

Contributions to the  
**12<sup>th</sup> Conference of the European Wildlife Disease Association (EWDA)**

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## Foreword

Dear colleagues,

Welcome to the 12<sup>th</sup> Conference of the European Wildlife Disease Association (EWDA) jointly organised by the European Wildlife Disease Association (EWDA) and the Leibniz Institute for Zoo and Wildlife Research (IZW) Berlin. This meeting brings together wildlife specialists of different disciplines from 39 different nations covering all continents to discuss and talk about “*Wildlife health and conservation challenges in a changing world*”. The selected main topics “*Wildlife health – emerging diseases*”, “*Wildlife conservation & management*”, “*Causes and consequences of anthropogenic environmental changes*” and a “*Special focus: Neglected host species*” are envisioned as a base for stimulating and creative discussions and to pave the way for new ideas and international as well as interdisciplinary collaborations.

The conference is preceded by the *EWDA Wildlife Health Surveillance Network Meeting* followed by a number of workshops, which will hopefully already tune everybody into a productive and joyful atmosphere. More than 190 scientific contributions were submitted for the main conference and to allow enough time to all participants whose submission did not fit into the oral presentation schedule to present their work, the poster session got its own format as a *Poster Party* with beverages and light food on Sunday evening. Every day’s programme will start with a plenary talk to introduce the session topic, except for the excursion day on Tuesday when we will leave at lunch to the *Spreeforest biosphere reserve* located 1.5 hours south of Berlin – where also the *EWDA Auction* will take place.

These proceedings contain in their first section the abstracts of oral presentations including the abstracts of invited plenary speakers, followed by the abstracts for poster presentations. Abstracts were printed as submitted. To help you to find abstracts of interest we have included an index of first authors, a taxonomic index, and an index of keywords at the end of the volume.

We thank the Conference Scientific Committee for their help in the preparation of the conference and its scientific programme. We are very grateful to the organisers of the workshops and the session chairs as well as to all the colleagues who have kindly reviewed all submitted contributions.

Berlin, August 2016

Gudrun Wibbelt, Anke Schumann, Steven Seet, Alex D. Greenwood, Heribert Hofer

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## CONTRIBUTED TALKS

### **PLENARY TALK: A global One Health Approach to wildlife surveillance**

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With humans and animals moving around the world at unprecedented rates, new encounters and interactions with wildlife are providing opportunities for repeated spillover events and the emergence of potentially pandemic pathogens. For the past six years, the PREDICT Project of the US Emerging Pandemic Threats Program has been using a One Health approach to standardise surveillance for zoonotic viruses in key wildlife species where spillover events to humans and livestock are likely. Currently active in over 30 countries of Africa and Asia, a global network of wildlife professionals has come together for horizontal and vertical capacity strengthening activities that push field surveillance and laboratory activities closer to where the action is: the developing country settings. With a primary focus on primates, bats, and rodents, the One Health teams are working at key interfaces associated with land conversion, animal production systems, and animal value chain to conduct aligned surveillance in wildlife, livestock, and humans. In the past five years, the PREDICT consortium has sampled over 56,000 wild animals, detected more than 800 novel viruses, and trained over 2,500 people around the globe. Because the project has focused on viral emergence from wildlife in remote areas in some of the most resource-constrained countries, we have by necessity developed a more sustainable approach to viral discovery and diagnosis of mystery illnesses that we have implemented across broad socioeconomic contexts. Employing simultaneous multiple-genus and family level consensus PCR, we have been able to quickly and accurately identify novel viruses, previously undiagnosed as potential pathogens, for further characterisation. Combining cutting-edge surveillance techniques with practical implementation of integrated One Health approaches, the global health community has contributed significantly to inexpensive diagnostic improvements that can benefit wildlife, livestock, and humans, while also realising step-wise improvement in the global knowledge of wildlife virology and the health infrastructure that is essential for early detection and response to outbreaks.

## **Viruses in water: herpesviruses remain stable and infectious for up three weeks**

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The stability of viruses in aquatic environments has been a contentious issue for many years. It is likely that there are many factors contributing to the persistence of viruses in water, however, these environmental conditions differ for each virus. Equine herpesvirus 1 (EHV-1) is a highly pathogenic virus to species in the equine family. However, viruses closely related to EHV-1 have been shown to lack host specificity and have been documented in many species of captive animals (Thompson's gazelles (*Eudorcas thomsoni*), llamas (*Lama glama*), black bears (*Ursus americanus*), polar bears (*Ursus maritimus*) and rhinos (*Rhinoceros unicornis*). As many of these animals never come into direct contact, we postulate that water may act as a vector for EHV-1. In order to establish the conditions promoting or hindering longevity, virulence and genomic stability of EHV-1 in water, we exposed EHV-1 to varied water environments (pH, salinity and sediment) in controlled experiments over 21 days. The presence and infectivity of the virus was confirmed with both RT-PCR and cell culture. Our results show that EHV-1 remains stable and infectious in distilled water for up to three weeks when at higher concentrations. Water with higher pH levels (pH 8-10) increased the length of time that the virus remained infectious by over a week. Our results also establish that when EHV-1 virus particles are in an environment with water and sediment they will associate with the sediment. Different salinity conditions did not have a significant effect on EHV-1 stability; however, our results indicate that environments with higher salinities (35,000 ppm and 3,000 ppm) are more favourable to viral stability. The results from this study challenge the dogma that herpes viruses are unstable in water, and suggest that aquatic environments may be a source of infection for EHV-1 without direct contact among animals, which may apply to other emerging infectious viruses.

## Novel retrovirus associated with ethmoidal tumours in moose (*Alces alces*)

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Ethmoidal tumours in moose (*Alces alces*) cause a typical hole-in-the-head lesion as the tumour mass erodes bone tissue in the upper nasal cavity, often leading to penetration of the ethmoidal and frontal bones. The disease has been noted as sporadic cases since at least a century, but appears to have been found in moose only in Sweden and Norway. The tumours are predominantly carcinomas, originating in the nasal mucosa adjacent to the ethmoidal bone. Viral aetiology as a cause for the tumour formation has previously been suggested, due to similarities with nasal tumour of sheep and goats. Illumina MiSeq high-throughput sequencing technology was used to search for virus in archived frozen tumour tissue samples from 24 necropsied moose cases from Sweden and Norway. Nucleotide sequences related to the enzootic nasal tumour virus of goats, ovine enzootic nasal tumour virus or Jaagsiekte sheep retrovirus were identified in all samples. These results suggest that a virus, tentatively named moose ethmoidal tumour retrovirus, is associated with the ethmoidal tumours of moose. If this virus causes the tumours leading to moose hole-in-the head-disease, it seems to be endemic in the moose population of the Scandinavian peninsula, as this specific type of tumour has not been reported in moose from other countries or continents.

## **Altered immune cell function associated with KoRV infection and season in koalas**

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Wild koala (*Phascolarctos cinereus*) populations are increasingly vulnerable and one of the main threats is chlamydial infection. Koala retrovirus (KoRV) has been speculated to be an underlying cause of the koala's susceptibility to Chlamydia and high rates of lymphoid neoplasia, however the regionally ubiquitous, endogenous nature of this virus suggests that KoRV A infection is not sufficient for immune suppression to occur. A recently discovered exogenous variant of KoRV, KoRV B, has several structural elements that cause increased pathogenicity in related retroviruses and was associated with lymphoid neoplasia in one study. The present study assesses whether KoRV B infection is associated with alterations in immune function. Cytokine gene expression by mitogen stimulated lymphocytes of KoRV B positive (n = 5 - 6) and negative (n = 6) captive koalas was evaluated by qPCR four times (April 2014 - February 2015) to control for seasonal variation. Key immune genes in the Th1 pathway (IFN $\gamma$ , TNF $\alpha$ ), Th2 pathway (IL 10, IL4, IL6) and Th17 pathway (IL17A) along with CD4:CD8 ratio were assessed. KoRV B positive koalas showed significantly increased up-regulation of IL17A and IL10 in three out of four sampling periods and IFN $\gamma$ , IL6, IL4 and TNF $\alpha$  in two out of four. There was also marked seasonal variation in up-regulation for most of the cytokines and the CD4:CD8 ratio. The up-regulation in both Th1 and Th2 cytokines mirrors that seen in human and feline retroviral infection and is associated with immune dysregulation in these species. IL17A is an immune marker for chlamydial pathogenesis in the koala and increased expression of this in KoRV B positive koalas, along with immune dysregulation, may explain the differences in susceptibility to chlamydia and severity of disease seen between individuals and populations. This is the first report of altered immune expression in koalas infected with an exogenous variant of KoRV

## **Peste des petits ruminants virus transmission trials illuminate important differences in the role of ruminants, pigs and camelids in PPRV epidemiology**

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The recent rapid spread of peste des petits ruminants virus (PPRV) lineage 4 to Northern African and Asian countries bordering Europe poses a high risk of PPRV introduction to the naïve population of susceptible European animal species. PPRV pathogenesis in goats that are highly susceptible to PPRV infection has been thoroughly investigated. However, whether other wild and domestic Artiodactyla species, including camelids, might contribute to PPRV spread is scarcely understood. Nevertheless, this is important for the design of effective vaccination strategies with regard to the planned worldwide eradication of PPRV. For Old World camelids, PPR has recently been considered a novel disease, while the susceptibility of South American camelids (SAC) has never been investigated so far. Various animal trials were conducted to illuminate these gaps in knowledge: Three to six cattle, pigs, SAC and dromedaries were intranasally infected with the recent PPRV strain Kurdistan/2011 in four independent experiments. After PPRV infection, two contact goats were stabled together with the infected animals to examine whether they may transmit PPRV to those highly susceptible animals. Serum, whole-blood, oronasal, conjunctival and faecal swabs were collected in regular intervals and tested for PPRV antibodies, PPRV-RNA and infectious PPRV. Various pathological samples were collected at post mortem examination and examined by RT-qPCR, histopathological and immunohistochemical analysis. The animal trials revealed important differences in the excretion dynamics and pathogenesis of the different Artiodactyla spp. Seroconversion after PPRV infection in five of six SAC demonstrated their susceptibility for PPRV infection for the first time. The study results indicate that cattle and camelids are dead-end hosts for PPRV. Therefore, cattle and camelids probably do not play a role in the transmission and epidemiology of PPRV and do not have to be included in vaccination regimens. In contrast, this is the first study demonstrating that domestic pigs may transmit PPRV to goats and other pigs. Accordingly, pigs are possible maintenance or spillover hosts for PPRV and might play a role in the epidemiology of PPRV. Whether pigs should be vaccinated to facilitate the eradication of PPRV in affected regions has to be further investigated.

