematoda: Order Spirurida and cestodes species of genus *Diplopylidium*. Later on, in order to identify the natural hosts for these helminths, a total of 101 vertebrates (*Asio otus canariensis*, *Buteo buteo*, *Tyto Alba*, *Falco pelegrinoides*, *Canis lupus familiaris*, *Felis catus*, *Chalcides sexlineatus*, *Gallotia stehlini*, *Tarentola boettgeri*, *Crocidura russula*, *Mus musculus* and *Rattus norvegicus*) both, potential definitive hosts and preys, were dissected. Although cysticeroids of *Diplopylidium* spp. are usually reported in reptiles, its intermediate host remains unknown. In the snakes studied, these larvae were found mainly at the small intestine serosa, with a prevalence of 82,5%. Therefore, other reptiles such as *Tarentola boettgeri* showed larvae in six out of fourteen geckoes examined. Furthermore, in the island, twenty out of 44 cats necropsied presented adults in small intestine, meanwhile the 15 dogs studied were negative. This fact could confirm the role of the cats as definitive hosts. Concerning to nematodes, different larvae have been found in the snakes, all of them belonging to the Order Spirurida, probably of the Superfamilies Spiruroidea and Acuarioidea. The prevalence registered was 77,8%, mainly located at stomach and small intestine. Spirocercid-like larvae has been quoted in other island's native *Gallotia* specie, but there are no reports in *Gallotia stehlini* of Gran Canaria, that is the main prey of this invasive snake. However, these and acuariid-like larvae were found in 1 out of 14 and 3 out of 14 geckoes, respectively. Moreover, 2 out of 4 shrews (*Crocidura russula*) analysed also presented acuariid-like larvae. Finally, the Spirocercid nematode *Mastophorus muris* was found in all the rats dissected and also the acuariid nematode *Synhimantus* spp in all the owls of the present study. In conclusion, this study shows that snakes could be acting like paratenic hosts, being *T. boettgeri* the principal donor of *Diplopylidium*, spirocercid and acuariid-like nematode larvae. Our data confirmed that cats are the definitive host for *Diplopylidium* spp. Furthermore, this data suggest that *Asio otus canariensis* could be the final host for acuariid nematodes and *Rattus norvegicus* for Spirocercidae. This is the first report for larval spirurids in reptiles from Gran Canaria and in *Lampropeltis californiae*.

## Endoparasitosis in hedgehogs from the Valencia Community (West Spain)

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**Keywords:** Hedgehog, Parasites, Epidemiology, Coprological diagnosis

The study of parasites in wildlife is a useful tool to know the health status of populations and the pathologies by which they may be affected. The objective of this work is to obtain information about the diversity and prevalence of endoparasites in two protected species present in the Valencian Community, Spain: the European hedgehog (*Erinaceus europaeus*) and the Moorish hedgehog (*Atelerix algirus*). For this, coproparasitological studies were carried out on 150 animals (118 European hedgehogs and 32 Moorish hedgehogs) from the Wild Fauna Recovery Center of the province of Valencia and the University of Alicante. Fecal samples were collected and stored in SAF solution (sodium acetate, acetic acid and formaldehyde) at room temperature until processed in the laboratory. Each sample was evaluated by sediment observation and flotation with 33% solution of ZnSO<sub>4</sub>. The results obtained showed similar levels of prevalence in both species, being slightly higher in the European hedgehog (49%) than in the Moorish one (35%). Three types of nematodes were found: first stage larvae of *Crenosoma striatum*, eggs of *Capillaria* spp. and eggs of the order Spirurida unidentified. Eggs of trematodes (*Brachylaima* spp.), eggs of an unattached acanthocephala species and coccidian oocysts (*Isospora* spp.) were also observed. *Crenosoma striatum* was the most prevalent species, with a global prevalence of 36% (41.5% in *Erinaceus europaeus* and 15.6% in *Atelerix algirus*). Prevalence for other parasites was significantly lower in both species of hedgehogs: 14% for *Capillaria* spp., 7.3% for Spirurida eggs, 8% for *Brachylaima* sp., 4% for *Isospora* oocysts and 2% for acanthocephalan eggs. Results showed that *E. europaeus* had a risk 3.8 times that of *A. algirus* to be parasitized by lung nematode, *C. striatum*. Given the prevalence of parasites detected in the two species of hedgehog (especially the pulmonary pathogen *C. striatum*), the implantation and evaluation of suitable antiparasitic protocols during their stay at the Recovery Centers is recommended, in order to optimize the process of rehabilitation and subsequent release in the natural environment.

## **Helminthological status of Balkan Chamois from Rhodope mountains**

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**Keywords:** *helminthological status, Balkan Chamois, gastro-intestinal and lung nematodes*

A herd from Balkan Chamois in different ages has been studied ante-mortally to clarify their helminth infection status. The origin of animals is from the territory of Rhodope Mountains and this species are caught for reintroduction in Vitosha Nature Park. Low to medium rate of infections with gastrointestinal and lung nematodes has been established. The species of parasites include representatives of the genera *Strongyloides*, *Trichostrongylus*, *Haemonchus*, *Ostertagia*, *Nematodirus*, *Protostrongylus* and *Muellerius*.

## **Paratuberculosis infection in wild Ruminants in Bulgaria**

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**Keywords:** *M. avium, subsp. paratuberculosis, wild ruminants*

The aim of the study was to prove *Mycobacterium avium*, subsp. *paratuberculosis* in diagnostic materials from wild ruminants in Bulgaria and to determine the part of animals affected by paratuberculosis in the population. With modern diagnostic methods, 61 materials (small intestine and mesenteric lymph nodes) were examined from regularly shot and dead deers, roes and mouflons. Patomorphological changes characteristic of paratuberculosis were found in 5 mouflons and 3 deer which did not differ from those described in domestic ruminants. It has been found that *M. avium*, subsp. *paratuberculosis* grows well in the liquid Middlebrook medium 7H-9 and the solid Herrolds medium with mycobactin. The used diagnostic commercial kit “*Mycobacterium paratuberculosis Vet*”- Sacace Biotechnologies, appears to be suitable for isolating DNA of *M. paratuberculosis* from strains and tissue cultures. The results obtained from PCR coincide 100% with those obtained from pathomorphological and microbiological studies. It has been found persistence of *M. avium*, subsp. *paratuberculosis* in deers and mouflons on average in 13.1% of the studied wild animals in Bulgaria.

## **Clinical aspects and diagnostic investigation of a fatal outbreak in wild birds in reservoir of Karla, Thessaly, Greece**

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**Keywords:** wild birds, deaths, Karla

In August of 2016, massive deaths of wild avifauna were recorded by the Management Body of the Ecodevelopment Area of Karla-Mavrovouni-Kefalovriso-Velestino in the Karla reservoir. Following a short course of disease, presenting severe breathing difficulties and various neurological clinical signs (lethargy, incapacity of flight and indifference to external stimuli), the affected animals were dying. In total, 88 dead individuals of waterfowl and wading bird species were recorded, including 60 pelicans (*Pelecanus crispus* & *P. onocrotalus*). Eight birds were transferred to the NGO 'Action for Wildlife' for provision of veterinary care. During summer of 2017, a fatal outbreak of smaller extend, was recorded again in wild birds. In total, seven dead wild birds were transferred to the Laboratory of Microbiology & Parasitology, Faculty of Veterinary Medicine, University of Thessaly as part of the 2016 and 2017 fatal outbreaks investigation. Tissue samples, and content from the intestinal tract, the urine bladder, the gall bladder and the pericardiac effusion were collected. Macroscopically, a significant amount of liquid into the pericardial sac and absence of content in stomach were detected in all the individuals. Aerobic and anaerobic conventional microbiological tests were conducted on selective and less selective media, in different tissue samples. The isolated bacteria were subsequently identified using 16S rRNA gene sequencing. Furthermore, molecular techniques were implemented for the detection of viruses classified to the genus *Flavivirus* and *Influenza virus A*. Among the bacteria recovered from the bacterial cultures, the isolation of *Moraxella* spp. and *Staphylococcus* spp deserve to be mentioned. *Influenza A virus* and *Flaviviruses* were not detected in any of the samples examined.

## Blood parasites in passeriform birds in Belgium

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**Keywords:** blood parasites, avian, passeriforms

Information on avian blood parasites in Belgium is scarce. To collect data on their prevalence, 990 blood smears from passeriform birds were collected from May to November 1996 in a ringing station. After fixation and staining (Haemacolor®, Merck KGaA, Darmstadt, Germany), they were screened by light microscopy at magnification 10x100 using immersion oil. Blood parasites were identified to the species level, using the experience acquired during a training in an avian hematozoa expert centre in South-Africa in 1994. In 2017, due to new insights in the taxonomy of avian hematozoa, we reviewed our early identifications, and converted them to the current taxonomy according to Valkiūnas (2005). Altogether 9,4 % of the smears contained gametocytes of hematozoa. Among these, the genus most frequently found was Haemoproteus (86%), followed by Leucocytozoon (12%), Trypanosoma (2%) and Plasmodium (1%). Mixed infections (3%) included: two Haemoproteus species (1x), Haemoproteus + Leucocytozoon (1x), and Haemoproteus + Trypanosoma (1x). *Sylvia atricapilla*, the bird species with most samples, appeared to be also the most infected one (*H. belopoloskyi* 28%), followed by *Sylvia borin* (*H. belopoloskyi* 24%, including one mixed infection with *L. majoris*), *Sylvia curruca* (*H. belopoloskyi* 9%; *L. majoris* 9%), *Fringilla coelebs* (*H. fringillae* 10%, including one mixed infection with *H. magnus*; *L. fringillinarum* 5%), *Parus major* (*L. majoris* 6%; *P. relictum* 2%), *Turdus merula* (*H. minutus* 3%; *L. dubreuilii* 3%), and *Erythacus rubecula* (*H. fallisi* 1%, *H. neseri* 3%). (Percentages rounded to the unit). No haematozoa were found in *Passer domesticus*, *Prunella modularis*, *Carduelis spinus*, *Cyanistes caeruleus*, *Troglodytes troglodytes*, *Regulus ignicapillis* and *Regulus regulus*. Bird species with less than 10 samples were not included. The sensitivity of light microscopy, if performed properly, is comparable to molecular PCR techniques (Valkiūnas et al., 2008). Moreover, light microscopy allows the identification of avian haematozoa up to the species level. The prevalence of blood parasites depends on a complex combination of factors, including the vector activity (distribution, climate, season, pollution, insecticides), bird age and body size, breeding behaviour (in colony or solitary, nest type and location, duration of stay in the nest), and migration pattern. Migrating birds mix up with local populations, impacting on the prevalence of blood parasites. We found haematozoa in both long- (*Sylvinae*) and short-distance migrators (*Fringilla coelebs*). Bird species in which we found no parasites were non-migrating, except *P. modularis* and *C. spinus* (short-distance i.e. within Europe migration). Interestingly, *H. neseri*, known only from South Africa, was observed in *E. rubecula*, the morphology matching entirely with the description (average 30 pigment granules/gametocyte, dumbbell shaped gametocytes). In conclusion, the most frequently found blood parasites in passerine birds in Flanders were *H. belopoloskyi* (in *Sylvia* spp.), *H. fringillae* and *L. fringillarum* (in *F. coelebs*), and *L. majoris* (in *P. major*). Infections with other blood parasites were marginal (<5%), or inconclusive given the small sample size per species. (References available on request).



## Field and laboratory investigations to infer potential wildlife reservoir hosts for *Borrelia Miyamotoi*

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**Keywords:** Tick-borne zoonoses, *Borrelia miyamotoi*, Wildlife reservoir host

The enzootic cycle maintaining the agent of Lyme borreliosis, the most common tick-borne zoonoses in the world, comprises a variety of wildlife such as white-tailed deer, white-footed mice, shrews, voles and birds. In the eastern USA, the blacklegged tick, the major vector of Lyme borreliosis, also transmits a relapsing fever group spirochete, *Borrelia miyamotoi*, which has been implicated as a human pathogen in North America and Eurasia. Although *B. miyamotoi* can be transmitted vertically, it is suspected that horizontal transmission is necessary for long term enzootic maintenance. Thus, we conducted two studies to elucidate the reservoir hosts for *B. miyamotoi*. First we estimated *B. miyamotoi* infection prevalence in small mammals captured from 2010 to 2012 in central Wisconsin, where multiple pathogens vectored by the blacklegged tick are endemic. We screened 1221 ear tissues and 516 blood samples and observed 0.4% and 3.1% *B. miyamotoi* infection prevalence, respectively. For the 409 samples for which we had matching blood and biopsy samples, *B. miyamotoi* prevalence was significantly higher in blood samples (2.4%) than ear biopsies (0.2%; Fisher's exact test,  $P = 0.0112$ ). We detected *B. miyamotoi* in 5 species by blood or tissue assay, including the masked shrew, northern short-tailed shrew, southern red-backed vole, white-footed mouse, and eastern chipmunk. Infection prevalence among host species varied (0.1 ~ 18.8%). Eastern chipmunks (18.8%,  $n = 16$ ) had a greater infection prevalence than white-footed mice (2.7%,  $n = 444$ ) by blood assay, potentially indicating greater contributions of the host species to the enzootic maintenance of *B. miyamotoi*. The highest spirochetemia rate (7.4%) was observed in June, corresponding with the peak questing activities of larvae and nymphs in WI, highlighting the importance of public health messages 1) to emphasize late spring/early summer as a risky period for exposure to *B. miyamotoi* in addition to the Lyme borreliosis spirochete, and 2) to be vigilant of tick bites from both larval and nymphs. For our second study, we are conducting serum complement sensitivity assays, in which we will compare killing and growth inhibition effects of serum complement of various host species on *B. miyamotoi* spirochetes. Data from the two studies together will inform future ecological and laboratory transmission studies required to determine the reservoir hosts that help maintain *B. miyamotoi* in nature.

## Prevalence and distribution of vector-borne parasites in Pyrenean chamois from the eastern Spanish Pyrenees

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**Keywords:** Pyrenean chamois, *Rickettsia* spp., *Bartonella* spp.