## **New insights of Lagovirus infection in wild rabbits and hares**

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Rabbit Haemorrhagic Disease (RHD) and European Brown Hare Disease (EBHS) are similar diseases caused by highly related but phylogenetically distinct Lagoviruses, family Calciviridae. RHDV emerged in mid '80 and during its worldwide spread evolved in six genotypes of which genotype 6 is an antigenic subtype (RHDVa) prevalent in some countries, including Italy. RHDV seems to rapidly co-evolving to match increased disease resistance in rabbits. In fact, in 2010, a new lagovirus having a genetic capsid protein identity with RHDV of "only" 80 %, and able to cause RHD also in vaccinated and new-born rabbits was identified in France. This virus spread rapidly within Europe (but it was also reported in Australia), apparently replacing former strains (RHDV/RHDVa). It has a very distinct antigenic profile and variable mortality rates (5 - 70 %) depending on the viral strains, being the recent ones we found the more virulent, also in experimental condition. Additionally, we showed that RHDV2 causes an EBHSV-like disease in Cape hares in Sardinia and a single case in Italian hares in Sicily. More recently we identified some sporadic cases of RHDV2 in Brown hares both in Spain and Italy. These specific features suggest that RHDV2 could represent a new viral emergence from an unknown source, including a possible species jump of lagoviruses between species of lagomorph genus. In addition to virulent RHDVs, non-pathogenic rabbit RHDV-like viruses (RCVs) have been identified in rabbits in Europe and Australia. The genetic diversity among RCVs varies from non-protective, partially or fully protective strains so that there is a gradient of cross-protection in rabbit populations following their circulation.

Since first description in Sweden (~1980), EBHS distribution has been restricted to Europe, affecting primarily brown hares and, less frequently, Mountain hare and Italian hare, but not other European species such as Iberian hare, Broom Hare and Cape hare. Moreover we proved the natural and experimental susceptibility of Eastern cottontail to EBHSV, occasionally resulting in EBHS-like disease. A single viral serotype of EBHSV is known, but a number of different genogroups were identified. The distribution of EBHSVs suggests that they slowly evolved in their area of origin without any dramatic changes in structure and pathogenicity. As in the case of RHDV, the existence a non-pathogenic EBHSV-like virus was put forward and we recently succeeded in the identification of such non-pathogenic Lagovirus in captive and wild hares, named "Hare Calcivirus" (HaCV). However, the epidemiological meaning of this "new" lagovirus is still to be clarified.

## **Experimental infection with highly and low pathogenic strains of border disease virus in Pyrenean chamois (*Rupicapra p. pyrenaica*) gives light to the epidemiological diversity of the disease**

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Since 2001 epizootic mortalities of Pyrenean chamois (*Rupicapra p. pyrenaica*) have been described in the Pyrenees associated to Border Disease Virus genogroup 4 (BDV-4), being the first description of BDV causing high mortalities in a wildlife population. Different epidemiological scenarios are present along the Pyrenees. Indeed, in the eastern Pyrenees no mortalities have been reported so far despite a BDV is detected since 1996 in healthy chamois. We hypothesised that this apparently low-pathogenic BDV strain is protecting the chamois population against the entrance of a more pathogenic BDV. To demonstrate this, an experimental infection was carried out at the Level-3 Biosafety Laboratory of the IRTA-CRESA (Barcelona, Spain). Fifteen chamois were captured in the wild and divided into two groups (A and B). Group A (n = 7) consisted of two antibody negative (Ab-) pregnant females, two Ab- nonpregnant females, one Ab- male, and two Ab+ pregnant females. This group was inoculated with a highly virulent BDV-4 strain (Cadi-6) isolated from a natural infected diseased chamois. Group B (n = 8) consisted of two Ab- pregnant females, one Ab- nonpregnant female, three Ab-males, and two Ab+ pregnant females. This group was inoculated with an apparently low virulent BDV-4 (Freser-5) isolated from a foetus of a healthy female chamois from the eastern Pyrenees. In Group-A, all seronegative chamois but one developed viraemia which was present up to the end of the experiment (26 days post inoculation). Clinical changes in this group were mainly prostration and lymphopenia and, at necropsy, most of them have different degree of haemorrhagic diathesis. The absence of viraemia and clinical changes in seropositive chamois from this group indicated cross-protection between non-homologous BDV. In Group-B all chamois seroconverted and clearance of the virus was confirmed by PCR. No clinical changes were seen in adult animals from Group B. Interestingly, all foetuses from seronegatives chamois of both groups were found dead at necropsy. The present results demonstrate the pathogenic and immunologic differences between these high and low virulent BDV-4 strains and contribute to the understanding of the different epidemiological scenarios of the disease observed in the last years. This disease can have a huge impact in local

conservation for some populations of chamois. Further knowledge related to the molecular differences among these viruses could help in the future to predict the impact of circulating viruses in a population.

## **Detection of the new emerging rabbit hemorrhagic disease type 2 virus (RHDV2) in European brown hares (*Lepus europaeus*) from Spain and Italy**

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Rabbit hemorrhagic disease virus (RHDV), a member of the genus Lagovirus, causes rabbit hemorrhagic disease (RHD), a fatal hepatitis of rabbits. In 2010, a new RHDV – related virus, called RHDV2, emerged in Europe. In addition to rabbits, this lagovirus causes disease and mortality in *Lepus capensis* (Cape hare) and *Lepus corsicanus* (Italian hare). However, RHDV2 infection has not been reported in European brown hare (*Lepus europaeus*), the most common hare species in Europe. Since arrival of the virus to Italy and Spain (probably in 2011) we have found four cases of mortality in this species caused by RHDV2. These cases had macroscopic and microscopic lesions consistent with RHD or European Brown Hare Syndrome. Macroscopic findings included epistaxis, disseminated visceral haemorrhages, moderate splenomegaly and a discoloured pale liver. Microscopic findings were most severe in the liver in the three hares examined, and consisted in extensive to massive hepatic necrosis with both widespread single-cell coagulative necrosis and multifocal areas of lytic necrosis. Acidophilic bodies also were scattered throughout the sections. There was moderate to marked fatty degeneration of hepatocytes and multinucleate hepatocytes occasionally were seen (8 per 10 high power fields). Also, occasional necrotic hepatocytes containing intracytoplasmic basophilic granules were seen. Two independent ELISA tests, both based on the use of specific MAbs produced against RHDV, RHDVa, RHDV2 and EBHSV were first used in order to narrow down the etiology. Second, the presence of EBHSV and RHDV2 RNA was tested by RT-PCR. We found that different RHDV2 strains caused a RHD-like disease in European brown hares. Our findings expand the known host range of RHDV2 and provide further support for the capability of RHDV2 to infect hosts other than rabbits. They also improve our knowledge about the distribution and epidemiological characteristics of this new lagovirus.

## **Emergence of Rabbit Haemorrhagic Disease Virus-2 (RHDV2) in wild and domestic rabbits (*Oryctolagus cuniculus*) in Sweden**

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Rabbit haemorrhagic disease (RHD) is a highly contagious, often fatal disease of rabbits (*Oryctolagus cuniculus*) caused by rabbit haemorrhagic disease virus (RHDV) which targets the liver. A new, related, pathogenic Lagovirus called rabbit haemorrhagic disease virus 2 (RHDV2) was first identified in France in 2010 and spread through Europe. Here we report the emergence of RHDV2 in Sweden. From 2011 to 2015, eleven wild rabbits (*Oryctolagus cuniculus*) collected from six separate incidents were diagnosed with RHD based on necrotising hepatitis. Animals came from Skåne and Stockholm counties and from the islands of Gotland and Öland. Animals were in normal (n = 8) to poor (n = 3) nutritional condition and all but one were mature. Frozen and formalin-fixed samples were saved from each incident. Liver also was available from a domestic rabbit that died of RHD in Gothenburg in 2015. Grossly, rabbits had a light brown-yellow, friable liver, often with a distinct reticular pattern and three rabbits were icteric. The lungs often were edematous and congested or haemorrhagic. Microscopically, there was moderate to severe, coagulative hepatocellular necrosis that extended from and bridged portal areas, and often spared hepatocytes around centrilobular veins. Occasionally, thrombi and necrosis could be seen in kidneys and spleen, respectively. Immunohistochemical staining consistently demonstrated virus within the nucleus and cytoplasm of degenerating hepatocytes. Genetic material from RHDV2 was detected in the liver of rabbits from all incidents by quantitative PCR. The VP60 capsid region of the virus was sequenced and preliminary phylogenetic analysis (n = 6) groups the Swedish isolates into two clades: one containing incidents from Gotland and one with those from the mainland. These findings suggest that RHDV2 is widespread throughout the southern half of Sweden and may have arrived as early as December 2011 in Skåne county. Similar to France and the Iberian peninsula, RHDV2 may be replacing classic RHDV strains. RHDV2 causes disease similar to RHDV and has infected certain hare species in southern Europe. Presence of RHDV2 in Sweden may have implications for all wild lagomorph populations.

## **Pathogenic potential of Bagaza virus, an emerging flavivirus in Europe, in ring-necked pheasants**

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In 2010, the first disease outbreak of Bagaza virus (BAGV), a flavivirus belonging to the Ntaya serocomplex, was reported in Europe (Spain) affecting red-legged partridges (*Alectoris rufa*) and ring-necked pheasants (*Phasianus colchicus*). It enabled the first isolation from a game bird species. Detection of specific BAGV neutralising antibodies in 2011 and 2012 in the same area, including juvenile birds, has suggested continued circulation of the virus in Southern Europe.

Previous experimental infection in red-legged partridges showed that this species is a competent host highly susceptible to BAGV infection and disease, and able to transmit this virus by direct contact.

In this study, an experimental inoculation of BAGV was performed in ring-necked pheasant, aimed to determine to what extent BAGV might affect this species and to define its potential role as a natural reservoir.

A group of 14 female adult pheasants was inoculated with an infectious dose of BAGV, and 4 non-inoculated pheasants were caged together as contact control group. Ten additional pheasants were sham-inoculated with diluent and located in a separated place. Birds were observed daily for clinical signs and a follow-up of viral load in blood, feathers, oral and cloacal swabs, and neutralising antibody titres was made up to 15 days post-infection (dpi). Four inoculated pheasants were euthanised at different dpi to study viral distribution through the body.

Viral inoculation triggered a systemic infection in all birds. BAGV infection caused a mild weight loss but no mortality in the course of the study. All infected birds developed neutralising antibodies at 7 dpi and maintained high antibody titres up to the end of the experiment. Viremia was lower and less prolonged than in red-legged partridges, indicating that pheasants are less competent for BAGV mosquito-borne transmission. Pheasants do not seem to be competent hosts for BAGV direct contact transmission, since neither virus nor seroconversion were detected in contact birds. However, the observed clinical symptoms in infected birds raise the question of sub-lethal effects and potential alterations of susceptibility to predation in the wild.

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## ***Neospora caninum* vertical transmission in roe deer, wild boar and red fox**

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*Neospora caninum* is an important cause of abortion in cattle and vertical transmission has been proved to occur frequently in this species. It is reported to infect wild mammals with high seroprevalences but reports of vertical transmission in wildlife is limited to captive animals. Seroprevalence in dogs differs from different areas, but it is higher in dogs who live in rural or wild areas, suggesting wildlife could play a role in the epidemiology of this parasite. Actually there is little evidence of vertical transmission in wild species even if this transmission route could be important in *N. caninum* maintenance in both intermediate hosts and within the sylvatic cycle of the parasite.

The aim of this study was to assess the existence of vertical transmission of *N. caninum* in three wild species: red fox *Vulpes vulpes*, roe deer *Capreolus capreolus* and wild boar *Sus scrofa*.

Skeletal muscle, kidney and central nervous system (CNS) were collected from 61 gravid females (n = 17 fox; n = 16 wild boar; n = 28 roe deer) and from 190 of their foetuses (n = 67 fox; n = 72 wild boar; n = 51 roe deer). A species-specific region of *N. caninum* ITS1 region was amplified by PCR using primers Np6plus and Np21plus. Twenty-four females (39.34 %) tested positive by PCR (6/17 35.29 % fox; 6/16 37.50 % wild boar; 12/28 42.86 % roe deer) while 53 out of the 190 foetuses tested, were positive by PCR (19/67 28.36 % fox; 18/72 25.00 % wild boar; 16/51 31.37 % roe deer). In all tested species, there was a positive correlation between foetal age and *N. caninum* positivity (p = 0.007).

Sequences obtained from 19 positive amplicons showed 100 % homology with those reported in wild-captured rodents from the same geographical area.

Our results evidence that vertical transmission occurs in wild species such as fox, roe deer and wild boar and that vertical transmission could be an important source of *N. caninum* maintenance in the sylvatic cycle independently from the excretion of oocysts by definitive hosts. The homology between strains found in wild animals with the ones previously reported from both wild and domestic rodents suggests that overlapping of sylvatic and domestic cycles is likely to occur.

## **Molecular epidemiology of infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae* in European countries**

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Infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae* may be associated with high mortality in wild Caprinae. Domestic sheep have been considered as the main source of infection of wild populations so far. However, recent studies have shown that *M. conjunctivae* also persists in wild populations. So far molecular analyses have been performed at local scale only and long-term data did not include sheep. The aim of this study was to acquire an overview of the dynamics of *M. conjunctivae* infections in European countries in multiple hosts, with a focus on the Alps. Samples from 114 diseased and healthy animals (62 Alpine and 26 Pyrenean chamois, *Rupicapra rupicapra*, *R. pyrenaica*; 23 Alpine ibex, *Capra ibex*; 3 sheep, *Ovis aries*) collected in Switzerland, France, Italy and Austria from 2007 to 2012 and tested positive by qPCR were analysed by DNA sequencing of the variable part of the gene *lppS*. Sequence data of these strains were compared with data of 59 previously published sequences, originating from the same countries and species (2000 - 2009) as well as from mouflon (*Ovis gmelini musimon*) from Spain (2006) and from sheep from Croatia (1995). A cluster analysis was performed and sample distribution was mapped considering epidemiological units based on animal movements, natural and anthropogenic barriers. Strains from the Alps were unrelated to those from the Pyrenees and Croatia. Within the Alps, 26 different clusters were identified, including 17 in Switzerland, seven in France, two in Austria and four in Italy, with four clusters distributed over country borders. A single cluster was detected over a linear distance of up to 115 km and within an area of up to 17.000 km<sup>2</sup>. Three different strains could be identified in all three considered species. In France, a given cluster was associated with a specific epidemiological unit but in Switzerland cluster distribution was not always consistent with the defined units, with sheep possibly playing a role as carriers across distinct areas. For nine clusters, strains were identified in both diseased and healthy animals. Furthermore, the results revealed

that a same cluster can persist during at least 12 years in wildlife within the same unit. In conclusion, *M. conjunctivae* is maintained in local wildlife populations over long time periods throughout the Alps and geographical barriers and animal movements determine the spread of particular strains. Interspecific transmission likely also influences disease dynamics but sample size in sheep should be increased to further investigate their carrier role among populations.

## **Clinical description of the experimental infestation of Iberian ibex with *Sarcoptes scabiei***

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Sarcoptic mange is a contagious skin disease caused by the mite *Sarcoptes scabiei*, affecting different mammalian species worldwide. Sarcoptic mange is able to cause high mortality rates (up to above 90 %) in the populations of Iberian ibex (*Capra pyrenaica*), a medium-sized ungulate endemic to the Iberian Peninsula.

Twenty-five Iberian ibexes from eight different haplotypes of the major histocompatibility complex class II DRB1 gene were experimentally infested with *Sarcoptes scabiei* for 131 days, whereas other 14 ibexes were maintained as controls for the same period. Clinical signs were monitored and blood samples were collected at days 0, 4, 13, 26, 33, 46, 61, 75, 103, 131 post infestation. Previous IgG levels against *Sarcoptes scabiei* were also measured as an indicative of previous contact with sarcoptic mange.

The clinical outcome and evolution of the infested ibexes showed different trends throughout the infestation period. Three of the 25 infested ibexes developed mange lesions in less than 50 % of the body surface, whereas 16 progressed to severe stages of the disease (i.e., lesions covering over 50 % of the skin surface). Conversely, four of the infested ibexes showed a reduction of the skin lesions during the infestation period, two of them recovering completely. Two of the infested ibexes died during the infestation due to other causes. Clinical, haematological, serum biochemical and immune (IgG) variables of the infested ibexes were analysed and compared with controls, in order to determine the reasons for the different evolution of the sarcoptic mange in the studied individuals. This knowledge will allow a better understanding of sarcoptic mange in Iberian ibex populations.

## Multi-locus sequence typing of *Trichomonas gallinae* strains from Bonelli's eagle in Spain

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The Bonelli's eagle (*Aquila fasciata*) is an endangered raptor species distributed in Europe across the Mediterranean region. The Iberian Peninsula harbours more than 80 % of the European population and different conservation programmes, including LIFE projects (at present LIFE NAT/ES/000701: integral recovery of Bonelli's eagle population in Spain) have been implemented. Human related causes, mostly electrocution and collision with power lines or direct persecution, are known to be the main reasons of population decline. In addition, the reduction of their preferred prey species, mainly rabbits (*Oryctolagus cuniculus*) and red legged partridges (*Alectoris rufa*), due to infectious diseases or conflicts with hunters interests, has introduced changes on its diet composition. The abundance of columbiforms in certain regions has been used by the eagles as a secondary food resource, increasing the numbers of this prey species on their diet. This shift in dietary composition has increased the risk of avian trichomonosis, an important parasitic disease that could be potentially fatal. In this context, 103 birds were sampled from 2010 to 2015: 72 nestlings, 4 juveniles and 27 adults. Animals were either admitted at wildlife recovery centres or captured on their habitat as part of other conservation projects. Gross lesions that ranged from superficial ulcers to caseonecrotic granulomas were detected at the oropharyngeal cavity of 36 birds: 33 nestlings, 1 juvenile and 2 adults. A total of 19 isolates of *Trichomonas gallinae* were genotyped by multi-locus sequence typing analysis that included ribosomal

regions ITS1/5.8S/ITS2 (ITS), small subunit rRNA (SSU) and Fe-hydrogenase gene (Fe-hyd). An association between the presence of macroscopical lesions at the oropharyngeal cavity of the birds and the genotype of the parasite was found. Animals with lesions harboured genotypes ITS-Tg-1, Fe-hyd A1 or A2 and SSU-VI, with one bird that did not displayed gross lesions at the moment of sampling. Mixed infections were also detected in three cases of birds with lesions. Birds without lesions harboured ITS-Tg-2, Fe-hyd C4 and SSU-Genbank accession EU215373. We have detected genotypes of the parasite that were described in different mortality outbreaks in Europe and America, including birds of prey, passerines and columbiform hosts. These findings indicate that pathogenic strains are found circulating among Bonelli's eagle population, being an important factor to consider in the evaluation of nestling mortality in conservation programmes and when supplemental feeding strategies using barrier lofts (pigeon lofts built to prevent eagle predation on racing pigeons) are employed.

## Parasite sharing as a preliminary indicator of multispecies connectivity

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At a time characterised by unprecedented ecological change, monitoring wildlife disease at the population, community and ecosystem levels is essential to direct surveillance efforts. In a landscape where the key to disease emergence is a change in transmission dynamics within or between host populations, the extent to which they are interconnected is fundamental. The lower Kinabatangan floodplain in Sabah (Malaysian Borneo) is exceptionally biodiverse and it is home to 10 species of primates, including some endemic and endangered species such as the Bornean orangutan. However, these habitats are suffering fragmentation through rapid land conversion, largely to agricultural oil palm plantation. Furthermore, global gap analyses indicate insufficient knowledge of parasites infecting these primates, making Sabah an area in critical need of further attention. To understand connectivity in host-parasite dynamics in a multi-species system, we assessed the genetic structure of *Strongyloides* sp., a model gastrointestinal helminth with a cosmopolitan distribution, across six sympatric primate hosts living at various degrees of fragmentation. We collected faecal samples (n = 146) and selected larvae from coprocultures (n = 51) microscopically assigned to the genus *Strongyloides*. Larval DNA was extracted and amplified using fragments of the *cox1* and *18S rRNA* genes. Preliminary phylogenetic analyses indicate *Strongyloides* moves easily across host communities, creating a genetic connectivity across the landscape. Parasite genetic structure provides insight into movement across heterogeneous environments and contact between host populations, providing a better understanding of parasite transmission and host connectivity in complex landscapes.

## ***Toxoplasma gondii* in red deer (*Cervus elaphus*): epidemiological investigation and alternative sera sampling method**

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Red deer (*Cervus elaphus*) can be intermediate host of *Toxoplasma gondii*, representing a potential public health concern. Indeed, its intense culling and the increasing consumption of raw or undercooked meat, may pose risks for humans. The need is therefore to investigate *T. gondii* in this host species. Moreover, the definition of alternative sampling methods for sera may help to solve diagnostic concerns related to logistical field activities and availability/suitability of sera.

A sero-epidemiological survey was performed in red deer from Italian Central Alps to investigate (i) the spread and the dynamics of *T. gondii*, (ii) the reliability of sera obtained from blood through intracavernous venipuncture (IV) as an alternative to sera from blood of major vessels (MV) in ELISA test.