*Rickettsia* spp. and *Bartonella* spp. are zoonotic intracellular bacteria of public health concern and veterinary significance. Ticks are the main vector for *Rickettsia* genus and they have also been related to *Bartonella* transmission. Therefore, the epidemiology of these bacteria is determined by environmental factors such as the altitude, climate and host community. Although subclinical *Rickettsia* and *Bartonella* infections in wildlife are quite common, there is no consensus about their role as population drivers. Wildlife, however, is considered of great importance in the epidemiology of these infections. In recent years, several tick species are expanding towards higher altitudes due to global warming. Information about the occurrence of these pathogens in mountain ecosystems will be useful to improve our understanding of the complex interactions between climate, landscape, host community and pathogen diversity. The aims of the present study were to evaluate the prevalence of *Rickettsia* spp. and *Bartonella* spp. in different populations of Pyrenean chamois (*Rupicapra p. pyrenaica*), a typical mountain dweller from the alpine ecosystem. We used the spleen of 333 Pyrenean chamois hunter-harvested between 2013 and 2016 in four different National Game Reserves (NGR) from the eastern Pyrenees, Spain (Alt-Pallars NGR, Cadí NGR, Cerdanya-Alt-Urgell NGR and Freser-Setcases NGR). The presence of bacterial DNA from *Bartonella* spp. and *Rickettsia* spp. was analysed in spleen through two real-time PCR. Pairwise comparisons of prevalence between the different NGRs were conducted with a Wilcoxon rank sum test. *Bartonella* spp. was only detected in Alt-Pallars NGR (29.9%; CI95%: 18.7-44.0) and Freser-Setcases NGR (1.9%; CI95%: 0.1-4.9) but only Alt-Pallars NGR was statistically different (all  $p < 0.05$ ) from the other NGRs. *Rickettsia* spp. was only detected in Freser-Setcases NGR (5.8%; CI95%: 3.4-9.9) but no statistical difference was detected among the NGRs. This study reveals the exposure to *Bartonella* spp. and *Rickettsia* spp. in Pyrenean chamois from the eastern Pyrenees. The differences between our study areas needs more in-depth analysis regarding ecological factors and herbivores densities in each NGR.

## Mortality of captive azure-winged magpie nestlings caused by Usutu virus

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**Keywords:** nestling, Usutu virus, mortality

Usutu virus (USUV), a mosquito-borne flavivirus, emerged in 2001 in Vienna causing high mortality mainly in blackbirds. In the subsequent years the virus spread in Europe, leading to deaths of wild and captive-held birds. Since the first outbreak, surveillance for USUV has been carried out. During our routinely performed necropsies we got four nestlings of the corvid species azure-winged magpie (*Cyanopica cyanus*; native to eastern Asia) belonging to a cognitive biology research group at the University of Vienna. The birds were kept in groups and originated from zoo populations in Europe or had hatched directly at the facility. They were housed in an aviary in an open courtyard in the city of Vienna. In 2017, the research group reported a higher nestling mortality compared to previous years in the first and second clutch of eggs. The most prominent finding in all four nestlings was a severely enlarged spleen. Pathohistological examination revealed a non-purulent myocarditis (3/4) as well as liver (4/4) and spleen (2/4) necrosis. Additional virological tests using RT-PCR were positive for Usutu virus and negative for West Nile virus. The performed immunohistochemistry showed also a strongly positive result in three nestlings. The complete genome sequence of one USUV and partial nucleotide sequences (E, NS5 and 3'UTR regions) of the others were determined. Phylogenetic analysis revealed the closest genetic relationships with USUVs of the “Africa 3” genetic cluster. Until recently only USUVs of the “Europe 1” and “Europe 2” genetic clusters were detected in Austria. Under these special conditions with a closely monitored breeding behavior (i.e., via nest cameras), it was for the first time possible to provide evidence of USUV-associated nestling mortalities, presence of USUV in a corvid species, as well as the introduction of a new USUV strain in Austria.

## **Pathogens of zoonotic importance detected in ticks from wild mammals in North-West Italy**

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**Keywords:** ticks, wild mammals, *Anaplasma*, *Rickettsia*

Bacterial pathogens from the genus *Anaplasma*, *Borrelia* and *Rickettsia*, and Tick-borne Encephalitis virus (TBEV) may be associated to various tick species. Ticks are hematophagous ectoparasites that can transmit a wide variety of pathogens to vertebrates, humans included, which makes them potential horizontal vectors. Over the past few years, as recorded by the ECDC, an increased incidence of tick-borne diseases has been reported worldwide. Besides *Anaplasma*, *Borrelia* and *Rickettsia* spp., the presence of TBEV in Northern Italy is also confirmed. The aim of our study is to provide an overview of the infection rate of tick-borne agents by monitoring wild ticks collected from wild mammals in the Liguria region between 2016 and 2017. Liguria's territory is characterised mainly by mountains and an intense urbanization of the terminal part of the alluvial plains. In these areas, a large variety of tick species that may act as pathogens' host can be found. After ticks identification and total nucleic acid extraction, simplex PCR assays were performed to detect *Anaplasma*, *Borrelia*, *Rickettsia* spp. and TBE virus. By project's design, all ticks belonging to a given species from the same animal and collection date were pooled and treated as a single sample. During the study period, we collected and identified more than 200 ticks from 100 wild mammals. In particular, we sampled 124 *Ixodes*, 68 *Dermacentor*, 34 *Haemaphysalis* and 9 *Rhipicephalus* spp. from 49 roe deer (*Capreolus capreolus*), 41 wild boars (*Sus scrofa*), 7 chamois (*Rupicapra rupicapra*), 2 fallow deer (*Dama dama*) and 1 marten (*Martes foina*). PCR results revealed 36 positives for *Rickettsia* spp. and 3 for *Anaplasma* spp.; all samples were negative for *Borrelia* spp. and TBEV. *Rickettsia* spp. were found in 32 *Ixodes* and 4 *Dermacentor* samples, while *Anaplasma* spp. were related to 2 *Haemaphysalis* and 1 *Ixodes* (co-infected with *Rickettsia*) samples. About mammal species, the 15% of wild boars and 61% of roe deer sampled were carrying ticks infected with *Rickettsia*, while the 29% of chamois carrying ticks infected with *Anaplasma*. At present, all PCR positive samples found during this study are under confirmation by the National Reference Centre for *Anaplasma*, *Babesia*, *Rickettsia* and *Theileria* (C.R.A.Ba.R.T. of Palermo, Italy). One similar paper from Otranto et al. 2014 on ticks collected on humans reported a 24% *Rickettsia* prevalence in *I. ricinus* species in Liguria. Our findings confirm the circulation of *Rickettsia* and *Anaplasma* also in ticks from wild mammals and the need to continue the monitoring of tick-borne agents.

## Wild bird surveillance in North-West Italy: West Nile and Usutu findings from 2015 to 2018

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**Keywords:** West Nile Disease, Usutu virus, wild birds, surveillance

The well known role of wild birds as natural hosts and carriers of diverse zoonotic diseases, e.g. Avian influenza, zoonotic flavivirus encephalites or chlamydiosis, deserves specific surveillance efforts from Public Health Institutions. Italy, located along the major bird flyways crossing Asia, Africa and Europe is considered a stop over site for many migratory species. In order to early detect the occurrence of pathogens, our Institute put in place a surveillance system on wild and synanthropic animals in the north western italian regions of Piemonte, Liguria and Valle d'Aosta. Here we report our findings on zoonotic flaviviruses, such as West Nile virus (WNV) and Usutu virus (USUV) on migratory and resident avifauna from 2015 till 2018. Wild birds were collected by passive surveillance from found dead animals in good post mortem conditions. An active surveillance on killed black crows, magpies and jays was scheduled since 2015 during the high risk season, i.e from July to November, in the context of the West Nile Disease (WND) surveillance plan. Moreover, an active collection of cloacal swabs mostly from the Orders Anseriformes and Passeriformes was also performed by trapping animals in wet areas. The collection of the target tissues for WNV occurred during necropsy. For each animal, if available, two samples were analyzed: brain and the pool of lung, heart, spleen and kidney. Viral RNA purification was performed using magnetic beads on the Maxwell 16 Instrument (Promega). Samples were tested using two Real-time one step RT-PCR assays: a duplex assay able to differentiate between Lineage 1 and 2 WNV and a simplex one specific for USUV.

All WNV and USUV positive samples were sent for confirmation to the OIE National reference Centre Laboratory for WND in Teramo. During the surveillance period, 2666 wild birds were analyzed: 1092 from the wild bird passive surveillance plan and 1574 from the WND plan, for a total of 5321 samples tested. Positivities for WNV Lineage 2 were found in 13 wild birds: 1 eagle owl found dead in Piemonte and the rest sampled under the WND plan. One live kestrel resulted positive from blood testing and the other 11 were culled black crows, three in 2015, six in 2016 and two in 2017 in different provinces of Piedmont region. USUV was confirmed in only two blackbirds in 2015, one from passive surveillance and one from WND plan. No cloacal swab resulted positive to the WNV/USUV screening.

Lineage 2 WNV circulates in wild birds in Italy since 2011 and is the most frequently detected Lineage in Italy. Of interest remains our sole Lineage 1 WNV identification in 2013 from an eurasian hobby found dead in Liguria. From a public health prospective, veterinary surveillance of wild birds and vectors allowed the early identification of WNV introduction at province and regional level, followed by the timely enforcement of WNV screening on blood donation sacks in Piedmont, recognized as WND endemic region since 2016.

## **Thelaziosis in wolf, foxes and brown bear from Greece**

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**Keywords:** *Thelazia callipaeda*, wildlife, Greece

*Thelazia callipaeda* (Spirurida, Thelaziidae), a nematode parasite that inhabits the eyes and their associated tissues, has been increasingly reported from domestic (dogs, cats and rabbits) and wild animals (wolves and foxes) in many European countries, including Greece. Additionally, reports of ocular infection in humans strengthen the medical importance of this parasitic infection in several countries. In Europe, the vector and intermediate host of this worm are male drosophilid flies *Phortica variegata* (Drosophilidae, Steganinae), which display a zoophilic behaviour feeding around the eyes of mammals. Clinical signs of thelaziosis range from subclinical to moderate or severe ocular disorders (e.g., epiphora, photophobia, conjunctivitis, keratitis, and corneal ulcers), which are mainly due to the lateral serration of the parasite cuticle being responsible for mechanical damage of the conjunctival and corneal epithelium. Here we report *T. callipaeda* infection in a wolf and two foxes and for the first time in a brown bear in Greece. At necropsy, adult worms were removed from the eyes during examination of conjunctival sacs and underlying nictitating membrane. All nematodes collected were morphologically identified based on the shape of the buccal capsule, presence of transversally striated cuticle and cloacal papillae, morphology of the spicules in males and the position of the vulva in females and further confirmed by molecular analysis. Sequencing of the partial mitochondrial cytochrome c oxidase subunit 1 gene (cox1) confirmed that only one haplotype of *T. callipaeda* was present, same with the one detected in domestic dogs, cats and rabbit (the only one haplotype circulating in Europe). The findings of this study support data of wolves and foxes as hosts of this nematode and represent the first report of brown bears as a competent host. Further, results indicate wildlife play a role in maintaining and spreading this eyeworm infection in domestic animals and possibly humans in endemic areas. Therefore, infected wildlife may pose a threat to animal and human populations living or having activities in forested and meadow areas. However, the role of the increased mobility of dogs mostly due to hunting and international tourism in the appearance and emerging of thelaziosis, needs to be assessed. Therefore, an increased medical and veterinary communities' awareness of thelaziosis is of major importance to limit further infections in domestic animals, wildlife and humans in Europe.

## Schmallenberg virus exposure in wild ruminants in Spain, 2010-2016

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**Keywords:** Schmallenberg virus, Wildlife, Serosurvey, Spain

Schmallenberg disease (SBD) is an emerging vector-borne disease that affects domestic and wild ruminants in Europe. A long-term serosurvey study was conducted to assess the exposure of wild ruminants to Schmallenberg virus (SBV) in Spain. Blood samples from 1216 wild ruminants, including 307 red deer, 246 Iberian ibex, 217 fallow deer, 194 roe deer, 118 mouflon, 98 chamois and 36 Barbary sheep, were collected between 2010 and 2016. Sera were tested for antibodies against the N protein of SBV using a commercial blocking ELISA (INGENASA, Madrid, Spain). Overall prevalence of antibodies was 27.1% (330/1216; IC95%: 24.6-29.6). By species, the seroprevalence was 45.6±6.7% in fallow deer, 31.6±5.2% in red deer, 28±8.1% in mouflon, 22.2±13.6% in Barbary sheep, 19.9±5.0% in Spanish ibex, 17.5±5.4% in roe deer, and 10.2±6.0% in chamois. Seropositive animals were not found before 2012, when the first outbreak of SBD was reported in Spain. In contrast, seropositivity was uninterrupted detected during the period 2012-2016, with values ranging between 23.3% in 2015 and 42.4% in 2013. To the authors' knowledge, this is the first report of SBV seroconversion in both Barbary sheep and Iberian ibex. A generalized estimating equations model identified the species (fallow and red deer) as the main risk factor for SBV infection. Our results evidence endemic and widespread circulation of SBV among wild ruminant populations in Spain during the last years. Serosurveillance for these species could be a useful tool for monitoring of SBV in Europe, especially in areas where wild ruminants share habitats with livestock.

## **Protocol for the rehabilitation and release of badgers (*Meles meles*) in England, with consideration of Bovine tuberculosis (*Mycobacterium bovis* infection)**

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**Keywords:** *Badger, rehabilitation, tuberculosis*

The badger (*Meles meles*) is an ancient, indigenous and protected mammal in the United Kingdom (UK). Badgers may be infected with *Mycobacterium bovis* and maintain the infection with the risk of transmission to cattle. Around 400 badgers are rehabilitated and released in England each year. This includes adult badgers, frequently admitted for reasons of anthropogenic trauma, and orphaned or abandoned badger cubs. Many adult badgers that come in to captivity are unsuitable for release back to the wild and around 70% of these animals are euthanased at the first possible opportunity for welfare reasons. Badger cubs are usually clinically well but require a long rearing and rehabilitation process before release. Adult badgers are released where found, for territorial and disease prevention reasons. Badger cubs are grouped together and require new sites to be found for their release. The Department for Environment, Food and Rural Affairs (Defra) published The Strategy for achieving Officially Tuberculosis Free Status for England in 2014. This is a 25 years plan and includes measures for disease control in both cattle and wildlife. Badger rehabilitation is not specifically mentioned in this strategy, but it is clear that some control of this practice is required to prevent disease transmission. In 2017 Defra and wildlife groups worked together to update the Badger Rehabilitation Protocol first published in 2003. This provides guidelines about all aspect of how badgers should be handled, cared for, rehabilitated and released. Disease prevention is at the core of the Protocol and appropriate controls for *M. bovis* infection, including testing of badgers are detailed. This reduces zoonotic risks in those handling badgers, and the risk of disease transmission to other animals, including livestock. Maintaining the confidence of landowners providing release sites for badger cubs is key to the rehabilitation of these animals. The only validated and commercially available serological test for badgers in the UK is the Dual Path Platform (DPP) VetTB assay (Chembio Diagnostic Systems). This measures specific antibody response to antigenic targets MPB83, ESAT6 and CFP10 and has a relatively low sensitivity (55.3%) but is highly specific (97.5%) in badgers. No routine testing of adult badger casualties is carried out under the Protocol, as they are maintained in isolation during captivity and released where they were found. A single test is not considered sensitive enough to be of significant benefit and these animals are not in captivity long enough to allow for multiple testing. Badger cubs are grouped in captivity for behavioural and social reasons and are released in these groups into a different location to where they were found. Badger cubs are tested using DPP on three occasions prior to release, in order to maximise test sensitivity. Badger cubs testing positive to any one of the three tests are euthanased and sent for standard post mortem examination and *M. bovis* culture. The outcome for remaining cubs in the group is determined by these findings. Badger cubs are also BCG vaccinated prior to release.



## A severe case of red deer Tuberculosis caused by *Mycobacterium microti*

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**Keywords:** red deer, *Mycobacterium microti*, Austria

This report describes a case of *Mycobacterium* (*M.*) *microti* tuberculosis in a free-ranging red deer (*Cervus elaphus*) originating from the province of Vorarlberg, Western Austria. In this alpine region tuberculosis caused by *M. caprae* is common in domestic animals and wildlife. Red deer represent a reservoir for *M. caprae* tuberculosis in cattle due to supplemental feeding in winter time and population densities beyond normal habitat carrying capacities. Strong efforts are implemented in the affected region to control and reduce the disease in red deer by means of disease surveillance and monitoring and by reduction of population densities. In 2017, the lung, heart and several lymph nodes (including medial retropharyngeal, tracheobronchial and mediastinal lymph nodes) originating from a 9 year old hunted stag were sent for TB diagnosis to the laboratory during the ongoing surveillance activity. The stag was highly emaciated and was shot due to a broken hind leg. Post mortem examination revealed multiple enlarged lymph nodes without typical abscessed purulent granulomas. The lung sections showed severe pyogranulomatous pleuropneumonia with multifocal to confluent cavernous granulomas from 2 to 10mm diameter, consisting of yellow purulent necrotic material. Multifocal to coalescing granulomas of 4-25mm diameter were observed on the surface of the epicardium. Histopathological examination appeared as typical tuberculous granulomas with central areas of necrosis and mineralization surrounded by epithelioid macrophages and a few multinucleated Langhans giant-cells. Lymphocytes, plasmocytes and occasionally well-differentiated fibroblasts were surrounding the granulomas. Using Ziehl–Neelsen staining, a few acid-fast bacilli were identified occasionally in the pulmonary granulomas. Samples from lung were subjected to bacterial culture and molecular diagnosis. Direct molecular testing of lung showed a positive PCR result for MTBC DNA in the *M. tuberculosis* complex specific Real-Time PCR. Culture on solid Stonebrink medium containing antibiotics was positive after six weeks. The diagnosis of *M. microti* was finally achieved from the culture material by the GenoType MTBC Ver 1.X kit which allows the discrimination against other common species of the complex. *M. microti* is a member of the *M. tuberculosis* complex and can cause infections in humans and different mammals. In Austria this agent was isolated only once in a red fox without visible lesions which was sent to the laboratory in 2016 within the ongoing TB surveillance scheme. This fox was located only 30 km in distance from the reported *M. microti* positive stag. Further molecular analyses are in progress to compare the genetic relationship of both isolates.

## **Tuberculosis and wild boar: frequentist and bayesian approaches to evaluate diagnostic tests when *Mycobacterium bovis* is present in wild boar but at low prevalence**

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**Keywords:** *Sus scrofa*, tuberculosis, diagnosis, serology, PCR, bacteriology

The Eurasian wild boar (*Sus scrofa*) is increasingly considered as a relevant actor in the epidemiology of bovine tuberculosis (TB). For that reason, monitoring TB in this species is becoming a key tool for establishing comprehensive control schemes for this disease still present in Europe. No data was available on the characteristics of TB direct and indirect diagnostic methods in wild boar in epidemiological contexts such as in some areas in France where TB is still endemic in cattle and detected in wild boars but at low prevalence. We aimed to estimate and compare sensitivity and specificity values of bacterial culture, PCR and three commercial ELISAs, the TB ELISA-VK® (using bPPD antigen), INgezim TB Porcine® and IDEXX Mbovis Ab Test® (both using MPB83 and MPB70 antigens) in wild boar under French field conditions. We use frequentist methods, with bacteriology as gold standard, and a Bayesian formulation of the latent class analysis (LCA) with no consideration of a gold standard. Submandibular lymph nodes and sera from 495 hunter-harvested wild boar from the core of three endemic areas (Aquitaine, Burgundy and Corsica), were collected between 2014 and 2016. Only 8 individuals were positive for *M. bovis* by bacteriology (1.61%; CI95% 0.70-3.51%). Both ELISAs using MPB83 and MPB70 antigens showed a very good specificity (above 98%) and good sensitivity (around 80% [60-95] when using LCA. With a specificity set up as perfect, the bacterial culture exhibited a limited sensitivity (around 45% [20-70]), the sensitivity here being the probability of giving a positive result in an animal exposed to *M. bovis*). PCR and ELISA using bPPD showed very good specificities (above 96%) but lower sensitivities compared to other ELISAs. These results suggest that ELISAs using MPB83 and MPB70 antigens could be used to detect and monitor TB exposition of wild boar populations to TB in French field conditions.

## First test of delivery of candidate baits for oral vaccination of badgers against bovine tuberculosis in France

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**Keywords:** badger, bovine tuberculosis, bait delivery

Although France is has an officially free bovine tuberculosis (bTB) status in cattle, *Mycobacterium bovis* infection has re-occurred and persists at low level in several regions in cattle and wildlife. bTB positive badgers have been found in the vicinity of re-infected cattle farms despite management measures targeting both cattle and badgers. In this context, vaccinating badgers appears as a promising strategy to prevent *M. bovis* transmission between badgers and cattle and subsequent environmental contamination. A licensed injectable Bacillus Calmette-Guérin (BCG) vaccine has been used in the UK and Ireland. However, using this vaccine requires to capture live badgers which limits its deployment scale for practical reasons. Alternatively, an oral vaccine consisting of live BCG contained in an edible bait is currently under development by APHA in collaboration with European partners. This bait has to be palatable to badgers in the field and compatible with the storage and survival of BCG. This study aims at evaluating the feasibility and efficiency of deploying the candidate bait (but without BCG) in a French area infected with bTB. The deployment efficiency is estimated as the proportion of badgers consuming baits marked by the biomarker Rhodamine B, per badger territorial group. The uptake of the biomarker marked baits is identified by detecting fluorescence in hair collected on hair traps positioned nearby the setts. Collected hairs are also genotyped to differentiate individual badgers and calculate the number of marked animals among the identified individuals within each group. Bait uptake by non-target species is also investigated and hairs collected from species other than badgers (including cattle) are examined for fluorescence marking. In addition, we use camera-trapping to monitor sett badger activity and visits by other species. The study is carried out at 15 badger setts, most of them being selected in close vicinity of pastures where cattle were found infected with *M. bovis* in Côte d'Or département (Burgundy region in north-eastern France). Between six and 20 pre-baits (i.e., without biomarker) per sett were delivered per day inside the holes for 8 days followed by 15 to 30 baits per day (containing biomarker) per sett for 4 days, once in spring and once in summer 2018. This study is the first one conducted in France on bait deployment in a badger population of medium density after intensive culling and coexisting with many potential non-target species, i.e., different conditions from UK and Ireland where this deployment is presently tested. Data collection and analyses are ongoing. Results are expected to provide valuable information regarding potentials limitations and success factors towards a realistic deployment of oral vaccine baits to support the development of an “exit strategy” to control TB in badger populations following culling.

## Surveillance of bovine tuberculosis in wildlife in France: methodological constraints to monitor prevalence in at-risk areas

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**Keywords:** bovine tuberculosis, *Mycobacterium bovis*, badger, wild boar, France

In 2001, *Mycobacterium bovis* infection was first evidenced in wildlife in France. Thereafter, infected wild animals (mainly badgers and wild boars) were regularly detected in the vicinity of domestic bovine tuberculosis (bTB) outbreaks and within areas where the disease was found to be endemic in cattle. In 2011, a national surveillance system for bTB in non-captive wildlife was created, named “Sylvatub”. The surveillance process targets on badger, wild boar and red deer and relies on different combinations of passive and active surveillance protocols depending on the level of risk of each administrative unit. Three levels of risk are defined and evaluated qualitatively twice a year. Different actors are involved in the field implementation of this program and coordinated by the local state veterinary services: hunters, trappers, veterinarians, technicians of the Environnement, laboratories agents. When bTB is detected in these populations, monitoring the disease prevalence is necessary (i) to evaluate the efficiency of control measures applied both in cattle and wildlife populations, and (ii) possibly to compare the levels of infection among different areas. Here, through analysis of the data obtained from Sylvatub from 2014 to 2017, we observed some diversity in the field implementation of the national protocols and we had to consider that simple random sampling assumptions were not always satisfied. Then, we questioned the feasibility to build a relevant indicator of infection rate for the two species mainly addressed in the surveillance system, wild boar and badger, and propose to adapt the surveillance protocol to meet the objective of monitoring the prevalence in at-risk areas.

## Contact networks between cattle herds: structure and contribution to bovine tuberculosis transmission

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**Keywords:** bovine tuberculosis, network analysis, badger-cattle interface

Bovine tuberculosis transmission may be represented by contact networks with nodes as epidemiological units and links as interactions allowing the transmission of *Mycobacterium bovis* between these units. We analyzed the implication of four contact networks in tuberculosis transmission between cattle farms of south-western France where several *M. bovis* strains (molecular types) circulate in a multi-host system. Using data from 1946 cattle farms (nodes) of known infection status, we built four empirical networks based on cattle trade data (trade network) and on spatial neighborhood relationships between farms, either direct (pasture neighborhood network) or badger-mediated, when two farms neighbored the same badger home range (cattle-badger network), or when two farms neighbored two distinct but neighboring badger home ranges (badger-badger network). Each network was described and its association with *M. bovis* transmission was assessed using a non-parametric test. A logistic regression model allowed evaluating the relative contribution of each network to *M. bovis* infection risk for the direct neighbors of infected farms. When merging the four networks, all the infected farms were located in the same component, thus allowing explaining *M. bovis* transmission among these farms. A significant association was observed between the farm infection status and each of the four networks when all the molecular types were simultaneously considered. It was not the case for the trade and pasture networks when considering only cattle-specific molecular types. The logistic regression model showed a significant association between *M. bovis* infection in direct neighbors of infected farms and the connection by trade, cattle-badger and badger-badger links. The corresponding attributable risk fractions were of 12%, 33% and 83%. Our results indicate a multifactorial bTB transmission between cattle farms of the studied area, with varying implication levels of the trade, pasture and badger networks according to the molecular type.

## **Mycobacterium bovis transmission between cattle and free-ranging wild ungulates in Eastern Pyrenees**

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**Keywords:** Tuberculosis, *Mycobacterium bovis*, Cattle, Wildlife, Pyrenees

Between March and April 2015, simultaneous outbreaks of bovine TB affecting 4 cattle herds were detected in Eastern Pyrenees (Catalonia, Spain). After slaughtering tuberculin skin tests positive reactors, tissues with TB-like lesions were collected and standard mycobacterial isolation and identification procedures were carried out. In the 4 herds, *Mycobacterium bovis* spoligotype profile SB0828 ([www.Mbovis.org](http://www.Mbovis.org)) was identified in all isolates. Afterwards, the epidemiological investigation elucidated that the 4 herds shared a common pasture during the last summer. The outbreak area is a woodland of coastal Mediterranean climate with well-preserved wildlife habitats (a Natural park and adjacent mountain areas), without fencing. Free-ranging wild boar, red deer and other ungulates (such as fallow deer and chamois) are abundant in this area. One month later, a red deer was found dead in this area. The necropsy revealed a generalized TB and *M. bovis* SB0828 was also isolated. Since that date to the present, specific culling and sampling of wild ungulates were conducted within the 4 municipalities that defined the TB focus area. Head (submandibular and retropharyngeal) and pulmonary (bronchial and mediastinal) lymph nodes were collected when possible, and subsequently examined for presence of TB lesions and mycobacterial culture. From May 2015 to March 2018, 5 out of 16 sampled wild boar showed TB lesions, 3 of them showed positive culture results (2 *M. bovis* SB0828 and 1 *M. bovis* SB121). In addition, another red deer without visible lesions showed a positive culture (*M. bovis* SB0828). We present here, evidenced of a multi-species TB outbreak in Eastern Pyrenees involving cattle, wild boar and red deer. The Eurasian wild boar and red deer are considered the main wild reservoirs of TB in the Iberian Peninsula. Nevertheless, only a few sporadic cases wild TB have been reported in Catalonia, and this is the first case of TB in red deer of the last decade. Even though some wildlife TB cases have been reported in French Atlantic Pyrenees, co-localized with bovine TB outbreaks, to our knowledge, this is the first reported case with a clear epidemiological relationship between infected cattle and wildlife in the Mediterranean Pyrenees. Measures for controlling emergence of TB at the wildlife/livestock interface in Mediterranean regions need to be addressed and surveillance plans in wildlife reinforced.

## **Tuberculosis outbreak in Montseny Natural Park involving free-ranging wild boar and domestic goats**

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**Keywords:** Tuberculosis, *Mycobacterium caprae*, Goats, Free-ranging wild boar

In 2012, the wildlife health surveillance plan started in Catalonia by sampling serum during the hunting seasons from various tuberculosis (TB) susceptible species. A cluster of 2 out of 4 seropositive wild boar was detected in 2013 in a municipality of the Montseny Natural Park, one with a dense population of wild boar. Later in 2015 and 2016 samples of lymph nodes with TB-compatible lesions were submitted from two different game meat processing facilities within the area through SESC (the slaughterhouse support network: [www.cresa.cat/blogs/sesc](http://www.cresa.cat/blogs/sesc)). Both were confirmed as *Mycobacterium caprae* (spoligotype SB0415). The whole Natural Park and the adjacent natural area of Guillerics were established as a geographical focus area for intensified sampling of wild boar lymph nodes to assess the extent of the outbreak. The main source of tissue samples were game meat processing rooms (either through active sampling by the agriculture department staff or submission of TB compatible lesion through SESC, i.e. passive surveillance, by meat inspectors). During the hunting seasons 2015-16 and 2016-17 a total of 148 wild boar lymph node samples were analyzed by gross lesion evaluation and, in case of a positive or non-conclusive result: Histopathology, Ziehl Neelsen stain, direct PCR, mycobacterial culture and DVR-spoligotyping was also performed. A total of 14/148 samples had TB compatible lesions, 12 of which were confirmed as *M. caprae* (spoligotypes SB0415 n=11, SB1908 n=1) giving an estimated prevalence of 5.8 to 15.2% (95% confidence interval) in the studied region. All cattle herds in the focus area were tuberculin skin test negative, so a recommendation was issued to investigate small ruminants, particularly caprine herds. During the 2017-18 season 44 caprine herds of the focus area were tested and two of them had skin test reactors which were confirmed later by necropsy and further analysis of TB compatible lesions, and *M. caprae* (SB0415) was isolated. Four additional wild boar with TB lesions were also confirmed in the area during that season (SB0415 n=2, SB1908 n=2). Even though it has not been possible to establish the source of the outbreak, the molecular identification of the circulating strain indicates a direct or indirect interaction between goats and wild boar. Follow-up surveillance on the area will be performed to assess whether removing the infected goats reduces TB prevalence in the wild boar population or, instead, is able to maintain the infection in the region.