Overall 242 sera were collected during two culling management plans (2014 and 2015). In 2015, 75 sera were obtained by both IV and MV techniques. Samples were tested by a commercial ELISA kit (IDVET, Montpellier, France) and results were analysed through Generalized Linear Models.

An overall prevalence (p) of 21.5 % emerged. Calves (p = 5 %) were less infected than 1-year-old (p = 23.5 %) and > 2-year-old (p = 31.5 %) deer. Subjects of low anthropised area (p = 8.6 %) were less infected than those of the high (p = 33.7 %) and moderate (p = 15.7 %) anthropised ones. Subjects of 2014 (p = 16 %) were less infected than those of 2015 (p = 29 %) and seropositive deer of 2015 showed serological titres higher than those of 2014. Prevalence of 30.7 % and 26.7 % from MV and IV, respectively, showed an "Excellent agreement" (K value = 0.9024) between the two techniques.

The effect of age class and anthropisation on infection supports horizontal transmission as the main route. The difference in prevalence and serological titres between study years leads to recent infections of deer of 2015 and thus to a new introduction or reintroduction of *T. gondii* in the study area. This finding should be considered even in relation to the potential zoonotic risk. The concordance between results supports the use of IV as a useful alternative for sera field sampling.

## The integrate West Nile Disease surveillance in Lombardy region, Italy

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In 2013, the circulation of West Nile virus (WNV) was detected in Lombardy and between 2014 and 2015 a surveillance programme was activated with the aim to detect WNV circulation in mosquitoes and wild birds as early as possible and before the occurrence of human cases. We present the results of the integrate WND surveillance adopted in Lombardy, based on both entomological and wild birds screening. The plan territory of Lombardy was split into square areas of 20 km<sup>2</sup> and one CO<sub>2</sub>-CDC trap was placed in each square for performing night trap sessions every 15 days from May to September. In addition, the monitoring of hunted wild birds (*Pica pica*, *Corvus corone cornix*, *Garrulus glandarius*) was performed and distributed either spatially, according to the size of each province, and temporally from April to October. After identification, *Culex* spp. mosquitoes were divided in pools, each of 100 specimens maximum. Organs (brain, spleen, heart, kidneys) from each bird were taken at necropsy and pooled. The samples were subjected to two distinct real-time PCR protocols for the detection of WNV and USUV respectively and to a screening PCR for the presence of flaviviruses, which targeted 250 nucleotides of the conserved region of the NS5 gene. The obtained amplicons were sequenced for virus identification. More than 100.000 *Culex* spp., divided in 628 pools, and 2,800 wild birds were tested in 2014 and 2015. The virus were detected in 30 pools of *Culex* spp. and in 23 wild birds. This WNV surveillance system precisely identified the areas and provinces affected by the virus and detected the viral circulation at least two weeks before the occurrence of onset of human cases. For this reason, as recommended by the Ministry of Health, human blood transfusion tests for WND (NAT-PCR) started immediately after the notification of WNV circulation detected by the integrated surveillance system. Note that the average time taken from sampling to getting the final results was less than five days. The capability of detection of WNV through mosquitoes and birds surveillance programmes is coherent with the One health approach and gives benefits in terms of cost-effectiveness and public health safety. In particular, it enables a more targeted blood unit testing strategy and reduces the probability of virus transmission via blood and organ donation systems, totally in line with a One Health methodology. Considering the endemic presence of WNV in the Lombardy, the entomological and wild birds surveillance represents an effective support for the early identification of WNV circulation, thus providing a useful tool for the public health authorities in the application of measures to prevent human infection.

## Surveillance and first detection of Chronic Wasting Disease (CWD) in Norway

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Chronic Wasting Disease (CWD) is a fatal neurodegenerative disease of cervids that belongs to the group of transmissible spongiform encephalopathies (TSE). The disease is endemic in North America, where natural infections occurs in mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), elk (*Cervus elaphus nelsoni*), and moose (*Alces alces shirasi*). CWD has never been reported outside North America except in captive deer in South Korea caused by the importation of CWD-infected animals.

The surveillance for CWD in Europe has been limited, and the European Food Safety Authority (EFSA) has stated that the occurrence of CWD could not be excluded in cervids in Europe, especially in remote and presently unsampled areas. In Norway, approximately 1,400 cervids have been tested for CWD in the period 2004 - 2015. Less than 20 of these have been wild reindeer (*Rangifer tarandus tarandus*).

Norway is the last refugium for wild tundra reindeer in Europe, counting roughly 25,000 animals in winter population. The species is found in fragmented sub-populations in remote alpine regions of South Norway.

In March 2016, a young adult female reindeer belonging to the Nordfjella sub-population was found dying. It had divided out from a herd of 400 reindeer followed by helicopter during research capture. The carcass of the animal was submitted for necropsy. The main finding was consistent with exertional myopathy. Brain tissue was routinely tested for TSE. The animal was positive for the detection of abnormal prionprotein both by the first routine test (TeSeE ELISA, Bio-Rad) and in two supplementary tests, TeSeE Western Blot Bio-Rad, and in-house immunohistochemistry.

The wild reindeer from Norway represents the first detection of CWD in Europe and the first detection of a natural infection in reindeer worldwide. This CWD case and plans for the follow-up surveys of this disease in the Norwegian wild reindeer populations (and other cervids) will be presented.

## **Review of Chronic Wasting Disease in North America**

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Chronic wasting disease (CWD) of deer was first identified as a spongiform encephalopathy of captive and wild mule deer in the Rocky Mountain region of northern Colorado and southern Wyoming as early as 1980. It really gained the attention of Wildlife Management agencies and the public when it was found to be caused by a prion, and identified in wild white-tailed deer in Wisconsin in 2002. By 2002 CWD was found in wild deer and/or elk in five States and captive elk in six States. Most of the locations were adjacent the original nidus of infection or linked epidemiologically to transport of animals from there. By 2016 CWD has been found in wild cervids in 22 States, and in 75 captive deer or elk herds in 16 States. The prion that causes CWD has been found in wild and captive cervids in the Canadian Provinces of Alberta and Saskatchewan, and captive cervids in South Korea. Now it has also been identified in reindeer and elg (moose) in Norway. The big questions are, how and why is this seemingly immobile prion protein spreading?

Natural and anthropogenic factors have contributed to the geographic spread and survival of CWD prions and spreading of infection in North America. Natural factors include prolonged incubation, multiple routes of prion shedding, the prion's environmental persistence, and migratory and dispersal movements of wild cervids. Anthropogenic factors include legal and illegal movements of infected live animals (and perhaps infectious tissues and other materials), concentrating susceptible host species, and other artificial wildlife management practices. With current tools and technology, given its extensive distribution and epidemiological attributes, eradicating CWD appears infeasible. But, adaptive approaches for containing foci and reducing infection and transmission have shown some promise and deserve further attention. Early detection and aggressive culling, followed by intensive harvest and surveillance, appear to have limited outbreaks in Michigan and New York.

There are several important reasons to continue to try to contain and control the spread of CWD in North America. Several modelling efforts and published studies suggest that heavily-infected cervid populations will not thrive in the long-term and that hunting opportunity will diminish. The public perception that deer and elk are a potential source of disease may reduce their perceived value and support for their conservation. And, the life-history of CWD and other animal prion diseases suggest minimising human exposure to these agents is prudent.

The primary source for this review was the SCWDS Briefs of April 2016.

**Discovery and partial characterisation of *Ranid herpesvirus 3* (RHV3), a novel virus associated with disease in free-ranging wild common frogs (*Rana temporaria*)**

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Amphibians have undergone significant losses worldwide with dramatic impact on biodiversity. Several factors have been implicated in this global decline including habitat loss and exploitation, illegal harvesting and pollution. More recently, the role of specific infectious agents in the drastic reduction of the amphibian populations in the wild has also been considered. Fungal agents such as *Batrachochytrium dendrobatidis* and *B. salamandrivorans* have been shown to serve as primary infectious agents responsible of fatal diseases in amphibians. Similarly, *Ranavirus* has surfaced as a significant threat to multiple amphibians species worldwide. During the last five years at the Centre for Fish and Wildlife Health (FIWI) of the University of Bern, we focused on the detection of emerging diseases in free-ranging wild amphibians in Switzerland in collaboration with the Swiss Amphibian and Reptile Conservation Program (karch) and a local reptilian and amphibian veterinarian. During the early spring of 2015, two common frogs (*Rana temporaria*) with obvious skin lesions were observed in a pond and were brought to the FIWI for full examination. The skin of the animals was characterised by multifocal, variably extensive, grey patches that histologically were consistent with areas of severe epidermal hyperplasia and necrosis associated with intranuclear eosinophilic to amphophilic inclusions associated with minimal inflammatory response. Transmission electron microscopy revealed the presence of particles with size and morphology consistent with a herpesvirus. Ongoing molecular characterisation supports the initial tentative morphological identification of the observed viral agent as a previously undescribed member of the order *Herpesvirales*. The newly discovered virus is the third herpesvirus partially characterised at the molecular level that has been identified in frogs and has been tentatively named *Ranid herpesvirus 3* (RHV3). Although it is not yet clear the pathogenic potential of RHV3 in the wild populations of frogs, the extent and severity of the lesions

observed in the examined frogs are consistent with a clinically relevant disease. During the early spring of 2016 only few individuals could be found in the same pond where the affected frogs were originally collected.

## **Epidemiological tracing of *Batrachochytrium salamandrivorans* infection in European private amphibian collections**

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Amphibian chytridiomycosis, an emerging infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), has caused widespread biodiversity loss on a global scale over recent decades. The recent emergence and discovery of *Bd*'s "sister fungus", *Batrachochytrium salamandrivorans* (*Bsal*), in Western Europe identified a second chytrid fungus causing mass mortalities of urodeles (newts and salamanders). This study follows the discovery of *Bsal* in the United Kingdom, infecting animals recently acquired by a zoological collection. Using contact-tracing, eleven epidemiologically-linked private amphibian collections across Western Europe were identified and all potentially-susceptible urodeles were tested for *Bsal* using quantitative polymerase chain reaction. Seven of these collections contained animals testing positive for *Bsal* infection, including the identification of *Bsal* in Spain for the first time. The death of *Bsal*-infected individuals was observed in several collections and chytridiomycosis was diagnosed in three collections from which carcasses were obtained and suitable for post mortem examination. Our results indicate that *Bsal* is prevalent within the hobbyist trade, at least in Europe and probably elsewhere. These findings are important for informing policy decisions regarding strategies to minimise the risk of *Bsal* spread.

## Disease outbreak in red squirrels *Sciurus vulgaris* in Germany

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The red squirrel (*Sciurus vulgaris*) is distributed throughout large parts of Europe and Asia. Its distribution in certain regions of Europe is endangered by the invasive neozoon grey squirrel (*S. carolinensis*). In 2013, a novel squirrel associated adenovirus was found in a red squirrel from Germany which suffered from acute diffuse catarrhal enteritis. During the 2015/2016 winter-season, a large-scale disease outbreak was observed in red squirrels at rescue centres for wildlife in different regions of Germany. This outbreak affected particularly young animals, causing high mortality. Two different symptom complexes were observed: In the majority of animals diarrhoea was diagnosed, whereas other animals showed severe respiratory symptoms.

Necropsy revealed catarrhal enteritis in 9 of 15 cases, suppurative bronchopneumonia in 7 of 15 cases (including two cases exhibiting both lesions), and no definitive morphological change in one case. Possible etiological agents identified within the intestine by histopathology included *Eimeria* spp. and *Trichuris* spp. The topographic distribution of lung lesions and demonstration of florid bacterial colonies suggested that aspiration was the ultimate reason in many cases. Bacterial culture approach for a lung sample indicated an extreme infection level with *Escherichia coli* O78. In addition, *Citrobacter koseri*, *Streptococcus* spp. and *Brevibacillus reuszeri* were detected. By using a newly developed real-time PCR targeting the adenoviral polymerase gene, viral DNA was detected predominantly in the intestine, but also in liver, lung and kidney samples of affected animals. In addition to German squirrels, the new adenovirus was also detected by PCR in samples collected from red and grey squirrels in Scotland, UK. Sequence analysis of the complete hexon gene, the most variable genome region of adenoviruses, indicated a very high similarity between the German and British samples and a close relationship of the new squirrel-associated adenovirus to sequences of Equine

Adenovirus B, but a strong divergence to Murine adenoviruses. Currently ongoing Next-Generation Sequencing investigations aim to identify the degree of identity among squirrel adenoviruses detected in different parts of Europe, and its relationship to other rodent-associated adenoviruses and further members of the genus *Mastadenovirus* based on the full-length genome.

In conclusion, the recent disease outbreak in red squirrels indicates a broad distribution of squirrel adenovirus and highlights the necessity of continuous wildlife surveillance.

## **Antimicrobial resistance prevalence in bacteria isolated from harbour seal pups (*Phoca vitulina*) stranded in the Netherlands: a first screening**

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Antibiotics are the most successful drugs used for fighting bacterial diseases in humans and animals. Its use in humans and animals leads to the selection of resistance genes. Similar resistance genes found in humans are also found in pristine ecosystems without any record of antibiotic contamination. The Netherlands are a good example where human populations in coastal areas continue to increase. Therefore, coastal ecosystems may become increasingly important as reservoirs of or sentinels for infectious organisms and antimicrobial resistance (AMR). Since many marine mammal species share the coastal environment with humans and consume the same food, they also may serve as sentinels for ocean and human health. Little is known on the antimicrobial susceptibility patterns in free ranging and/or stranded marine mammals, more specifically in harbour seals in the Netherlands. In this study, we revealed the prevalence of AMR in bacteria isolated from the rectum of harbour seal pups and weaners admitted for rehabilitation at the Seal Rehabilitation and Research Center (SRRC) in Pieterburen, the Netherlands. Rectal swabs for bacterial culture were collected during health assessment from 100 harbour seals including pups and weanlings during summer 2015. All animals had stranded alive along the Dutch coast and islands and were transported to the SRRC. Within 48 hours after collection swabs were streaked onto different plates to screen for Methicillin Resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta-Lactamase (ESBL) and B-lactamase hyper producing gram-negative bacteria, and Vancomycin Resistant Enterococci (VRE). ESBL-producing organisms were isolated from 4 pups but no MRSA and VRE were found. Subsequently, the whole genome of the four isolates was sequenced on the MiSeq and the genomes were compared to genomes obtained from human isolates by a core genome multi-locus sequence typing (cgMLST) approach. In addition, the presence of resistance genes was determined. *E. coli* isolates of this study contained genes causing resistance against aminoglycosides, beta-lactams, macrolides-lincosamide-streptogramin, sulphonamides, tetracyclines and trimethoprim. Furthermore, cgMLST revealed that two of the four *E. coli* isolates cluster together. These isolates were obtained

from seals admitted on the same day and stranded at the same place. Moreover, isolates from seals differ only in 40 of 2,764 analysed genes from human *E. coli* isolates. In conclusion, prevalence of MRSA, VRE and ESBL-producing *E. coli* in harbor seal pups is low. *E. coli* isolates found in them are closely related to those found in human and contain resistance genes that are also found in human isolates.

## **The Vampire Bat Virome: evolutionary implications in an immunological context**

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Vampire bats feed exclusively on blood placing them in regular contact with blood borne pathogens of their food species and putting both vampire bats and their prey at risk of viral pathogen cross-species transmission. To investigate the non-rabies virology of vampire bats and the consequences for their immune systems we applied pan viral screening and metagenomic approaches to sequence viruses and immune genes of two species of vampire bats (*Desmodus rotundus* n = 29, and *Diphylla ecaudata* n = 3) from three populations in Mexico (Tlaltizapan, Morelos; Soledad Doblado, Veracruz and Estado de Mexico, Mexico). We also screened their main cattle food source (n = 31) in these locations using identical metagenomics approaches. We obtained full length vampire bat sequences of the viral sensing Toll-Like Receptors (TLRs, TLR3, 7, 8, and 9). The evolution of bat TLRs in general was unique in several aspects compared to other mammals exhibiting episodic diversifying selection on several TLRs in important functional domains associated with recognition of viral pathogens. Consistent with their unique immunogenetics, the vampire bats exhibited unusual virology. A novel retrovirus (*Desmodus rotundus* endogenous retrovirus, DrERV) which demonstrated a complex evolutionary history was identified. It was most similar to retroviruses found in conspecific squirrel monkeys (the squirrel monkey retrovirus SMRV). However, DrERV was not found in *Diphylla ecaudata* and the evidence suggests the viral group integrated in *Desmodus* millions of years ago while a potentially still circulating exogenous relative may have very recently infected squirrel monkeys and rats. Multiple gammaherpesviruses ( $\gamma$ HVs) were identified in both tested vampire bat species. When the bat  $\gamma$ HVs known to date were compared to all of the known mammalian  $\gamma$ HVs, we obtained evidence that bats, including vampire bats, are historical superspreaders of this viral group, a surprising break with virology dogma that generally considers herpesviruses to exhibit strong species specificity. The data we are obtaining from our metagenomic analysis suggests that vampire bats have a unique viral tolerance genotype to blood borne viruses and as a consequence historically, along with other bat species, have hosted viruses capable of being transmitted among all major mammalian groups.

## **A comparative and computational study of the affects of population size and group size on pathogen richness in bats**

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The number of pathogen species that infect a host species has important ecological consequences as well as being one factor to consider when prioritising wild species for zoonotic disease surveillance. Population density, absolute population size, geographic range size and social group size are all thought to increase pathogen richness.

However, these factors are intrinsically linked; all else being equal, increasing density or group size directly increases population size.

We have used metapopulation SIR models to test whether it is density per se that increases the ability of a newly evolved pathogen to invade and persist in a population as opposed to population size, geographic range size and social group size. We studied the probability that a newly evolved pathogen would invade and persist in a population in the presence of an identical, competing pathogen.

Using 102 bat species as a case study, we tested the predictions from these simulations using phylogenetic regression to test for associations between colony size, geographic range size and viral richness while controlling for study effort and body mass. We found that increased group size increases the chance that a new pathogen will invade into a population to the largest extent. Both group size and population size promote pathogen richness more than population density.

However, in the comparative analysis, only study effort is found to be associated with viral richness.

These results suggest that population density and population size are not interchangeable and care should be taken when using them in regressions of pathogen richness. Furthermore, as group size has the strongest affect on pathogen invasion probability, this factor should be included in future models of disease competition in wild animal species. Finally, by far the largest predictor of virus richness in wild bats is study effort; therefore more unbiased studies of pathogen richness in this group are needed.

## Have small mammals been neglected as *Coxiella burnetii* reservoirs?

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Several wild species are potential hosts of *Coxiella burnetii*, the agent of Q fever in humans and other mammals. Those of wide geographic distribution reach high population densities, are abundant, gregarious and live in humanised environments most prone to be relevant reservoirs. Previous studies on *C. burnetii* in Iberia targeted on dense, widely distributed and gregarious species such as red deer (*Cervus elaphus*) and European wild rabbit (*Oryctolagus cuniculus*). Many other wild animals may be good candidates as reservoirs of *C. burnetii*. We analysed the status of *C. burnetii* infection in wild small mammal species to estimate their role in the ecology of *C. burnetii*.

From 2004 to 2015, spleen samples were collected from 10 species of small mammals captured along different research projects. Small mammals were collected from 16 locations covering peninsular Spain from south to north. Eight-hundred and sixteen samples from 571 (70 %) common voles (*Microtus arvalis*), 138 (17 %) wood mice (*Apodemus sylvaticus*), 47 (5.8 %) black rats (*Rattus rattus*), 27 (3.3 %) greater white-toothed shrews (*Crocidura russula*), 17 (2 %) Algerian mice (*Mus spretus*), 10 (1.2 %) house mice (*M. musculus*), 2 (0.2 %) yellow-necked mice (*A. flavicollis*), 2 (0.2 %) garden dormice (*Eliomys quercinus*), 1 (0.1 %) European water vole (*Arvicola amphibius*) and 1 (0.1 %) red squirrel (*Sciurus vulgaris*) were analysed by real-time semi-quantitative PCR (qPCR) and a sub-sample characterised by single nucleotide polymorphism (SNP) and PCR/RLB analysis.

*Microtus arvalis* displayed the highest average infection prevalence (Mean (M): 10.9 %; 95 % Confidence Interval (CI): 8.4 - 13.7; Inter-population prevalence range (IPR): 0.0-23.3) followed by *A. sylvaticus* (M: 8.7 %; 95 % CI: 4.6 - 14.7; IPR: 0.0 - 21.2), *C. russula* (M: 7.4 %; 95 % CI: 0.9-24.3; IPR: 0.0-33.3) and *R. rattus* (M: 6.4%; 95 % CI: 1.3-17.5). *Coxiella burnetii* DNA was absent in the rest of small mammal species. Prevalence ranges in *M. arvalis* and *A. sylvaticus* - surveyed in 8 and 13 of the study locations, respectively - widely diverged among locations, suggesting - as evidenced from red deer long-time series data - that local population and environmental factors influence the spatio-temporal dynamics of *C. burnetii*.