## Searching for lagoviruses in Flemish hares

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**Keywords:** hare, lagovirus, EBHS

Different lagoviruses are known in rabbits and hares in Europe. Next to EBHSV, also RHDV2 and non pathogenic HaCV have been found in European brown hares (*Lepus europeus*). In Flanders, information about the prevalence of lagoviruses in hares is marginal. We screened livers from healthy hares, hunted in 2004 and 2017. Liver tissue samples from hares shot at random locations in Flanders were collected *secundum artem* in a game processing plant in 2004 (n=22) and 2017 (n=16), and frozen at -20°C until analysis. A sandwich typing ELISA based on the use of specific MAbs and capable of distinguishing between EBHSV and RHDV2 with a specificity >98%, was run. One single sample (1/16; 6%) from the 2004 series was EBHSV positive whereas all hares sampled in 2017 tested virologically negative. No other lagoviruses were detected. Antibodies against lagoviruses were searched on liver homogenates, using IgG-ELISA (cross reactive lagovirus test) and cELISA specific tests respectively for EBHSV and for RHDV2. For the antibody search, the use of liver homogenate instead of serum (when not available) decreases the sensitivity (factor 8-16). This means that samples negative at the first dilution of 1/40 could correspond to a serum titer range between neg-1/320. The IgG-ELISA on liver homogenates resulted in 50% with titers equal or below the dilution 1/40 for the 2004 series. Conversely, the 2017 series showed 100% positives with medium titers (from 1/80 to 1/1280). By using the more specific cELISA, the 2004 samples showed 32% EBHS antibody positives with titres from 1/10 to 1/80 whereas in 2017 only 12% of sera resulted positive with very low titre (1/10) close to the threshold value. In hunter shot healthy hares, the absence of ELISA detectable virus was as expected. Nevertheless, the 2004 sample showing a weak positive signal for EBHSV, could reveal a hare having survived acute EBHS at least 4-8 days before shooting. Antibody detection showed a clear difference between the 2004 and the 2017 results: IgG ELISA indicates a general high circulation of lagovirus, possibly a non-pathogenic one such as HaCV, in Flemish hare populations in 2017, but cELISA EBHSV suggests a higher circulation of EBHSV in 2004. Taken together with the EBHSV positive liver, a circulation of EBHSV with a high population susceptibility is probable for the period 2002-2004. These findings, together with the apparent phylogenetic evolution of lagoviruses in Europe (emergence of RHDV2) underline the need for continued surveillance in order to monitor the population health and immunity of hares and rabbits.



## Estimating Apicomplexan parasite exposure in Icelandic arctic foxes (*Vulpes lagopus*)

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**Keywords:** arctic carnivore, *Toxoplasma gondii*, immunoblot analysis

The arctic fox (*Vulpes lagopus*) is the only native terrestrial mammal in Iceland. The population comprises both “coastal” and “inland” fox ecotypes, with regard to food resources. While coastal ecotype foxes mainly feed on sea birds and eggs, invertebrates and marine mammal carcasses, the inland foxes feed on ptarmigans, migrating waterfowl, eggs and wood mouse. Because of the relatively low biodiversity within arctic ecosystems and the involvement of the species in both marine and terrestrial ecosystems, Icelandic arctic fox population could serve as sentinels for overall ecosystem health of Iceland. It was demonstrated that coastal arctic foxes have higher levels of mercury, helminth burden and richness compared to the inland populations, indicating that the two ecotypes are quite separate and distinct. However, the presence of Apicomplexan parasites has not been reported in Icelandic foxes, yet. Using immunoblot analysis, we tested serum samples from 37 arctic foxes for the presence of antibodies to *Toxoplasma gondii*, *Neospora caninum* and *Besnoitia besnoiti*. A seroprevalence of 72.9% to *Toxoplasma gondii* antigens was found, whereas no antibodies were detected against the other two Apicomplexan parasites. There was no difference in exposure between gender and ecotype groups, however adult foxes had a significantly higher seroprevalence than juveniles (90.9% and 46.6%, respectively). Compared to previous studies from Svalbard, Canada and from Medny Island (51.7%, 40% and 5%, respectively), the seroprevalence in Icelandic arctic foxes is the highest. Since the seroprevalence in human population is low (10%), further studies on the ecology and epidemiology of *Toxoplasma gondii* in Iceland are warranted.

## Identification in European hare of new RHDV2 recombinant virus

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**Keywords:** hare, lagoviruses, recombination

Rabbit haemorrhagic disease (RHD) is a highly contagious and acute hepatitis of the European rabbit, caused by a lagovirus (RHDV). In 2010, a new phylogenetically and antigenically distinct virus, RHDV2, was identified in both rabbits and four hare species. Recently, the sequencing of the entire genomes of RHDV2 strains shown the occurrence of multiple recombinant events. To investigate the occurrence of recombination in Italy we amplified and sequenced a region over the RdRp-VP60 junction, of ~30 RHDV Italian RHDV2 strains between 2012-2015, including the first RHDV2 strain identified in 2012 in hare (RHDV2\_Bg12). Positive samples were originating from wild, domestic, rural and industrial farmed rabbits from whole Italy, including those detected from 2012 in Bergamo province, where the RHDV2-positive hare was found. This area is characterized by a high density of hares for hunting purposes, some industrial and many rural rabbits farms, and few confined close populations of wild rabbits. Overall, we identified four RHDV2 recombinant strains from rabbits, all detected on 2015 in Central Italy. These strains showed a recombination event with a RHDV-G1 strain and a very high nucleotide identity i.e. about 98%, for vp60 and p58 with the recombinant strain identified in Algarve on 2014. By analyzing the complete genome sequence of RHDV2\_Bg12, the sole Italian RHDV2 identified in brown hare till today, the vp60 gene sequence showed an 97.2% identity (98.4% at amino acid level) with UD11 strain the first Italian RHDV2 identified in farmed rabbits in 2011. Moreover, the nucleotide identity of the genomic region upstream the vp60 gene, showed an average of 86% to EBHSV, 70% to RHDV, RHDV2 and RCV and 81% to HaCV. Phylogenetic and SimPlot analysis performed with the lagovirus sequences present in GenBank, showed a recombination event between the non-structural proteins of an EBHSV-like virus and the structural protein of RHDV2 virus. In order to determine the nature of the "parental" lagovirus involved in the recombination event, by using RT-PCR with specific primers designed on the p37 gene (NSP region) of RHDV2\_Bg12, we examined 19 EBHSV and 11 HaCV strains identified in hares in Italy from 2012 onwards, but we didn't get any positive amplification, making the nature of such virus apparently not referable to any known lagovirus. The occurrence of recombination among RHDV2 strains, as shown in other European countries, was confirmed, but we also identified a new lagovirus generated by recombination between RHDV2 and a still unknown hare lagovirus. The origin of RHDV2\_Bg12 remains indefinite but the lack of further detections in hares and rabbits suggests a likely origin outside Italy followed by an accidental introduction. In this case, albeit viable, the emerged new virus was not able to persist in the lagomorph populations, likely due to its low fitness more than to the absence of susceptible hosts. In conclusion, the ability to generate new genomes and viable lagoviruses throughout recombination mechanisms, even between pathogenic and non-pathogenic lagoviruses, could help to understand the genetic mechanism underpinning the emergence of new strains/variants/serotypes with different tropism, fitness and virulence, as assumed for other caliciviruses.

## **Brucella melitensis shedding in Alpine ibex: age and sex heterogeneity**

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**Keywords:** epidemiology, transmission, bacteriology

In Europe, brucellosis has been eradicated in domestic ruminants from most countries (including France) and wild ruminants had not been considered important reservoirs so far. However, a high seroprevalence of *Brucella melitensis* infection (38% in 2013, 95% CI [28.2; 47.8]) has been identified in a French population of Alpine ibex (*Capra ibex*), after the occurrence of brucellosis in 2012 in a dairy cattle farm and two human cases [1]. Determining the routes of transmission and the categories of individuals that present the most infectiousness is of utmost importance for efficient disease control [2]. To reach these goals, assessing the health status of individuals based on serological assays is not sufficient. Instead, it is necessary to assess within-individual pathogen distribution to infer a shedding pattern and individual heterogeneity in infectiousness [3]. Our study aimed to (i) identify possible transmission routes based on *Brucella* distribution in organs, (ii) determine the frequency of bacterial carriage in seropositive individuals and (iii) analyse the potential variations of excretion with sex and age. Test-and-cull and selective culling operations allowed to sample 127 seropositive ibex between 2012 and 2017, among which 88 were necropsied and bacteriologically analysed. A total of 516 culture results were obtained from organs with a shedding potential or with gross lesions. These results were compatible with the existence of four possible transmission routes, as described in domestic ruminants: horizontal transmission through urogenital excretion, vertical transmission in utero, pseudo-vertical transmission through milk consumption, and venereal transmission. Among 88 seropositive ibex, 58% were positive for at least one sample, and were considered as having active infection. In *B. abortus* naturally-infected cattle, proportions of active infections in seropositive animals have been reported between 45 and 50%. Using generalised linear mixed models, we found that the probability of positive bacteriological culture was at the highest level for the youngest animals in our sample (i.e., 2 years old) and then decreased with age in both genders. *Brucella* infection often leads to abortion following the first infection, with high shedding in associated urogenital excretions. Our results thus suggest that the probability of bacterial shedding should be at the highest in young females, possibly corresponding to abortion during the first pregnancy following infection, and should decrease during subsequent pregnancies, as reported in domestic ruminants. The probability of positive culture was slightly higher in males than in females, probably as a result of anatomic/physiological differences among sexes. This description provides a better understanding of disease epidemiology, which is critical for future development of management strategies. In our case, targeting young seropositive females may increase the efficacy of test-and-cull strategies since they are the ones the most at risk of shedding *Brucella*.

## **TSE found in a Finnish moose *Alces alces***

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**Keywords:** *Transmissible spongiform encephalopathy, moose, Finland*

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative prion diseases of mammals. The only TSE known in wild animals is chronic wasting disease (CWD) which affects several cervid species. CWD has occurred in North America for decades but it was only recently, in 2016, found in a wild reindeer *Rangifer tarandus* in Norway, making it the first case of CWD in Europe. The finding and subsequent further Norwegian cases raised concern in European countries with wild and semi-domesticated cervid populations susceptible to CWD. In Finland, CWD testing has been part of general wildlife disease surveillance since the early 2000's but annual sample sizes have been small (10-20). Increased surveillance of CWD targeting dead and diseased cervids, including road-kills and predator-killed individuals, has been implemented in Finland according to the EU Commission Regulation 2017/1972 starting in 2018. The target species are the semi-domesticated reindeer, the wild forest reindeer *R. tarandus fennicus*, the moose *Alces alces*, the roe deer *Capreolus capreolus* and the white-tailed deer *Odocoileus virginianus* which was imported from the USA in the 1930's. In late January 2018, a female moose was found dead in forest in a remote location in eastern Finland, ca. 10 km from the Russian border. The nutritional state appeared poor. The head of the moose was submitted to the Finnish Food Safety Authority Evira for CWD sampling and pathological examination. The age of the moose was determined from cementum annuli of an incisor to be ca. 15 years. The obex and retropharyngeal lymph nodes were analyzed using a commercial enzyme-linked immunosorbent assay test (IDEXX HerdChek Bovine Spongiform Encephalopathy-Scrapie Antigen Test Kit, EIA). The test was strongly positive for the obex but negative for the lymph node. Western blot (Bio-Rad) assay that was performed as confirmatory test was also positive for the obex. A sample of the obex was sent to the TSE EU reference laboratory (Animal and Plant Health Agency, Weybridge, UK) for final confirmation. The result was TSE which was not similar to the North American CWD. This is the first case of TSE in Finnish cervids. The export of live cervids from Finland was banned after the confirmation of TSE but other measures have not been implemented as yet. Further studies on the prion isolate and the disease status of the cervid populations in the affected area are needed to evaluate the significance of the finding.

## Chronic Wasting Disease surveillance programme for Sweden 2018-2020

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**Keywords:** CWD, Sweden, surveillance

The surveillance of CWD in Sweden has since the first finding of CWD Europe in 2016, in wild reindeer and moose in neighbouring Norway, mainly focused on reported clinical suspicions and submitted fallen or euthanized diseased cervids. So far, no positive case of CWD has been found among the just over 400 cervids tested. EU Commission regulation 1972/2017 amending EC Regulation No 999/2001, is the regulatory basis for how a three-year surveillance programme for chronic wasting disease in cervids in Estonia, Finland, Latvia, Lithuania, Poland and Sweden, is to be carried out during the years 2018 - 2020. These member states have populations of moose or reindeer. Other cervid species to be tested are red deer, roe deer, and white-tailed deer. A minimum number of 6 000 animals are to be tested, with half the number of samples from wild and semi-domesticated deer, from 100 randomly selected primary sampling units (PSU) throughout the country. The other half are to be from 100 PSU with farmed deer. As the minimum number of samples is the same for all countries involved, the sensitivity of the surveillance will vary between countries with different cervid populations. But the regulation allows for some national adaption. In Sweden, there are large populations of wild moose and roe deer, fewer wild red deer (in all, approximately 650 000) spread over most, or the entire country. Limited to the northern half of Sweden, the reindeer herding area contains 250 000 free-ranging semi-domesticated reindeer, that share the area with wild cervids of various densities and distribution. There are around 5 000 farmed red deer in just over 100 farms. Dividing the samples between farmed cervids in one category and wild and semidomesticated in the other, would mean sampling of 60% of all farmed red deer in Sweden, whereas only 0.03% of wild cervids together with reindeer, would be screened at the minimum surveillance level. The sampling plan for 6 000 cervids has therefore been adapted to proportions more representative for Swedish populations. All deer farms with red deer will in total test 500 animals. From 50 PSU covering the entire country, 2 750 wild cervids will be tested, and from 50 PSU covering the entire reindeer herding area, 2 750 semi-domesticated reindeer will be tested. If CWD is present in Sweden, we assume it may occur at a low prevalence and maybe clustered. Therefore, cervids from the entire land area are included for testing and not only randomly selected areas. Higher risk areas for possible presence of CWD, with present knowledge, such as proximity to the Norwegian border, is also taken into consideration when creating the PSU areas. By focusing on testing animals with a higher risk of being affected by CWD (e.g. fallen animals, roadkills), we increase the possibility to find a positive case. The funding available is only for the minimum level of the EU regulation surveillance, this may limit the possibility to detect the disease in Sweden if it is present at a very low level.

## **A multi-disciplinary approach to investigations of supplementary salt-licks as transmission hot-spots for CWD and endoparasites in Norway**

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**Keywords:** CWD, prions, endoparasites, reindeer, ungulates, sheep, salt-lick stones

“Classic” chronic wasting disease (CWD) has recently been diagnosed in the Nordfjella subpopulation of wild reindeer in Norway. To avoid spread of the disease, the subpopulation has been depopulated and the area will be let fallow for a long period before reintroduction of a new herd. Even so, infectious prions may still persist in the environment. This presents a risk of spread to surrounding wild ungulate populations, free-ranging domesticated sheep, and other animals. Most importantly it poses a potential reinfection risk for reintroduced reindeer. Risk of prion transmission may be further increased by presence of salt-lick stones in these areas. Salt-lick stones are used by farmers and reindeer herders both for supplemental nutrition and as a gathering point for easier monitoring and/or collection of their animals. However, these sites are also used intensively by wild ungulates. Even after removing the salt stones, the salt in the soil of these areas remain attractive to ungulates for years. These areas, therefore, represent potential hot-spots for disease transmission, both directly between animals and indirectly through environmental contamination. Although supplementary feeding for wild ungulates has been banned throughout the country, salt-lick stones for free-ranging sheep in mountain areas are still present. Our current CWD knowledge is derived from North America, where ungulate species, climate and environment, and wildlife management strategies differ greatly from that of Norway. Both regions have limited knowledge regarding the environmental transmission of prions and other pathogens, such as endoparasites. This presentation will describe our newly started multi-disciplinary project which aims to define the importance of salt-licks for disease transmission in Norway. This study is taking place in three representative Norwegian reindeer areas, one of which is the CWD-infected Nordfjella. These areas encompass a variety of habitat and land-use types. We will use camera traps on active salt-lick areas to monitor which species are visiting the stones. Using both camera traps and current/historic data from GPS collared reindeer, we will determine the frequency and duration of animal visits to these sites and how this may vary spatially and temporally. Soil samples will be collected from both sites with a stone present and sites where the stone has been removed to analyse for presence of prions. Because we do not expect a high level of prions to be present, we will also analyse these samples for the presence and intensity of endoparasites. These parasites are common pathogens of ungulates and, thus, could be used as a proxy for prion transmission. In addition, these soil samples will be analysed for mineral content, which may indicate how attractive these areas are even after salt-lick stone removal. To perform this project, we have gathered a team of researchers with expertise on a range of topics, including veterinary science, parasitology, prions, soil science and general and spatial ecology of ungulates. Disease transmission in the wild is a multi-faceted problem, and we believe such a multi-disciplinary approach is vital to produce scientific insights to these problems.

## **Not CWD: Diagnoses from cervid heads submitted for CWD surveillance in Sweden**

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**Keywords:** *Cervid, Surveillance, Pathology*

Surveillance for Chronic Wasting Disease (CWD) was resumed in Sweden after CWD was diagnosed in wild reindeer in 2016 in Norway. Between June 2016 and April 2018, brain and retropharyngeal lymph nodes from 334 moose (*Alces alces*), 28 roe deer (*Capreolus capreolus*), 26 reindeer (*Rangifer tarandus*), 13 red deer (*Cervus edaphus*), and eight fallow deer (*Dama dama*) have been analyzed for CWD. Samples were taken from all adult cervids submitted to the general wildlife disease surveillance program at SVA, either as full carcasses, or as only the head of, animals found dead or euthanized because of poor body condition, abnormal behavior or other signs of disease. Sixty of the samples were taken from heads from presumed healthy moose hunted in the region adjacent to the Norwegian county where three positive CWD cases in moose had been found. All analyzed Swedish samples were negative for CWD. A post-mortem examination was carried out on a subset of the submitted heads from animals with signs of disease. In 88 of 272 examined cases (32%), a diagnosis that could be associated with the clinical signs or constitute the cause of death was found during postmortem examination of all heads. In moose, 28 had cataracts, 13 had ethmoid tumors, 12 had dental attrition or other inflammatory or degenerative changes in the oral cavity, two had lymphoma and 2 had non-suppurative encephalitis. Cataracts had resulted in partial or total blindness. Ethmoid tumors had eroded parts of the skull and affected the brain. The cause of the non-suppurative encephalitis has not yet been determined. One moose with lymphoma had such enlarged lymph nodes around the pharynx that it had had difficulties swallowing, which resulted in emaciation. Additional incidental findings in the investigated moose heads included *Chorioptes* spp. in ears and nasal bots (*Cephenemyia ulrichii*) in the pharynx. In roe deer, eight had dental attrition or other inflammatory or degenerative changes in the oral cavity and one had an osteoma in the nasal cavity. In reindeer one had an ophthalmitis. In red deer, one had dental attrition due to malocclusion and one had osteomyelitis in the jaw. In fallow deer, there was one case with dental attrition, one meningioma, one non-suppurative encephalitis, one keratitis, and one lymphoma. In the fallow deer with the lymphoma, the infiltrative tumor also compressed the optic chiasm, resulting in blindness. In one third of the investigated cases an informative diagnosis was obtained from the examination of the submitted heads. Extended investigation of heads in association with sampling for CWD has thus contributed to general wildlife disease surveillance and diagnostics.

## **Monitoring of the Rabbit Hemorrhagic Disease virus 2 (RHDV2) epidemics in European wild rabbit (*Oryctolagus cuniculus*) in Andalusia (Spain), 2013-2017.**

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**Keywords:** Wild Rabbit, RHDV2, Epidemiology.

Rabbit hemorrhagic disease (RHD) is a highly infectious and fatal disease of the European rabbit (*Oryctolagus cuniculus*), caused by a virus belonging to the genus *Lagovirus* (family *Caliciviridae*). In 2010, the new pathogenic RHD virus (RHDV2) variant emerged in France, affecting both domestic, even vaccinated for classical RHDV subtype, and wild rabbits. Subsequently, RHDV2 was identified in other European countries. Here, we describe the spatio-temporal evolution and main findings of the RHDV2 epidemics in wild rabbits in Andalusia (Southern Spain) between 2013 and 2017. By the beginning of summer 2013, abnormal mortalities were detected in wild rabbit populations in Southern Spain. An emergence health program was launched by the Regional Government of Environment of Andalusia. A total of 190 death rabbits from 75 affected hunting states and protected areas were collected during the period 2013 and 2017. The most common clinical symptoms and lesions were: epistaxis, hemothorax and petechiae of internal organs. Mortality was found in rabbits of different age classes including kittens. Liver samples were sent to National Reference Laboratory for RHDV (Central Veterinary Laboratory at Algete, Madrid, Spain) for diagnostic analysis. RNA-RHDV2 was confirmed in 185 of the 190 analysed rabbits (97.4%) using RT-PCR. Phylogenetic analysis of two RHDV2-positive samples showed the highest nucleotide identity (96-97%) with RHDV2 strains previously detected in Portugal. The spatio-temporal distribution of the RHDV2 outbreaks was not homogeneous. The first outbreak was observed on June 2013. Since then, the number of cases sharply increased between 2013 and 2014, while a decreasing tendency was observed during the following years. Mortality was found along the year and peaked in winter (95/185; 51.4%) and spring (59/185; 31.9%). Most of outbreaks were located in western part of Andalusia. Cases confirmed during the last five years suggest an endemic circulation of the new RHDV2 variant in wild rabbit populations in Spain. The results constitute an important step for understanding RHDV2 emergence and spread in this country and will provide valuable information for the development of surveillance programmes in Europe.



## **Tapeworms on the rise. Increasing prevalence of *Echinococcus multilocularis* in eastern Austria**

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**Keywords:** *Echinococcus multilocularis*, *alveolar echinococcosis*, *vulpes vulpes*, fox

Larval stages of the fox tapeworm *Echinococcus multilocularis* cause one of the most serious helminthic diseases of humans in the northern hemisphere. Final hosts are foxes (*Vulpes vulpes*), furthermore racoon dogs (*Nyctereutes procyonoides*), wolves (*Canis lupus*) and racoons (*Procyon lotor*). Domestic dogs and cats may also rarely serve as final hosts whereas rodents are intermediate hosts. Regional hot spots of this disease in western and middle Europe are Switzerland, Germany, Austria and France. From 2014-2017 we investigated 275 dead foxes, using the shaping and scraping method for demonstrating the tapeworm. The mean prevalence reached from 24% (2014) up to 39% (2017) with peaks as high as 48% in some regions. Compared to data from 2003 when only 10% foxes were found positive in similar regions, we demonstrate a marked increase in the prevalence of the fox tapeworm in eastern Austria in recent years, which mirrors the increased incidence in humans, with up to e.g. 15 reported clinical cases in 2015. We were able to demonstrate increase of this serious zoonosis in Austria. It is very important to know about spread and prevalence of this disease as well as to inform exposed persons, as hunters, loggers and farmers.

## Molecular detection of human pathogenic *Leptospira* in small mammals

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**Keywords:** *Leptospira*, small mammals, human health

Pathogenic *Leptospira* are zoonotic agents and cause leptospirosis worldwide. Currently, 22 genomospecies of these gram-negative bacteria are known and can be subdivided into a pathogenic, a non-pathogenic and an intermediate group. Numerous mammals, including domestic and companion animals, can be infected by leptospires, but rodents and other small mammals are considered as the main reservoir. Human leptospirosis outbreaks occur sporadically in temperate zones. For example, field fever outbreaks in strawberry pickers, due to infection with *Leptospira kirschneri* serovar Grippotyphosa, were reported in 2007 and 2014 in Germany. Our investigations focused on identification of the most commonly occurring *Leptospira* genomospecies, sequence types and their small mammal hosts in Europe and German military bases in Afghanistan. PCR-based analyses resulted in the identification of the genomospecies *Leptospira kirschneri*, *Leptospira interrogans* and *Leptospira borgpetersenii*. Common voles (*Microtus arvalis*) in Germany and Spain were infected with *L. kirschneri*. Furthermore, sequence type (ST) 110 was exclusively detected in common voles and field voles (*Microtus agrestis*) in Germany. This ST was the causative serovar in the previously observed leptospirosis outbreaks in Germany. Similarly, Norway rats in Europe were exclusively infected with *L. interrogans* ST 17. In contrast, DNA of more than one genomospecies was detected in bank voles (*Myodes glareolus*), yellow-necked mice (*Apodemus flavicollis*) and common shrews (*Sorex araneus*). In addition, leptospires were also identified in house mice (*Mus musculus*) at the military bases in Afghanistan. Therefore, results of our analyses confirm a broad geographical distribution of *Leptospira* in small mammals and suggest them to be of important public health relevance in human settlements. Currently, we are evaluating several real-time PCR assays for the detection of leptospires and other human pathogenic agents in Europe transmitted by rodents. Perspectively, we intend to design a diagnostic platform, which enables a comprehensive and timesaving molecular bed-side and pen-side diagnostics of *Leptospira* and other pathogens causing similar symptoms.

## The host age related occurrence of *Alaria alata* in wild canids in Latvia

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**Keywords:** *Alaria alata*, *metacercariae*, *adult trematodes*, *wild canids*, *Latvia*

The trematode *Alaria alata* has a complex life cycle where carnivore mammals may become infected by feeding on mesocercariae infected second intermediate hosts or paratenic hosts. Afterwards, young flukes migrate through various organs of the definitive host, including the diaphragm and the lungs, before reaching the small intestine. The aim of the present study was to establish the prevalence and infection intensity of *A. alata* in carnivore mammals and host age related occurrence of different *A. alata* development stages in particular animals in Latvia. Overall, samples from 992 animals (539 red foxes, 411 raccoon dogs and 42 grey wolves) were examined using sedimentation and counting techniques to observe the presence of *A. alata* metacercariae and adult trematodes in the particular host. The present study, based on data collected over five hunting seasons (2010/2011-2014/2015), is a comprehensive study on the circulation of *A. alata* in wild canids in Latvia. *A. alata* adult trematodes were found in 855 animals (88%). All sampled host species were found to carry *A. alata* adults with a prevalence reaching 92.9% in grey wolves followed by foxes (87.4%) and raccoon dogs (83.9%). The highest mean intensity was detected in raccoon dogs (755.0±118.7) and a significantly ( $p<0.05$ ) lower mean intensity was observed in foxes (193.1±18.2). *A. alata* metacercariae were detected in 216 animals (25.6 %) and the prevalence was significantly higher in raccoon dogs (49.5%;  $p<0.0001$ ) followed by red foxes (10.7%) and only one grey wolf was found to be infected with five *A. alata* metacercariae. A significantly higher ( $p<0.05$ ) mean intensity was found in raccoon dogs compared to other animals. A significant positive correlation was observed between the intensity of *A. alata* metacercariae and adult trematodes in raccoon dogs ( $r_s=0.27$ ,  $p=0.001$ ) and red foxes ( $r_s=0.17$ ,  $p=0.0002$ ). A significantly higher ( $p=0.003$ ) *A. alata* metacercariae mean intensity was observed in juvenile raccoon dogs compared to that observed in adult raccoon dogs and red foxes from both age groups. There were no significant differences found in the prevalence of *A. alata* adult and metacercariae between different host sexes. However, a significant positive correlation was observed between raccoon dog population density and adult *A. alata* prevalence ( $p=0.01$ ) as well as the intensity of *A. alata* metacercariae ( $p=0.007$ ) per game administrative units. The results of present study show that *A. alata* is endemic in Latvia and all the wild canid species present in Latvia are highly susceptible to *A. alata*. A number of features of the biology and host/parasite relationship of *A. alata* were identified, such as host age dependent parasite intensity, load of both *A. alata* development stage specimens present in the definitive hosts, and the relationship between host population density and occurrence of parasites. Hence, understanding the life cycle of this kind of parasite is essential to identifying the infection routes for definitive and paratenic hosts and the parasite transmission in natural conditions.

## **Malignant lymphoma in lesser hedgehog tenrec (*Echinops Telfairi*) – Case series**

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**Keywords:** *Echinops telfairi*, malignant lymphoma, surgery

In this report, we present clinical significance in the treatment of malignant lymphoma in three specimen of closely related Lesser Madagascar hedgehog tenrec (*Echinops telfairi*) diagnosed during the seven years period. At the initial presentation all animals were in good clinical condition, however a soft, subcutaneous, oval shaped masses 11 mm x 13 mm on the left (patient #2), on the right (patient #3) and on both sides of the submandibular neck area (patient #1) were detected. Hematology, biochemistry investigations and fine needle aspiration of masses were performed. In all three patients, cytology results were consistent with diagnosis of malignant lymphoma. In patient #1, hematological variables were within reference values; however in other two patients marked lymphocytosis was detected. All other clinicopathological values were within physiological range. In patient #1, excisional biopsy of tissue mass was performed. Histology and immunohistochemistry analysis confirmed the diagnosis of malignant lymphoma with possible involvement of thyroid tissue and no other treatment was performed. This patient died four years after surgery due to the malignant mass in the abdomen not connected with lymphoma. Patient #2 and #3 were treated with methylprednisolone in a dose of 2 mg/kg orally every 24h for 24 weeks and four cycles of doxorubicin in a dose of 1 mg/kg injected intraperitoneally every six weeks. Tumors were slowly reducing in size and a complete remission was detected after third and fourth cycle of chemotherapy in patients #2 and #3, respectively. No tumor growth was seen in two years after the last therapy in both of these patients.