This study highlights the relevance of small mammals in the ecology of *C. burnetii*. The common vole - cyclic population changes with intense density peaks - in the transmission of *C. burnetii* to livestock and humans should not be dismissed and better understood.

## Occurrence of *Mycoplasma* spp. in free-ranging songbirds and doves

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*Mycoplasma* spp. are important pathogens in poultry and cause high economic losses for poultry industry worldwide. In other bird species (e.g. birds of prey, white storks and several waterfowl species) *Mycoplasma* spp. were regularly found in healthy individuals and hence considered apathogenic or part of the physiological microbial flora of the respiratory tract. However, in other wild bird species *Mycoplasma* spp. do cause severe respiratory signs. As they are absent in healthy individuals of some wild bird species (e.g. nightingales or tits) they might play a role as respiratory pathogen. Studies on the prevalence of *Mycoplasma* spp. in free-ranging bird species are scarce. To evaluate the relevance of *Mycoplasma* spp. in free-ranging bird species 130 clinically healthy individuals of 19 different species were screened for the occurrence of *Mycoplasma* spp. via culture and molecular biological methods. The birds were sampled via tracheal or pharyngeal swabs, respectively and examined via culture and genus-specific *Mycoplasma*-PCR. In case of a positive result the *Mycoplasma* species was determined by sequencing parts of the 16S rRNA and 16S-23S Intergenic Transcribed Spacer Region. The results demonstrate that the relevance of *Mycoplasma* spp. differs between avian species. Hence, a differentiation of the *Mycoplasma* species is essential for the interpretation of the role of *Mycoplasma* spp. as a potential pathogen in respiratory disease in free-ranging bird species. So far, it remains unclear if ecological and / or behavioural factors may explain the varying detection of *Mycoplasma* spp. in different avian species.

## ***Ophidiomyces ophidiicola*, the pathogen associated with fungal dermatitis detected in free-living snakes in Great Britain**

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Fungal pathogens are emerging across diverse taxa and are associated with severe population declines and extinctions on a global scale. Snake fungal disease (SFD), caused by the pathogenic fungus *Ophidiomyces ophidiicola*, is an emerging infectious disease of captive and free-living wild snakes in North America first recognised in 2008. There are published reports of SFD in free-living snakes from multiple states across eastern and midwestern USA, but there have been no confirmed accounts in wild snakes outside of this region to date. There is a general paucity of knowledge on the health conditions affecting wild reptiles due to their cryptic nature which makes disease surveillance studies challenging. An adult female grass snake (*Natrix natrix*) was observed at a study site in southern England in July 2015 showing signs of lethargy and abnormal behaviour, and it subsequently died. At post mortem examination, approximately 10 % of the scales of the head and body showed well demarcated areas of erosion and ulceration. Histopathological examination revealed presence of multifocal ulcerative dermatitis associated with mats of fungal hyphae and arthroconidia with a morphology consistent with *O. ophidiicola*. There was no evidence of systemic mycosis. Microbiological examination of the skin lesion isolated mixed bacterial and fungal growth. The fungal D1-D2 region of the large ribosomal subunit was directly sequenced from the lesioned skin, and was a 99 % match to *O. ophidiicola* isolates in Genbank. The cause of death in the grass snake could not be determined, however the animal was found to be dehydrated, with no avoidance behaviour and in thin body condition which may have been associated with SFD. In response to this finding, archived skin samples from 24 British free-living snake carcasses and 58 shed snake skins were screened for *O. ophidiicola* using a real-time PCR assay specific for the fungus. Four carcasses and seven shed skins tested positive for the fungal pathogen by PCR in addition to the index case described. Mild skin lesions were present in ten of these positive cases. An attempt at fungal isolation and further molecular characterisation of these samples is underway to compare the results with North American strains of *O. ophidiicola*. Here we report the first detection, to our knowledge, of SFD in free-living snakes in Europe. Further

research is required to understand the significance of this finding, for instance whether this represents a previously missed endemic infection, or an emerging condition in GB.

## **Characterisation of the antimicrobial peptide family defensins in the Tasmanian devil (*Sarcophilus harrisii*)**

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The Tasmanian devil (*Sarcophilus harrisii*) is a native Australian marsupial that is at risk of extinction because of the lethal contagious cancer Devil Facial Tumour Disease (DFTD). The recent sequencing of the Tasmanian devil genome has provided the opportunity to identify and characterise immune genes, which is a critical first step of understanding the devil immune response to disease and DFTD. Using genome and transcriptome mining we have characterised the devils defensin genes, an antimicrobial peptide family which selectively kill bacteria, viruses and fungi and have broad immunomodulatory roles including anti-tumour properties. The interaction of defensins to rapidly evolving pathogens, and their antimicrobial nature makes them a useful gene family to study adaptive molecular evolution, and makes them ideal targets to be used as templates for potential novel antibiotics.

We have identified 49 defensins in the Tasmanian devil genome, most of which have a cationic charge (between 1+ to 13+) and high proportion of hydrophobic residues (> 30 %). These properties are characteristic of defensins as they aid in the selective interaction and binding of defensin to their cellular targets. Phylogenetic analysis shows that devil defensins have undergone gene duplication events resulting in unique devil specific defensin lineages. Such expansions of antimicrobial peptides have also been observed in other marsupials, and it is possible that the unique marsupial reproductive biology, where altricial young are born immunologically naive, is involved in driving these expansions. Site specific selection analysis reveals that residues that are essential for structural stability of defensins are under strong purifying selection, while sites that are hydrophobic and positively charged are positively selected. This work provides the first steps in understanding the role of defensins in the Tasmanian devil immunity and DFTD, and also provides potential templates for novel antimicrobial development.

**PLENARY TALK: Thinking beyond silos – implementing conservation medicine and One Health approaches in a dynamic and uncertain environment, the real world**

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Understanding, containing and mitigating infectious disease threats at the Animal-human-ecosystem interface (AHEI), while considering human well-being, conserving biodiversity and the provision of ecosystem services, constitutes an international imperative (UN, MDG, 2016). Today practically all humans live in dynamic multi-use landscapes in which anthropogenic activities have become the main driver of environmental change. We have clearly left the unusually stable 10,000 years of the Holocene behind us and have firmly entered into the Anthropocene. Due largely to the reliance on fossil fuels, the expansion of industrial agriculture and land transformation in a society reliant on constant growth, the Earth's regulatory mechanisms, that have in the past provided regular temperatures, freshwater availability and guaranteed biogeochemical flows are being severely perturbed. Humankind is potentially, irreversibly, leaving the safe operating space for human existence on earth.

In this challenging environment, seemingly novel cross-disciplinary approaches to human, animal and environmental health have been developed, defined and branded. Conservation Medicine, One Health and Ecosystem Health are but a few of the concepts that are being widely discussed and equally hyped. However, on closer inspection, it is obvious that the impacts of these approaches have been insufficiently assessed. Recently a publication-based survey showed that, while the field of One Health was growing rapidly by nearly 15 % per year, its impacts clustered into three distinct communities: ecologists, veterinarians and a third community consisting of population biologists, mathematicians, epidemiologists and experts in human health. While intensive work within a discipline is essential when developing expertise, it is equally clear that solving problems that impact the continued development of human societies, including the maintenance of health and the provision of Ecosystem Services (ESS) as the basis for all life, necessitates research that bridges the traditional disciplinary silos.

The sustainable management of the AHEI, including amongst others biodiversity conservation and emerging infectious diseases clearly cannot be achieved within strict sectoral, disciplinary and administrative boundaries. Overcoming these boundaries has proven an elusive and arduous pursuit. Particularly the divide between natural and social sciences has proven to be especially enduring. But silos persist also within the natural sciences. One justification that has been brought forward to explain these boundaries is that natural science researchers operate at a variety of immensely different scales of biological organisation ranging from molecules and genes to landscape level ecosystem approaches. It is crucial to be

cognizant of the fact that research pertaining to emerging infectious diseases is particularly challenged in overcoming disciplinary boundaries, as it falls into the scope of the fundamentally segregated veterinary and ecological communities (Manlove et al., 2013). Addressing the complex problems emerging at the AHEI necessitates transcending these disciplinary boundaries while additionally integrating policy makers, administrators and stakeholders from civil society.

Developing and implementing a transdisciplinary research approach remains similarly challenging and elusive. In contrast to the more common multidisciplinary approaches, which are additive and preserve disciplinary boundaries, transdisciplinary approaches are integrative and transcend disciplinary boundaries (Roy et al., 2013). Various approaches have been described to engender transdisciplinary research. These include *inter alia*: developing early interdisciplinary breadth at the undergraduate level, creating an incubator for innovative information technologies and research tools, integrating performance measures in academia that account for and reward transdisciplinarity, establishing research and training grants that further multi-scale literacy in infectious disease.

The gaps in knowledge regarding the sustainable management of the AHEI highlight the fact that these issues involve highly dynamic and interconnected, rather than simplistic and straightforward processes. It appears essential to reconcile the dynamic and complex nature of the problems with specific and appropriate problem solving approaches. In contrast, solutions based on naïve simplification of interdependencies and the lack of ground-truthing leads to results that are ultimately not relevant when informing policy and implementing management. Furthermore, this author's experience in the past 25 years indicates that health issues at the AHEI and global biodiversity conservation are most likely so-called "wicked or even super wicked problems" implying the need for novel approaches when addressing these issues. This author also feels strongly that the usual backward looking method of investigating the past and generating selective and singular predictions, is only sufficient for "tame problems" but wholly inadequate for highly dynamic and interconnected environmental conservation and emerging infectious health issues. It appears necessary to apply a forward reasoning approach, which identifies possible future scenarios while integrating uncertainties.

Beyond the challenges of establishing and implementing transdisciplinary approaches, it appears necessary to reflect on the wealth of novel knowledge and tools available from the exponential growth of technological progress. The Singularity notion, a possible culmination and threshold in technological advances, is presently being extensively discussed and defined within a multitude of scientific disciplines and in society: e.g. "*Singularity is a break in human evolution that will be caused by the staggering speed of technological evolution*" (James Martin). A multitude of novel technologies are being mainstreamed on this path to Singularity: amongst others Virtual Reality (VR) and Artificial Intelligence (AI). However, we do not actually know what Singularity is or will be. It does however seem prudent to discuss and reflect, from within our own disciplines, on future scenarios, the related consequences and opportunities for the AHEI and biodiversity specifically. While Singularity may be perceived by some to be science fiction and best left to futurist forums, it is important to note that the European Commission's Future and

Emerging Technologies (FET) Flagship project: Human Brain Project, a comprehensive attempt to reverse-engineer and simulate the complete human brain, has been awarded 1.2 billion Euro in funding. Understanding, containing and mitigating infectious disease threats at the AHEI and biodiversity conservation regularly employs approaches and methodologies largely similar to those developed some 50 - 100 years ago. At the same time, in the segregated parallel world of technology, knowledge, tools and novel societal norms are being developed at an exponential rate. These tools and most importantly the emerging societal norms are only infrequently reflected on and subsequently only partially available to the field of infectious disease mitigation and biodiversity conservation. In this author's opinion it is imperative to build bridges and interlink these segregated worlds. Most barriers and boundaries are firmly entrenched in the heads of individual researchers, their respective groups and institutions. A minimum of open-mindedness towards other research cultures, terminology, methodologies and approaches would already greatly facilitate the initiation of value-added trans-disciplinary research ventures.