## **Toxoplasma gondii and Neospora caninum in wild boars: seroprevalence and potential biosecurity implications in areas with different levels of animal productions**

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**Keywords:** Wild boar, *Toxoplasma gondii*, *Neospora caninum*

The protozoa *Toxoplasma gondii* and *Neospora caninum* can cause relevant economic losses on small domestic ruminants and cattle, respectively. Furthermore, *T. gondii* may represent a serious issue for human health through the consumption of raw/undercooked meat or manipulation and handling of infected carcasses. In addition to domestic animals, many wild species can have a role in those protozoa lifecycles and, among wildlife species, wild boars could have a relevant importance due to their scavenger behaviour and omnivorous diet. Moreover, the increasing density of wild boars populations and their distribution, even in close proximity to pastures and farms, should raise the importance for a more focused monitoring to assess the potential risk for these protozoan infections. A sero-epidemiological investigation was carried out on wild boars' populations from Northwest Italy (province of Brescia) in order to evaluate: (i) the spread, (ii) the spatio-temporal dynamics of those infections within populations from areas characterised by different levels of large animal productions. Overall, 1516 sera samples were collected during three hunting seasons (n=number of samples), 2015-2016 (n=460), 2016-2017 (n=519), 2017-2018 (n=537), from wild boars of three macro-areas characterized by different levels of farms density: low (n=705), medium (n=193) and high (n=618). Sera were tested for *T. gondii* and *N. caninum* antibodies with an indirect ELISA (kit ID VET®, Grabels, France). Data were analysed through logistic regression. Average *Toxoplasma gondii* seroprevalence was 22.56%. Wild boars from the low farm density area were significantly less infected (13.90%) than individuals from both high (30.91%,  $p<0.001$ ) and medium (27.46%,  $p<0.001$ ) ones. Wild boars showed a significantly low seroprevalence in the hunting season 2016-2017 (19.08%) than in hunting season 2015-2016 (24.78%;  $p=0.008$ ) or in hunting season 2017-2018 (24.02%,  $p=0.010$ ). Overall, *N. caninum* antibodies were detected in five out of 1516 (0.46%) samples. Diffusion of *T. gondii* and *N. caninum* was very different within the studied wild boars sub-populations. The sporadic presence of *N. caninum* suggests that wild boars do not to play a relevant epidemiological role in the studied areas; conversely, *T. gondii* seems rather widespread. *Toxoplasma gondii* showed an increased seroprevalence in areas with high and medium density of farms. It is reasonable to hypothesize that this difference in seroprevalence is mostly attributable to the higher anthropization of those areas. Moreover, the large presence of pig smallholders (>60% of total pig farms) may further increase potential risks, which roaming wild boars can pose, in terms of biosafety. These findings highlight importance of biosecurity measures in farms (e.g. fencing, pest control) located in areas where wild boards are present. Differences in seroprevalence among hunting seasons should be interpreted cautiously. Although they could reflect yearly variations of *T. gondii* spread, this study timespan was limited. During the next hunting seasons, systematic monitoring should be implemented and annual sampling should be improved, in order to drawn solid evaluations on the role of wild boards in *T. gondii* epidemiology and as a zoonotic risk source in human-domestic-wildlife interface.

## Post-mortem findings in free- ranging European Brown Hares (*Lepus Europaeus*) from Schleswig-Holstein, Germany

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**Keywords:** *Lepus europaeus*, post-mortem examination, coccidiosis

For several years, hunters in Schleswig-Holstein, Germany have observed a decrease of European brown hares (*Lepus europaeus*) associated with an increasing number of diseased animals. In 2017, 111 post-mortem examinations on 60 hunted free-ranging hares from three hunting grounds (33 females and 27 males) and 51 deceased hares from all over Schleswig-Holstein (22 females and 29 males) were performed. From each of the three hunting grounds, 20 animals were chosen and blood samples were taken immediately after death. During necropsies, selected samples were collected for histopathology. Furthermore, routine bacteriological investigation of small intestine and lungs as well as parasitological screening of faecal samples were performed. Additionally, we screened for European Brown Hare Syndrome Virus (EBHSV), Rabbit Haemorrhagic Disease Virus Type 2 (RHDV-2) and *Francisella tularensis* (tularemia). Liver tissues were negative for tularemia (tul4-PCR, culture), EBHSV and RHDV-2 (PCR, antigen-ELISA). No antibodies for *Francisella tularensis* (SLA) were found [n=57]. Further serological testing for EBHSV- and RHDV-2-specific antibodies will be performed. Besides hunting-related injuries and agonal alterations, histopathological results included findings such as lympho-histiocytic, granulomatous or purulent hepatitis, hyperplasia of mesenteric lymph nodes and spleen, lympho-plasmacellular or granulomatous enteritis, granulomatous-necrotizing steatitis as well as interstitial nephritis and pneumonia. The parasitological analyses of 107 hares demonstrated marked intestinal coccidiosis in almost all cases (99%). Additionally, gastrointestinal strongylids (65%) and *Trichuris* spp. (8%) occurred in some individuals. *Yersinia pseudotuberculosis* [n=2], *Salmonella* sp. [n=1], *Pasteurella multocida* [n=1], *Staphylococcus aureus* [n=4] and *Yersinia enterocolitica* [n=4] were cultured during bacteriological investigations. In three hares serological and in at least two hares antigen (culture) analyses for *Brucella* sp. were positive. Furthermore, *Escherichia coli* were detected in many hares. This project contributes to the advancement in preventive protection of public health and food safety. A higher sample size is needed to verify our results. In future investigations non-infectious noxae should be included to elucidate the causes of the hunting bag decline of European brown hares in Schleswig-Holstein.

## **Preliminary study on gastrointestinal parasite community of urban brown rats (*Rattus norvegicus*), Vienna, Austria**

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**Keywords:** *Rattus norvegicus*, *Capillaria hepatica*, *Hymenolepis* sp.

**Background** Brown rats (*Rattus norvegicus*) have invaded all continents except Antarctica. Anthropogenic pressure is modifying the environment, including inter alia: urbanization, higher temperature, habitat fragmentation, and increased impervious surface cover. Brown rats are highly adapted to urban settings. They thrive on human garbage and benefit from improper waste management. Rats can serve as reservoir of zoonotic pathogens. Via their urines and faeces, they not only contaminate the environment, but also human and animal food. **Objectives** The study explored the gastrointestinal parasite community of urban brown rats in Vienna, Austria. Contamination rates of children playground were also investigated. **Materials and Methods** Live-trapping of *R. norvegicus* was conducted in Vienna, Austria, from 06/03/2017 to 20/06/2017. Each rat was sexed, aged, and fresh faeces were sampled from the rectum and stored in 10% formalin. Public sandpits on ten children playgrounds were investigated. Five samples per sandpit were collected both in June and October 2017. Sugar flotation method was performed on fecal and environmental samples. Statistics tests were used to explore the data. A network approach was used to investigate multiple parasite relationships. **Results** Fifty rats were captured. Eggs of three helminth species were identified from the faeces: *Capillaria hepatica* (22/50 rats, 44%), *Heterakis spumosa* (40%), and *Hymenolepis* sp. (26%). Oocysts of *Coccidia* spp. were observed in 66% of the samples. Twenty-six rats (52%) shed eggs (or oocysts) from more than one parasite species, among them, the mean number of parasite species was  $2.7 \pm 0.8$  (range = 2-4). The mean number of parasite species was not different between males and females but it was significantly higher in adults when compared to juvenile rats. Prevalence of *C. hepatica* was significantly higher in adult rats. Age and gender did not influence *Hymenolepis* sp. prevalence. The network analysis revealed no significant overall parasite association (observed connectance: 0.62,  $p=0.73$ ) but infestation with *H. spumosa* was significantly associated with infestation with *C. hepatica* and *Hymenolepis* sp. None of the environmental samples contained eggs from gastrointestinal parasites. **Discussion** We report two species of veterinary relevance (*Coccidia* spp. and *H. spumosa*) and two potentially zoonotic species (*C. hepatica* and *Hymenolepis* sp.) in which transmission to humans occurs via ingestion of eggs present in contaminated soil, water, or food. We show that adult rats represent a higher risk of environmental contamination with *C. hepatica*. Capillariasis and hymenolepiasis induce mild symptoms in humans, they are not considered as a major public health issue in Vienna, although homeless people may be at a risk. Within the rat host, the associations *H. spumosa*/*C. hepatica* and *H. spumosa*/*Hymenolepis* were significantly over-represented, probably due to the similar routes of transmission. Public children playgrounds presently represent a low risk of infestation. Further studies are planned to investigate the seasonality of excretion and variations in parasite communities. Genetic methods are required to explore if human cases are epidemiologically linked with rat parasites. Prevention of capillariasis and hymenolepiasis must focus on the control of rat-populations, particularly adult individuals.

## **Avoiding ruffled feathers – methods of reducing injuries and stress during waterfowl capture for disease sampling.**

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**Keywords:** *Waterfowl, animal welfare, banding, catching, injury, mortality rate, ringing, trapping*

Wild birds have been captured for a variety of reasons, by various techniques, over many centuries, for e.g. food, clothing, domestication, removal of pest species, and scientific study. For the latter, such studies include ringing (banding) waterfowl to allow estimates of survival, productivity, emigration, and immigration, and are therefore central to the development of population models on which sound conservation management can be based. The assumption that capture for ringing does not significantly affect the birds is essential, because it is the basis for generalising the data to unmarked birds. The Wildfowl & Wetlands Trust (WWT) has been capturing waterfowl for scientific study since its inception in 1946, and has changed and refined capture methods used, to improve their effectiveness and reduce the risks of injury and stress to birds captured. This is important both ethically and to ensure high-quality data. The emergence of several highly pathogenic avian influenza (HPAI) viruses in wild birds has resulted in increased disease surveillance and related research of waterbirds. The original wild bird H5N1 HPAI outbreaks in Asia and Eastern Europe in 2005, European Union (EU) Member States increased levels of live bird surveillance for avian influenza (AI) viruses in wild birds. Between autumn 2005 and December 2010, WWT significantly increased capture efforts and sampled nearly 19,000 live captured birds for AI under contract to the Department of Environment, Food and Rural Affairs (DEFRA). Birds were captured with the use of cage traps, duck decoys, swan pipes, cannon netting, and roundups. Buccal and cloacal swab samples were taken, and screened for AI viruses at the European Reference Laboratory, Weybridge, UK. These methods of capture are often regulated in countries with national ringing or banding programmes and are considered to be safe, and thus justifiable given the benefits to conservation. However, few published studies have addressed how frequently injuries and mortalities occur, or the nature of any injuries. In this study, rates of mortality and injury during captures with the use of these methods carried out by the WWT as part of conservation programmes were assessed. The total rate of injury (including mild dermal abrasions) was 0.42% across all species groups, whereas total mortality was 0.1% across all capture methods. Incidence of injury varied among species groups (ducks, geese, swans, and rails), with some, for example, dabbling ducks, at greater risk than others. This presentation will describe techniques used before, during, and after a capture to reduce stress and injury in captured waterfowl.



## Being a European or not: Searching for the reservoir of zoonotic Variegated squirrel bornavirus 1

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**Keywords:** squirrel, bornavirus, zoonosis

The Variegated squirrel bornavirus 1 (VSBV-1) was found to be associated with the death of three German squirrel breeders through a fatal encephalitis. Real-time reverse transcription-polymerase chain reaction (rt RT-PCR) analysis of about 800 squirrels of 18 species resulted in the identification of VSBV-1 positive individuals of the subfamilies Sciurinae and Callosciurinae. The highest prevalence was observed for Variegated squirrels (*Sciurus variegatoides*) and Prevost's squirrels (*Callosciurus prevostii*). Red squirrels (*Sciurus vulgaris*) from Germany and Grey squirrels (*Sciurus carolinensis*) from UK were all found negative. Due to the detection of closely related VSBV-1 sequences in squirrels of five species and two subfamilies, there are two alternative hypotheses about the origin of the virus: 1) VSBV-1 does also exist in Central America/Asia and was imported to Europe by one of the exotic squirrel species 2) VSBV-1 is endemic to Europe and a still unknown reservoir host spreads the virus to squirrels. Therefore the objective of our ongoing investigations is to identify the geographic and host origin of this zoonotic virus. For this purpose we are currently collecting squirrel samples from South East Asia (*Callosciurus* spp.), Central America (*Sciurus* spp.) as well as from introduced populations of such squirrels, and will screen them by pan-bornavirus rt RT-PCR and VSBV-1 specific rt RT-PCR. In addition we will analyse small mammals from all over Germany and in particular from zoological gardens and the surroundings of squirrel breedings for infections with this novel bornavirus.

## Epidemiological investigation on erysipelas in wild boars: spread, isolation and potential impacts at the wildlife-domestic-human interface

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**Keywords:** *Sus scrofa*, serology, *Erysipelothrix* spp.

The genus *Erysipelothrix* was originally associated with the species *Erysipelothrix rhusiopathiae* (ER) which may pose zoonotic and economic concerns due to its wide range of domestic and wild hosts. Furthermore, *E. tonsillarum* (ET) was described and differentiated from ER by fermentation of saccharose, serological and molecular techniques, and the lack of pathogenicity for pigs. Despite erysipelas outbreaks occur regularly among domestic animals, the source of infection is seldom investigated. The increasing popularity of animal friendly production systems leads to concerns of outbreaks becoming more prevalent in the future. Although domestic pigs are considered the main reservoir of *Erysipelothrix*s, many wildlife species could have a role in the spread of the infection and wild boar (*Sus scrofa*) have been suggested as an healthy host. Moreover, the increase of wild boar densities, the expansion of its ranges even in proximity to farms and the little information available about the distribution of the infection in this species should be considered. The need is therefore to investigate if wild boars could represent a risk of erysipelas at the wildlife-domestic-human interface. Wild boars from Northwest Italy were analysed to evaluate (i) the seroprevalence of ER and (ii) the prevalence of *Erysipelothrix* spp. isolates to disclose (iii) the epidemiological factors associated with the presence and the transmission of the infection. During two hunting seasons (2015/2016 and 2016/2017) 971 sera samples were collected from 8 macro-areas with different density of pig farms. Moreover, during 2016/2017 overall 223 wild boar tonsils were collected from the high and low density pig farms macro-areas. ER seroprevalence was evaluated through a commercial ELISA kit while *Erysipelothrix* spp. were isolated and confirmed by Gram stain, cell morphology, catalase activity, and H<sub>2</sub>S production on triple sugar iron agar medium. Results from serology and microbiology were analysed by multivariate logistic regression. An overall ER seroprevalence of 59.63% (579/971) emerged. Wild boars from high density pig farms macro-area showed the highest seroprevalence (71.4% (298/417),  $p < 0.0001$ ): in this macro-area the probability to be seropositive was 11.4 (6.05-24.5) times higher than in others. *Erysipelothrix* spp. was isolated from 58 wild boars (26.01%). No significant association were found between isolates and study factors. Partial 16S sequencing was performed on 10 of 58 isolates: 7 were ER while 3 were identified as ET. The increased seroprevalence in the high density pig farms macro-area could support the hypothesis of a relation between the seroprevalence of wild boars and the presence of farms, although the role of wild boar density cannot be ruled out. Further statistical analyses would be performed to deepen this aspect. However, as 60.41% of farms in this macro-area belongs to smallholders, wild boars could represent a biosecurity risk. Wild boars may pose zoonotic risks through ER infected carcasses while the importance of ET in erysipelas diagnosis emerged considering its lack of pathogenicity for pigs. Comparative whole-genome sequence analysis of isolates originating from wild boars and domestic pigs is ongoing and will provide further clues to better evaluate the potential economic and zoonotic risks related to wild boars.