## **Prioritisation of wild bird biological hazards to be targeted in European surveillance programmes using a novel expert-based qualitative risk assessment method**

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Surveillance of wild animal diseases is essential to control the health risks for domestic animals, humans and wild animals themselves. In order to optimise resources available for surveillance programmes, prioritisation works have been done in the past in Europe to identify pathogens to be targeted but none on wild birds. However, wild birds have biological features allowing the fast spread of pathogens over long distances and their transmission to several targets, making them an important animal class to consider in wildlife disease surveillance programmes.

In order to determine which biological hazards carried by which wild birds should be targeted in European surveillance programmes, we developed an expert-based qualitative risk assessment method.

Initially, 119 “biological hazard/wild bird order” pairs of hazards were identified for prioritisation (43 biological hazards – bacteria, viruses or parasites – and 17 wild bird orders). Experts were invited to score each pair based on six criteria in five distinct online surveys implemented in 2014. Experts were European specialists from different fields (wild bird diseases, ornithology, microbiology, farm animal health or public health). The mean survey participation rate was 20 % (range 12 % - 26 %). The criterion scores provided by the expert panels were combined using a qualitative matrix to estimate a health risk specific to each “biological hazard/wild bird order” pair, for each of the three targets (farm animals, humans or wild animals). Then, for each target, “biological hazard/wild bird order” pairs were ranked in decreasing order according to their risk estimate level. Pairs for which the risk estimate was higher or equal to “high to moderate” were identified as high priority for surveillance to control the risks for farm animals, humans or wild animals in Europe.

For the farm animal target, pairs identified to be monitored in priority combined five bacteria including *Salmonella enterica* with five wild bird orders mainly Charadriiformes and Passeriformes. For the human target, priority pairs combined foodborne disease bacteria including *Salmonella enterica* or the *West-Nile virus* with six wild bird orders, often Passeriformes. For the wild animal target, priority

pairs combined bacteria including *Salmonella enterica*, arboviruses including the *West-Nile virus* or *Trichomonas gallinae* with six orders mainly Passeriformes. This innovative work provides knowledge to improve wild bird disease surveillance in Europe and a novel, transparent and reproducible prioritisation method for animal diseases.

## **An epidemiological model for the European brown hare syndrome (EBHS)**

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In the last decades a progressive decline in the hare populations has occurred in Italy and other European countries. Among many other causes, this decline has been associated with the occurrence of the EBHS, a highly contagious disease of brown hare, emerged in the '80s and currently considered endemic in Europe. The EBHS infection can achieve almost 100 % morbidity, when it is introduced into a naive brown hare population, and the mortality can be about 60 % in infected adult. The disease is not observed in new-born hares that are infected without exhibiting any clinical sign and develops a long-lasting immunity. In order to gain new insights on the EBHS epidemiology, we developed a mathematical model considering both the hare biology and the EBHS infection dynamics in an age-structured population. The model aimed at investigating the influence of the hare population density on the EBHS epidemiology. A set of eight ordinary differential equations was used to describe a compartmental system, where individuals were classified into different age categories (new born = less than 3 months, young = 3 - 6 months, and adult = older than 6 months) and status with respect to the infection (susceptible/seronegative, infected and recovered/seropositive). The fluxes between different compartments were described by transfer functions representing development, fecundity, infection and recovery rates. The functions' parameters, including natural and EBHS-related mortality, were estimated from literature and from available observations. The model showed that EBHS transmission had a complex dynamic, strongly affected by hare density. In particular, at high-density value the virus was endemically maintained in the population, with a low mortality rate since most new borne or survivals had acquired the infection becoming seropositive. On the contrary, for low-density values, no constant transmission of EBHS between susceptible individuals was observed in the simulation and the virus was pushed to extinction within a short time. Therefore, low density hare populations did not support EBHS endemic persistence and were exposed to recurrent EBHS outbreaks due to a low or absent immunity. An intermediate epidemiological pattern was observed in the transition from low to high population density. These simulation results were consistent with the serological data collected in different protected but open areas in the province of Brescia (Northern Italy) over the last years. Model results suggested that the strategy of promoting the endemic circulation of the virus through density control mechanisms seems to be the best way to reduce the EBHS impact.

## Measuring the distribution and densities of wild mammals for disease control in the Balkans

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Successful campaigns to control diseases that involve a wildlife host require knowledge of the distribution and abundance of those hosts. We estimated mammal distribution and densities within 12 forest areas in Kosovo from camera trap data collected between July 2014 and December 2015 as part of the EU Control and/or eradication of animal diseases in Kosovo (DCE) project. Forest is Kosovo's largest land cover at 44 % of the land area. The study was carried out in conjunction with a wildlife rabies vaccination campaign to permit assessment of the status of the red fox (*Vulpes vulpes*) and grey wolf (*Canis lupus*) as potential hosts of the disease and the status of wild boar (*Sus scrofa*) as potential hosts of the Classical swine fever (CSF) virus. Data were also collected on species of conservation concern such as the European hare (*Lepus europaeus*) and brown bear (*Ursus arctos*). Wild boar, red fox, grey wolf and brown bear densities were found to be consistently high across the sites at which they were recorded and the mean Kosovo population sizes of grey wolf and brown bear were much higher than indicated by recent data from the same region. Mean fox densities estimated for Kosovo were above the threshold required to support a rabies epizootic, and the presence of the grey wolf may further add to the weight of infection in the potential wildlife host network. Wild boar were found at mean densities above the estimated thresholds required for CSF persistence. This study constitutes the first attempt to quantify the size and distribution of populations of medium to large sized mammals at the landscape scale in the Balkans, an area of Europe that is currently poorly represented in mammalian research. The methods that we used could inform similar disease control and/or wildlife management campaigns in this region and elsewhere.

## **Tasmanian devil microbiome - implications for conservation and management**

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The Tasmanian devil (*Sarcophilus harrisii*) is at risk of extinction due to a fatal contagious cancer known as Devil Facial Tumour Disease. The Save the Tasmanian Devil Program has established an insurance population, which now holds over 700 devils in captive facilities across Australia. One of the important goals of the programme is to ensure that the devils which are released back into the wild are robust and fit, both genetically and physically.

The microbiome plays a crucial role in the health of humans and other animals. Changes in the microbiome can impact on various aspects of host physiology and development. To improve our understanding of devils and facilitate management and conservation of the species, we characterised the microbiome of wild devils and investigated differences in the composition of microbial community between captive and wild individuals.

A total of 1,223,550 bacterial 16S rRNA sequences were generated via Roche 454 sequencing for 56 samples, including 17 gut, 15 skin, 18 pouch, and six oral, collected from 23 wild and eight captive devils. The microbiome of devils in captivity was found to significantly differ from that of wild individuals. Some zoo devils had considerably lower bacterial diversity in their gut flora, which can result in increased risks of obesity and thus lead to reduced success rate of captive breeding. As the microbiome is known to play an important role in shaping the host's immune and endocrine systems, there is a possibility that alterations in the microbiome of captive devils can have long-term impacts on their survival in the wild after reintroduction. Our findings highlighted the importance of monitoring and managing the microbiome of captive animals, not only for the Tasmanian devil, but also for the many other endangered species that are bred for release to the wild globally.

## **Production and characterisation of pan-avian monoclonal antibodies and their application in serological monitoring of wild birds**

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The role of the wild fauna as a reservoir for several infectious agents, including viruses, bacteria and macroparasites, may have a great impact on human and animal health. Wild birds may take part in the maintenance and dissemination of several pathogens, among which West Nile virus (WNV), Usutu virus (USUV), Avian Influenza virus (AIV), Newcastle Disease virus (NDV), *Salmonella spp.* and *Campylobacter spp.* might be some examples. Furthermore migratory birds could introduce emerging or neglected disease moving through different and distant areas. Serological surveys may be important in the identification of a pathogen circulation and in the knowledge of its epidemiology, although the lack of immunoreagents availability for the wildlife species often affects the feasibility of this type of investigations.

The aim of our work was the production and the characterisation of pan-avian monoclonal antibodies (mAbs), reactive against various avian species, in order to develop indirect ELISA tests, which may be useful in wildlife surveillance programmes. In the present study chicken's purified immunoglobulins and a pool of purified avian immunoglobulins belonging to four species' (*Columba livia*, *Pica pica*, *Gallus gallus* and *Coturnix coturnix*) were used to immunise Balb/c mice, in order to produce hybridomas following a standardised protocol. These hybridomas were firstly screened via an indirect ELISA test, using the homologous antigen. Then selected ones were characterised with an ELISA test, using immunoplates coated with avian immunoglobulins belonging to 39 serum samples of 14 avian orders (Anseriformes, Galliformes, Pelecaniformes, Ciconiiformes, Phoenicopteriformes, Struthioniformes, Falconiformes, Gruiformes, Charadriiformes, Columbiformes, Piciformes, Accipitriformes, Podicipediformes and Passeriformes) and also with mammalian immunoglobulins (using cattle, horse, sheep, goat, dog, cat, swine and human sera). This procedure allowed the characterisation of 105 hybridomas producing reactive mAbs against avian immunoglobulins, 31 of these were made with the avian immunoglobulins' pool antigen whereas the others 74 mAbs with the chicken's one. Two out of these cross-species reactive mAbs, which showed the higher reactivity (in OD value) with the avian sera and no or low reactivity with the mammalian ones, were selected, cloned, purified and then horseradish peroxidase (HRP) conjugated.

In conclusion, the main result of our study was the production of pan-avian HRP-

conjugated mAbs, which were used to set up indirect ELISA tests for the identification of specific antibodies against WNV, USUV and AIV in serum samples, which may be useful introduced into wildlife surveillance programmes.