## High prevalence of cephalosporin resistant Enterobacteriaceae with zoonotic potential in wildlife of Catalonia.

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**Keywords:** *bacteria, multiresistant, beta-lactamases*

In the last decades, an increase of opportunistic and antimicrobial resistant (AMR) bacteria associated with nosocomial infections has been observed in hospital settings. The overuse of antibiotics in human and veterinary medicine have led to the spread of AMR pathogens, becoming a global public health problem. Wildlife may serve as sentinel for assessing the level of AMR environmental contamination. The aim of this study was to determine the prevalence of AMR bacteria in wildlife of Catalonia. Between November 2016 and May 2017, faecal samples of 261 animals from 67 different species (birds=154, mammals=95, reptiles=12) were plated in blood agar. Only samples growing as pure culture were further characterised for antimicrobial susceptibility testing and also plated on MacConkey agar with ceftriaxone (1mg/L). Cephalosporin resistant (CR) genes were detected in isolates coming from the selective media. Pure cultures growing in blood agar were observed in 31% (81/261) of samples, identifying *E. coli* (43/81), *Citrobacter* spp (8/81), *Enterobacter* spp (6/81), *Proteus* spp (6/81), *Klebsiella* spp (5/81), and *Salmonella* spp (5/81). Most of these isolates exhibited resistance to ampicillin (86%), cephalexin (68%) and streptomycin (57%). In addition, 43% (35/81) of these isolates were CR *E. coli* (49%), *C. freundii* (23%), *K. pneumoniae* (17%) and *E. cloacae* (9%). Out of the 35 CR isolates, 20 harboured at least one CR gene: 52% CMY-2 (*E. coli*, *C. freundii*, *E. cloacae*), 28% SHV (*K. pneumoniae*, *S. enterica* subsp *diarizonae*), 12% CMY-1 (*K. pneumoniae* and *Providencia* spp), and 8% CTX-M (*E. coli* and *Providencia* spp). Additionally, 5 isolates contained two CR genes; two *K. pneumoniae* (CMY-1 with SHV), two *C. freundii* (CMY-2 with SHV) and one *Providencia* spp (CMY-1 with CTX-M). The most frequent SHV and CTX-M types were; SHV-12 and -28 and CTX-M-15 and -M-14 respectively. Most of the CR genes were detected in wild mammals (55%), principally in hedgehogs where 15% of the total analysed (6/41) were carriers of CR genes, followed by two American minks, and one individual of each, a beech marten, a European roe deer and a wild boar. The remaining positive isolates (45%) were detected in wild birds from different species, some of them migrant (tawny owl, Sardinian warbler, goldfinch, common blackbird, European serin, Eurasian collared dove and yellow-legged gull). In conclusion, wildlife in close contact with urban and farming areas of Catalonia carries a huge variety of zoonotic bacteria genetically resistant to cephalosporins with similar resistant genes to those found in livestock and human hospitals. Wildlife can contribute indirectly to the dissemination of resistance genes into natural areas increasing the prevalence of these genes in natural environments.

## **Ulcerative enteritis associated with *Clostridium perfringens* in an American kestrel (*Falco sparverius*)**

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**Keywords:** *Kestrel, Enteritis, Clostridium perfringens*

An American kestrel (*Falco sparverius*) died three days after exhibiting clinical signs of progressive weight loss, vomiting and lethargy. The post-mortem examination revealed mild to moderate emaciation, distended intestine with brown fluid and multiple small ulcers in the mucosa, especially prevalent in the proximal intestine. Histopathological examination evidenced severe necrosis of the enterocytes and villi with occasional transmural necrosis of the intestinal wall accompanied by fibrinosuppurative inflammation and the presence of rod-shaped bacteria scattered throughout the intestine. *Clostridium perfringens* was isolated from the intestine in pure culture on anaerobic media. Gram stain showed Gram positive bacilli and immunohistochemistry for *C. perfringens* also revealed positive bacteria lining the villi of the intestine. To the author's knowledge this is the first report of ulcerative enteritis associated with *C. perfringens* in a kestrel.

## **Cryptosporidium spp. and Giardia duodenalis in wild ungulates: zoonotic risk from the Alps?**

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**Keywords:** Wild ungulates, zoonoses, intestinal protozoa

Cryptosporidium spp. and Giardia duodenalis are ubiquitous parasites able to infect a wide host range, including humans, wild and farming animals, with severe zoonotic, economic and ecological concerns. The aim of this study was to investigate on presence of these protozoa in wild alpine ungulates as a potential source of transmission for humans and livestock. The investigation has been carried out in both hunting grounds and protected areas from three non contiguous territories: the first two areas lie in South-Eastern Alps in (i) Lombardy (Lecco and Sondrio provinces) and in (ii) Veneto (Belluno province); the last is located in North-Western Alps in (iii) Piedmont (Verbanco-Cusio- Ossola province). Faecal samples were collected from the rectum of animals culled during the regular hunting activity and a specific population control plan of red deer in Sondrio province. In protected areas samples were collected from the ground after localization of animal groups. From 2013 to 2015 faecal samples were collected from 348 chamois, 437 red deer (only from Piedmont and Lombardy) and 119 roe deer (sampled only in Piedmont). Samples were analysed by an immune enzymatic test for the detection of copro-antigen of Cryptosporidium spp. and G. duodenalis (RIDASCREEN® Cryptosporidium – RIDASCREEN® Giardia). Positive faecal samples with a sufficient amount of material, have been subsequently analysed through PCR in order to identify Cryptosporidium species and Giardia assemblages. In Veneto chamois showed a G. duodenalis prevalence of 4.4%, while in Lombardy a prevalence of 0.8% and 7.0% in red deer and chamois respectively was detected. In these areas all animals tested were negative to Cryptosporidium infection. In Piedmont G. duodenalis had a prevalence of 4.5%, 1.5% and 8.4% in chamois, red deer and roe deer respectively. Cryptosporidium showed a prevalence of 1.2%, 0.5% and 3.4 % in chamois, red deer and roe deer respectively. No animal was infected simultaneously by Cryptosporidium spp. and G. duodenalis. Concerning species and assemblages, isolates from chamois and roe deer clustered within the zoonotic genotype C. ubiquitum. As regard to G. duodenalis, the zoonotic assemblage A was detected in chamois and red deer. Both the protozoa detected are characterized by strong environmental survival, and the higher prevalence of G. duodenalis could be explained through the longer emission by infectious animals. Although the low prevalence recorded for both protozoa suggests a low risk of infection, considering the zoonotic species and assemblage detected, a potential role of wild ungulates as source of infections for humans and livestock cannot be excluded. In this sense, focused studies are desirable mainly in areas showing high densities of wild ungulates together with positive trend of human outdoor activity and livestock breeding.

## Free-ranging red deer contribution to environmental contamination of Shiga toxin-producing *Escherichia Coli* in Italian alps

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**Keywords:** *Red deer, Shiga toxin-producing Escherichia coli, antimicrobial resistance.*

Wild ungulates are increasing their density and distribution across Europe. At the same time, habitat fragmentation caused by human activities and infrastructures favours proximity with humans, livestock and other animal species. This critical issue for public health and for conservation, highlights the need for ecological studies of multi-host infections within an eco-evolutionary framework. Red deer (*Cervus elaphus*) is among the most representative species of such patterns: in the Stelvio National Park (SNP, central Italian Alps), for example, very high population density is associated with intense browsing impacts and cross transmission of pathogens with domestic ruminants. In turn, a culling plan has been initiated in the Lombardy sector of the Park, with the aim to reduce red deer density: this led to the introduction of some 37.3 tons of meat into the food chain over just 5 years, between 2011 and 2016, with potential consequences on human health. Among food-borne pathogens, herbivorous are the main reservoir of Shiga toxin-producing *Escherichia coli* (STEC). The microorganism is shed in feces and can persist in the environment, rising the risk of ingestion, intestinal colonization and fecal shedding in increased numbers of grass-feeding animals. This study aims to evaluate the prevalence, antimicrobial susceptibility and spatial distribution of STEC in red deer in the Lombardy sector of the SNP in January and February 2017. The analysis was based on the collection of feces from 65 red deer from three culling areas with different anthropization levels (low, moderate, high), for which radio-tracking showed low or absence of deer movements among areas. Feces were enriched into modified Tryptone Soya Broth, and DNA was extracted for stx genes PCR. Positive samples were plated on Levine-eosin methylene blue agar and 20 single *E. coli* colonies were tested by PCR to confirm STEC identification and evaluate the presence of eaeA gene. The isolates were tested for their susceptibility to 12 antimicrobial agents by disc diffusion. Twelve of 65 feces were positive for STEC, showing a prevalence in red deer of 18.4% (95% CI:10.8-29.5), with strains harboring stx2 (n=7) or stx1 (n=5). No strain carried both stx genes and eae gene was never detected. STEC positive calves were only detected in high anthropised area. The STEC isolates were resistant to at least one of the antimicrobial agents tested, mostly ampicillin. The present study showed that red deer shed STEC in environment during winter, with prevalence similar to cattle. Additional samples and characterization of STEC serotypes are needed to highlight the effects of environmental and human-mediated factors influencing the infection dynamics.

## Zoonotic *Campylobacter* species in sympatric wild and domestic herbivores from alpine ecosystems in the Pyrenees

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**Keywords:** Pyrenean chamois, *Campylobacter* spp., Livestock

The transmission of zoonotic pathogens at the livestock-wildlife interface is becoming a global issue of growing interest for human health and food safety. In recent years, the incidence of human campylobacteriosis has increased worldwide and as a result, *Campylobacter* is regarded as one of the most important food-borne pathogens. In alpine ecosystems, where wildlife and seasonal livestock share the same habitat and resources, farmers, hunters and consumers are concerned about the effect of this coexistence. However, the risks of transmission and the epidemiological role of these animals regarding zoonotic pathogens have been scarcely studied. The present study aims at assessing the occurrence and genetic relationship of thermophilic *Campylobacter* spp. across four herbivores species sharing the same grasslands in an alpine and subalpine ecosystem. From 2015 to 2017, fresh faecal samples were collected from Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) (n=83) and free-ranging livestock (n=73 cattle, 39 sheep and 30 horses) in the alpine and subalpine grasslands of the Freser-Setcases National Game and Wildlife Reserve (eastern Spanish Pyrenees). Samples were kept at 4°C until cultured (<48h) for *Campylobacter* spp., using mCCDA selective agar. *Campylobacter* identification at species-level was carried out by PCR and the genetic diversity was assessed by *flaA*-RFLP. *Campylobacter* was not isolated from Pyrenean chamois, whilst *C. jejuni* was isolated from 16 cattle (21.9%; CI95%: 13.9-32.7) and 3 sheep (7.7%; CI95%: 2.7-20.3). *C. coli* was recovered from 2 cattle (2.7%; CI95%: 0.8-9.5). Of these, one cow was co-infected with both species. A high genetic diversity was observed among isolates, since almost all *Campylobacter*-positive individuals carried a single and unique genotype. Certain host specificity was observed, as isolates from the same host species clustered together at different similarity levels, except a *C. jejuni* isolate from a sheep showing >90% similarity with a cow isolate. Livestock in the studied area is a reservoir of *Campylobacter* spp. and can spread enteric zoonotic bacteria of public health concern in the environment. Therefore, the contaminated pastures may constitute a source for the dissemination of these organisms. However, so far it seems that this has no effect on Pyrenean chamois, highlighting a possible spatial behaviour adjustment to avoid pastures when livestock is present, or a low contact rate with contaminated grasses due to the wide-open areas present in this habitat.

## Antimicrobial resistance of enteric bacteria in Eastern chimpanzee (*Pan troglodytes schweinfurthii*) and Olive baboon (*Papio anubis*) from Budongo Forest, Uganda.

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**Keywords:** antimicrobial resistance, *Pan troglodytes*, Uganda

The transmission dynamics of bacteria of public health concern among humans, livestock and wildlife is still poorly understood. Moreover, the World Health Organization has identified antimicrobial resistance (AMR) as a global threat to public and environmental health, including wildlife conservation. In fact, the increasing incidence of AMR in humans and livestock is suggested as the origin of AMR emergence in wildlife, and this is especially concerning for some resistance traits, such as cephalosporin resistance. In order to assess the anthropogenic effect as a risk factor for the presence of AMR in wildlife populations, *Escherichia coli* isolated from 62 Eastern chimpanzees (*Pan troglodytes schweinfurthii*) and 23 olive baboons (*Papio anubis*) were analysed for the presence of AMR against common antibiotics. Three communities of chimpanzees exposed to different degrees of anthropogenic pressure were sampled: Waibira (n=19) and Kaniyo-pabidi (n=6), with scarce human contact and Sonso (n=37), which regularly show crop-raiding behaviour in neighbouring local human communities. The baboons analysed inhabited areas around Budongo Conservation Field Station, being in indirect contact with human beings in the research camp. Samples were kept at 4°C until cultured (<48h) for *E. coli* in MacConkey agar with and without ceftriaxone (2 mg/L, a third-generation cephalosporin antibiotic). Antimicrobial susceptibility testing was performed in all *E. coli* isolates recovered from non-selective media. Minimal inhibitory concentration was performed on those isolates obtained in MacConkey agar with ceftriaxone, followed by PCR for the detection of cephalosporin resistance genes. The chimpanzees from Sonso carried *E. coli* with antimicrobial resistance against ampicillin (5.41%; CI95%: 1.5-17.1), sulfamethoxazole (10.8%; CI95%: 4.3-24.7), gentamicin (2.70%; CI95%: 0.5-13.8) and tetracycline (5.41%; CI95%: 1.5-17.1), while *E. coli* isolates from Waibira and Kaniyo-pabidi did not present resistance against these antibiotics. Interestingly, one chimpanzee from Waibira (4.55%; CI95%: 0.8-21.8) was resistant to ceftriaxone. Three chimpanzees from Sonso carried *E. coli* with antimicrobial resistance against two (n=1) and three (n=2) antibiotics. Resistance against chloramphenicol, enrofloxacin or ceftazidime were not detected in any of the isolates from chimpanzees. *E. coli* of baboon origin exhibited resistance against ampicillin (40.00%; CI95%: 21.9-61.3), sulfamethoxazole (36.84%; CI95%: 19.2-59.0), tetracycline (40.00%; CI95%: 21.9-61.3), chloramphenicol (5.00%; CI95%: 0.9-23.6), and ceftriaxone (42.11%; CI95%: 23.1-63.7). Eight isolates from baboons, including four isolates resistant to ceftriaxone, were multiresistant, defined as resistant to three or more families of antimicrobials. The present study confirms that, as expected, anthropogenic activities, i.e. livestock and/or proximity to humans are associated with the occurrence of AMR in wildlife. Predicting the spread of AMR at the human-livestock-wildlife interface in a "One Health" approach and understanding the underlying factors driving persistence of resistance should be the focus of future studies. Additionally, these results demonstrate the vulnerability of wild endangered species to pathogens of human and livestock origin.



## **Epidemiology of zoonotic *Campylobacter* at Gough Island (South Atlantic): the role of brown skuas (*Catharacta antarctica*)**

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**Keywords:** *Campylobacter*, public health, seabirds

Thermophilic *Campylobacter* spp. is a major cause of foodborne enteric disease worldwide. These bacteria colonize the intestines of a broad range of animal hosts that usually act as asymptomatic carriers, which can spread the pathogen in the environment. Brown skuas (*Catharacta antarctica*) are one of the major scavenging species in the Southern Ocean and thus, a seabird more likely to carry zoonotic agents than those feeding on marine prey. Gough Island is one of the most remote islands lying in the South Atlantic Ocean, about 2,700 Km from Cape Town. It is uninhabited, but the South African National Antarctic Program has continually maintained the personnel of a weather station on the island since 1956. Although human waste on Gough is currently not made available to the fauna of the island, enterobacteria may have been previously spread by scavenging seabirds in the past. We surveyed the presence of thermophilic *Campylobacter* in adult brown skuas present in the island at the onset of the breeding seasons, in September/October 2009, 2016 and 2017. Cloacal swabs were obtained from 112 skuas and *Campylobacter* was isolated using conventional culture methods. Species identity was determined by PCR and the genetic diversity of isolates was determined by PFGE-SmaI genotyping. Isolates were also compared with those from other localities of the Southern Ocean. Potential spreading of pathogens by skuas from Gough Is. was studied by deploying geolocators in 2009 to track their annual movements. We found a high variability in the frequency of *Campylobacter* species along the three sampling periods: 73% (2009), 4% (2016), 20% (2017), although the 2016 low prevalence was probably due to insufficient amount of sample collected. *C. lari* was by far the most frequent species, but *C. jejuni* was also isolated. A high diversity of genotypes was found, with each bird carrying a single and unique genotype. However, we found some similarities among isolates from 2009 and 2017. Also, same pulsotype was found among skua isolates from Gough and Falklands or Livingston Islands, and between a skua from Gough Is. and a domestic duck from Falkland Is. However, further (ongoing) analyses are needed to confirm these findings. Annual movements of skuas from Gough Is. showed these birds either stay at Gough or migrate to the western coast of South Africa. Overall, these results suggest skuas can spread *Campylobacter* across the Southern Ocean, contributing to the global spread of this zoonotic agent.

## Seasonal influence in parasite communities of feral cats *Felis catus*, in Gran Canary Island (Spain)

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**Keywords:** *Feral cats, Parasites, Zoonosis*

Gran Canaria Island is called the miniature continent due to its different weather conditions in just 1560km<sup>2</sup>. It has 21 municipalities, from the cloudy areas of the north to the dry and sunny regions down south, with the wetter midlands up to the Pico de Las Nieves right in the center. This weather diversity produces great contrast on the isle landscape, its flora and fauna and has an impact on the presence and development of feline parasites. The aim of this study was to determine the prevalence of parasites in feral cats in Gran Canaria. Two hundred and forty samples were collected from 30 different locations across the island from January to December 2017. Samples consisted on a pool of fresh feline faeces taken from the ground where uncontrolled groups of feral cats lived. The samples were analyzed within 24-48 hours of collection. Flotation techniques were used with NaCl solution and 33% Zinc Sulfate Solution, Formol-ether concentration method, Modified Mc Master test and, Baermann Test. Nematodes, Cestodes and Protozoa were present in the different samples analyzed. Nematodes were the most numerous parasites detected (48.3%). The hookworms, *Ancylostoma* spp., were the most abundant (29.6%), followed by lungworms: *Aelurostrongylus abstrusus* and *Troglostrongylus* spp. (23.75%). Roundworms (*Toxocara cati* and *Toxascaris leonina*) were isolated in 14.16% of cats studied. Protozoa was the second most isolated taxa, (24.16%): *Giardia* spp. (12.5%), *Cystoisospora* spp. (11.6%) and also *Entamoeba* spp. In 4/240 (1.6%) and *Toxoplasma gondii* in 2/240 (0.8%). The species of tapeworms identified in 47/240 samples (19.58%) were members from the family *Dipylidiidae* 41/240 (17.08%), followed by *Taenia* spp. 6/240 (2.5%). Interestingly, high number of samples showed multiple infections, mainly by two species (68/240), being the most common combination *Ancylostoma* spp. and lungworm in 45,6%. Moreover, 20 samples presented a combination of three parasites, such as *Dipylidiidae*, *Ancylostoma* spp. and lungworms. Considering the municipalities and the season, data were analyzed in order to elucidate if parasites could be under seasonal influence. According to the area of provenance, those with more probabilities of precipitation during the year showed higher numbers of lungworms and hookworms. Thus, the municipalities of the north and center of the island presented higher levels of infection. Nevertheless lungworms were also found in some areas on the northeast and the east, but only in extremely humid seasons (winter and spring). Protozoa were observed most frequently from October to May anywhere. Tapeworm infections were more frequent in the east and southeast locations, probably due to the greatest resistance of their dissemination elements. In general, a smaller number of parasites was observed on the south-east and south coast of Gran Canaria, where the temperature is higher and the humidity is lower all year round. In conclusion, our data suggest that weather conditions affect the distribution of feline parasites not only geographically, even seasonally. Epidemiological studies would be necessary to follow different strategies for prevention the risk of zoonosis that some of those parasites represent.

## **A survey on *Campylobacter* spp, *Salmonella* spp, and *Yersinia* spp in faecal samples of hunted wild boars (*Sus scrofa*) in Aosta Valley Region (North Western Italy).**

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**Keywords:** *Campylobacter* spp., wild boar, Aosta Valley Region

In order to assess the risk of contracting food-borne infections from wild game animals meat in Aosta Valley Region (North Western Italy), we investigated the presence of *Campylobacter* (C.) spp, *Salmonella* (S.) spp, and *Yersinia* (Y.) spp in fecal samples of wild boars (*Sus scrofa*) (N=1966) shot over the last three hunting seasons (2015-2018). To detect the three bacterial agents, 75, 50 and 50 animals were analyzed respectively (corresponding to a design prevalence of respectively 4% for C. and 6% for S. and Y.). As regards *Campylobacter* spp, the research was performed by bacteriological examination (culture in Bolton Broth for 24 h followed by CCDA Agar for 24-48 h, in microaerophilia at 42 ° C) with selection of the suspected colonies by Gram staining and oxidase test; the selected strains were identified by sequencing of the 16S rRNA gene with a Micro-Seq 500 system and the obtained sequences were compared with those from the Microseq 500 system data bank and the National Center for Biotechnology Information (NCBI - Rockville Pike, Bethesda, USA) Blast data bank. Detection of *Salmonella* and *Yersinia* was based on PCR Real Time. All samples tested negative for *Salmonella* spp. and *Yersinia* spp., while 19 tested positive for *Campylobacter* (crude prevalence = 25.3%, 95%CI 16.0-36.7). , distributed as follows: 4 *C. coli*, 2 *C. hyointestinalis*, 2 *C. fetus fetus*, 5 *C. lanienae*, 6 *C. spp.* (not further identifiable). In humans, *C. coli*, as *C. jejuni*, is considered an established pathogen for gastroenteritis as well as sometimes meningitis and acute cholecystitis, unlike *C. fetus* (cause of abortion in cattle and sheep) and *C. hyointestinalis* (cause of proliferative enteritis in swine) reported occasionally in cases of sepsis or intestinal diseases (Kaakoush et al., 2015); the pathogenic potential of *C. lanienae* in humans, although isolated from workers in abattoir (Logan et al., 2000) and from food-producing animals (Schweitzer et al., 2011), is currently unknown. The data we have observed agree with those from other more extensive monitoring campaigns performed in the wild boar in Spain (Carbonero et al., 2013) and in Japan (Sasaki et al, 2013), in both of which *Campylobacter lanienae* was found the most isolated species. The contamination of game meat is linked to faecal contamination during the gross evisceration by the hunters on the field, immediately after the killing of the animal. The larger risk of contracting *Campylobacter* infection compared to *Salmonella* and *Yersinia* from wild boar meat in our region is consistent with recent EFSA reports that indicate campylobacteriosis as the most frequent food-borne disease in humans in the European Union (200,000 cases every year), with the chicken products considered as the most likely source of infection.

## **Aural haematoma in a wolf (*Canis lupus*)**

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**Keywords:** *Wolf, Aural haematoma, Treatment*

Case report of the handling and medical treatment of a wolf (*Canis lupus*), showing an aural haematoma. Aural haematomas occur mainly in dogs, but have also been described in other species such as swine, cats and rabbits. The predisposing factors include accidental trauma to the pinna, a history of violent head shaking due to itching, acute or chronic otitis externa of bacterial or parasitic origin and bite wounds, causing fissures. Treatment of this disease normally is done under surgery with general anaesthesia, but this type of procedure may need intensive aftercare. Nonetheless, this can be a problem in wildlife canines due to the importance of reintroducing the animal as quickly as possible to the pack, avoiding the use of protective collar. In this report, we describe an aural haematoma in the left ear of a free-ranging, female wolf (*Canis lupus*), that was the alpha- female of the pack. In the clinical examination, there was inflammation that affected the entire pinna and the surrounded areas. Due to this problem, we decided to immobilize the animal using tiletamine and zolazepam (5mg/kg) as preanaesthetic medication. Later, the patient was prepared with an intravenous heparinised catheter in the cephalic vein. Propofol was used for maintenance. In order to avoid longer recovery, we followed the Kuwahara and Young techniques, aspirating the haematomal fluid in combination with topical and intravenous administration of corticoids. After one month under a combination therapy composed of corticoids and systemic antibiotics, the animal was completely recovered. Six months later, no signs of the disease were seen, but only a small deformation of the external ear was observed. To the authors knowledge, this is the first description of aural haematoma in a wolf (*Canis lupus*).

## Salmonella spp. and Campylobacter spp. in the livestock-wildlife interface in rural Uganda

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**Keywords:** bacterial zoonoses, Uganda, wildlife

Salmonella and thermophilic Campylobacter are the leading causes of human gastroenteritis worldwide. Their presence and transmission dynamics among livestock and wildlife in the African continent is still poorly understood. To assess the presence of these zoonotic bacteria in livestock and wildlife in rural Uganda, we collected fecal samples from domestic and wild animals in a range of geographical areas with different ecological characteristics and different levels of interaction among them and with humans. This included areas within and nearby three National Parks (Mgahinga - MGNP, Queen Elizabeth – QENP, and Murchison Falls - MFNP). Overall 365 animals were sampled in 2015: African buffaloes *Syncerus caffer* (n= 98), Ugandan kob *Kobus kob thomasi* (n= 33), cattle (n= 103), goat (n= 84), chicken (n= 27) and sheep (n= 20). Fecal samples were stored in ethanol 100% for molecular analysis and detection of both pathogens was performed by PCR. Salmonella spp. was detected in 3.6% (13/365) of the analysed samples. Two studied areas were positive for the presence of Salmonella: MGNP (2.6%) and QENP (3.9%). Different prevalences were found among most host species that were sampled in both study areas (MGNP-QENP): cattle (10%-0%), goat (0.0%-25.0%), buffalo (0.0%-1.8%) and chicken (0.0%-0.0%); sheep were only present in MGNP and Salmonella prevalence was 6.7%. Salmonella Typhimurium was identified in one cattle sample. Campylobacter spp. was detected in 21.6% (79/365) of the analysed samples. All three studied areas were positive for the presence of Campylobacter: MGNP (35.1%), QENP (18.1%) and MFNP (10.8%). Different prevalences were found among host species present in all three study areas (MGNP, QENP, MFNP): cattle (45%, 0.0%, 3.0%), goat (18.8%, 25.0%, 40.0%), chicken (0.0%, 16.7%, 0.0%) and buffalo (36.4%, 12.5%, 5.0%). Sheep was only sampled in MGNP and MFNP and Campylobacter prevalence was 93.3% and 20.0%, respectively. Kob was present in QENP and MFNP but only those from QENP were Campylobacter-positive (55.6%). Among the 79 positive samples, we detected *C. jejuni* and *C. lanienae* with similar frequencies (8.9% and 7.6%, respectively). Twelve of the 13 Salmonella-positive samples were also positive to Campylobacter spp. Overall results point to cattle, goat and sheep as main potential sources of Salmonella, while Campylobacter is more widespread in both livestock and wild ungulates. Thus, in rural areas not only livestock but also wildlife may be relevant as a source of zoonotic enteric bacteria and control measures to reduce the risk of infection should focus on both niches.-

## **Serological and molecular investigation of selected bacterial and parasitic pathogens in European brown hare (*Lepus europaeus*); Inferring the Ecological Niche of *Toxoplasma gondii* and *Leishmania infantum* in hares**

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European brown hare is considered a susceptible bioindicator of environmental changes and its epidemiological importance stems for the fact that it maintains the so-called home range thus being prone to many infectious diseases of natural nidality. Besides, its short life span makes it an excellent indicator for recent pathogen transmission in the area and its intensive contact with vectors such as ticks, mosquitoes and sandflies has implicated hare in the epidemiology of vector borne diseases. The epidemiological studies on wildlife are scanty in Greece while to our knowledge, there are no data available on the occurrence of pathogens in the hare population. In this study, the occurrence of infection and/or exposure to *Toxoplasma gondii*, *Neospora caninum*, *Leishmania infantum*, *Brucella* spp, *Coxiella burnetii*, and *Francisella tularensis* was investigated in European brown hares (*Lepus europaeus*) hunter harvested during the hunting periods 2012-2014 in northern and central Greece. From a total of 105 free-ranged hares, 105 serum and 52 liver samples were examined using serological (IFAT and ELISA) and molecular (PCR) techniques respectively. Geographical information system (GIS) together with the Ecological Niche Model (ENM) were used to define the geographical distribution of seropositive hares in relation to environmental parameters and to identify high-risk areas for hare exposure. Molecular analysis showed that 3.8% and 9.6% of the examined hares were *N.caninum* and *L.infantum* infected respectively, while, 5.7%, 0.95% and 12.4% of the hares reacted positively for the presence of antibodies against *T.gondii*, *N.caninum* and *L.infantum* respectively. None of the examined hares was PCR positive for *T.gondii*, *Brucella* spp, *C. burnetii*, and *F. tularensis* DNA or seropositive for *Brucella* spp, *C. burnetii*, and *F. tularensis*. Mixed exposure against both *T. gondii* and *L. infantum* was found in 2.9% of the hares examined. Rainfall indices and land uses were significantly influential for hare exposure to *T.gondii* and *L.infantum*. This is the first molecular and serological survey of protozoan and bacterial pathogens in European brown hare in Greece. Furthermore, we herein report the environmental parameters related to hare seropositivity and we present a risk map for hare exposure to *T.gondii* and *L.infantum* in northern and central Greece.

Hares may significantly contribute to the epidemiology of important pathogens of public health and veterinary concern due to cross border and long-distance movements possibly as part of live animal translocations, as the host of vectors and the prey of carnivores and omnivores that travel in great distances and get into contact with domestic animals and humans. The continuous surveillance of hare populations could provide information on the population health status and the pathogens circulating in the area posing risk for wildlife, domestic animals and humans.