## Field age estimation of the Eurasian lynx (*Lynx lynx*)

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The Eurasian lynx (*Lynx lynx*) was successfully reintroduced to Switzerland in the 1970's. By now the population can serve as a source for restoration programmes in Central Europe. The ability to age individuals is crucial to investigate population dynamics and disease epidemiology in wildlife. In the context of restoration programmes, age determination is necessary to choose suitable candidates for translocation. Exact age of lynx can be determined by counting tooth cementum annuli in extracted teeth (invasive and time-consuming) or calculated for individuals already identified as kittens (possible for selected individuals only, delayed results). The aim of this study was to develop a tool for aging lynx, which would be non-invasive, applicable under field conditions, economic, and would deliver immediate results. We took tooth pictures of 104 free-ranging lynx of known age from Switzerland. They included animals anaesthetised for marking procedures (n = 22), animals found dead submitted to necropsy (n = 43) and prepared skulls (n = 44). Tooth colour, number of incisors, dental calculus, extent of canine, premolar and molar wear, presence of fractures, open pulp cavities and enamel flaking were systematically recorded. A classification scheme was developed based on these criteria, distinguishing six partly overlapping age classes: < 1 year (n = 48), 1 - 3 years (n = 23), 3 - 6 years (n = 17), 7 - 9 years (n = 3), 9 - 12 years (n = 6), ≥ 13 years (n = 7). The suitability of the scheme was subsequently tested with the same lynx. It allowed to correctly classify 88/104 lynx (85 %). Lynx younger than one year (n = 47) were all aged correctly. Prepared skulls were most susceptible to wrong classification because not all classification criteria could be applied. When excluded, accuracy increased to 92 % (60/65). Lynx < 10 months (n = 38) showed deciduous dentition or signs of tooth replacement. Replacement sequence and corresponding age in months followed a homogeneous pattern in our material and were summarised in a separate aging chart for juveniles. Canine morphology and teeth colour were solid criteria in adults. Canine ridges were worn down in all lynx > 6 years old, and canine length was reduced to premolar size in all > 13 years old individuals. In conclusion, the proposed classification scheme is a promising approach to assign lynx to meaningful age categories. Individual variations and the low sample size available for certain categories currently limits the reliability of the current scheme. Nevertheless, it may be a useful tool when a non-invasive and immediate age estimation is needed and result in a harmonised methodology in the framework of international projects.

## Long-term dynamics of *Mycoplasma conjunctivae* infection in Pyrenean chamois (*R. pyrenaica*) and other sympatric hosts

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Infectious keratoconjunctivitis (IKC) is an ocular disease mainly caused by *Mycoplasma conjunctivae* in wild and domestic Caprinae. Chamois (*Rupicapra* spp.) is one of the most susceptible hosts which populations undergo sporadically severe IKC outbreaks that can affect its population dynamics. The potential reservoir role of other hosts for chamois has been extensively discussed.

The epidemiology of *M. conjunctivae* was studied in domestic and wild Caprinae from the Spanish Eastern Pyrenees during a seven-year period (2009 - 2015). Eye swabs from hunted wild Caprinae were collected from five different geographic units (Freser-Setcases, Cadí, Cerdanya-Alt Urgell, Alt Pallars and Vall d'Aran) and were analysed for the presence of *M. conjunctivae* by a qPCR. Species sampled were Pyrenean chamois (*R. pyrenaica*; n = 1030), mouflon (*Ovis musimon*, n = 77), red deer (*Cervus elaphus*, n = 109) and roe deer (*Capreolus capreolus*, n = 108). The highly variable part of the *lppS* gene of *M. conjunctivae* was sequenced to perform cluster analyses of the strains from selected qPCR-positive samples (Pyrenean chamois, n = 17; mouflon, n = 1) and from sheep (*Ovis aries*, n = 43 from 13 flocks) that seasonally graze in the alpine meadows from the same study areas. *Mycoplasma conjunctivae* was detected in 4 out of 5 study areas in chamois (2009 - 2015) and only in one study area in mouflon (2014). Prevalence by years and study areas in chamois ranged from 0 % (CI95 % 0.0 - 4.5) to 25 % (CI95 % 10.2 - 49.5) and was 33.3 % (CI95 % 12.1 - 64.6) in mouflon. No IKC epizootic conditions but isolated IKC cases were observed in chamois during the study period. *M. conjunctivae* strains from 2 different genetic clusters corresponding to 2 geographic areas (near Alt Pallars and Vall d'Aran as well as Freser-Setcases) were detected in chamois throughout the study period. Mouflon strain was different from that historically circulating in chamois from the same study area but closer to one sympatric chamois and to sheep strains. No shared strains of sheep with wild Caprinae were detected in this study. Although interspecific transmission of *M. conjunctivae* may sporadically occur, dynamics of the *M. conjunctivae* infection in chamois were not dependent on other sympatric hosts. Therefore, *M. conjunctivae* is endemic and self-maintained in chamois populations from the Eastern Spanish Pyrenees.

## **The effect of winter stress on heart rate variability in the Przewalski's horse (*Equus ferus przewalskii*)**

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Many large mammals living in habitats with harsh winter conditions exhibit pronounced seasonal changes in physiological and behavioural parameters. The body reacts to environmental stressors predominantly in two ways: by activating the hypothalamic-pituitary-adrenal axis, and by stimulating the sympathetic nervous system. To date, measuring environmental stress in wildlife has mainly focused on the quantification of glucocorticoids. In humans, heart rate (HR) and heart rate variability (HRV) have been used to investigate physiological and psychological stress. Healthy cardiac function is characterised by irregular time intervals between consecutive heart beats. This implies vagal dominance, because the sympathetic influence on the heart is too slow to produce rapid beat-to-beat changes. Hence, a low HRV indicates an increased sympathetic/decreased parasympathetic tone and has been associated with stress.

In order to investigate the effect of seasonal stress on changes in HRV in the Przewalski's horse (*Equus ferus przewalskii*), we evaluated HR and HRV in six GPS-collared pregnant mares using subcutaneous heart-rate telemetry units. The study was performed between October 2008 and December 2009 in the Hortobágy National Park in Hungary, providing an undisturbed and natural setting to the horses. We applied generalised linear regression models with HR and HRV as response variables and the time of the year as explanatory variable.

HR and HRV varied periodically throughout the year, showing lower values during the winter than during the summer months. HR peaked in April along with a pronounced drop in HRV.

The low HR during winter indicates a reduction of cardiovascular oxygen demands and metabolic rate. The concurrent decreased HRV indicates a reduction of the vagal tone and hence, stress due to energetic bottlenecks. The pronounced drop of HRV in April might reflect an additional stress response during the mating/foaling season.

In this study we show that, measuring telemetric HRV is a probate method to study undisturbed reactions of wild animals to their changing environment over the long term. Przewalski's horses experience a loss of complexity in cardiovascular dynamics during winter, indicating an endogenous seasonal stress response. The mating season is energetically costly and leads to a peak in stress response.

## **Biosecurity at the livestock-wildlife interface: farm characterisation and design of a field protocol**

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Extensive livestock farming is an important component of the landscape and a primary sector in south-central Spain. In this type of farms, water and food resources are scarce for a long period along the year, which conduct to wildlife (especially wild boar and red deer) and domestic livestock to share resources. Proper management of these interactions can help to reduce direct and indirect transmission of infectious pathogens at the interface. We visited 53 cattle farms in south-central Spain to assess *in situ* all the risk points for wildlife-livestock interaction, for which we developed a standardise field protocol to assess biosecurity. We proposed specific biosecurity plans for each farm to reduce interspecific contacts, which are mainly indirect. With the information obtained so far we have been able to conclude that:

- i) Complex situations where different hosts are present, in addition to the wild, are common. Extensive pig may act as epidemiological driver among wildlife and cattle.
- ii) In a not inconsiderable proportion of farms (almost one third), supplementary feeding is provided to wildlife for hunting purposes.
- iii) Among the measures proposed, apart from wildlife population control, the most frequent recommended practices included ensuring that animals drink together safely in each of the fences and the intelligent management of grazing.
- iv) The most expensive measures refer to the need for perimeter game fences or livestock fences. Regarding the cost per farm to implement a biosecurity program, provisional data indicate that in 70 % of the costs does not amount to more 100 per cattle.

In the near future, the efficacy and practical applicability of these programmes is going to be evaluated by means of different disease models, so as the design of biosecurity protocols for extensive pig and goat.

## **Social network analysis of Doñana National Park ungulates in an epidemiological context**

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Social Network Analyses (SNAs) are useful tools to describe the intra- and inter group interactions in order to understand the community behaviour and potential transmission ways of pathogens. SNA can lead to a better understanding of factors and agents involved in the dissemination of infectious diseases and help to implement more effective control strategies. Such studies become important for those multi-host diseases, such as bovine tuberculosis in ungulates from southwest of the Iberian Peninsula. We used proximity GPS-GSM collars and environmental proximity data loggers (PDL) to (i) describe intra- and interspecific interactions between wild and domestic ungulates in Doñana National Park (DNP); (ii) to identify individuals or species with highest contact tendency; (iii) and to study the spatio-temporal variation of these contacts. Wild and domestic ungulates were collared: red deer (RD; n = 4), fallow deer (FD; n = 4), wild boar (WB; n = 5) and cattle (C; n = 7). Additionally, 16 PDLs were set along different environmental resources. Each collared individual and PDL included in the SNA framework was defined as a node. We obtained a total of 11755 edges or contacts within a network of 35 nodes in which all species of ungulates and environmental PDL were represented; 2670 of these contacts occurred between individuals. Regarding contact frequency, the pairs of nodes with highest number of edges occurred between C – C, FD – FD and RD – FD. However, the most intense –in terms of closeness– contacts were observed between C – C and FD – C. In addition, four different communities of individuals and PDLs were identified along a continuous area; these communities were composed with individuals of the four species that interacted with the same environmental resources. In spite of intraspecific contacts being more frequent, we interestingly found that individuals composing different communities over the same area. This research provides understanding into the local factors affecting disease transmission and persistence in multi-host systems, evidencing that mitigation of both inter and intraspecific contact is key to control disease at the wildlife-livestock interface. Our future research aims to combine SNAs and molecular epidemiology.

## **Wildlife disease management in practice: oral vaccination to control wild tuberculosis**

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Disease management in wildlife is crucial in shared diseases with impact in conservation, economy and human health such as animal tuberculosis (TB). We report a field experiment on TB control in wild boar (*Sus scrofa*) - the main wildlife reservoir in Mediterranean scenarios - through oral vaccination in a highly prevalent area with an increasing TB prevalence trend.

The study was conducted in Montes de Toledo (Central Spain) during six years (two control years plus four years vaccinating) and involved 10,000 vaccinated ha and 50,000 control ha. Fifteen sites were used as controls (a mix of private and public hunting estates and protected areas). Vaccinated areas cover two situations: (1) a high initial prevalence (85 %), corresponding to a non-managed Natural Park (NP) and (2) a medium initial prevalence (55 %), corresponding to managed hunting estates (HE). In each of these settings, one site was treated with the live vaccine BCG and another one with a heat-inactivated *M. bovis* vaccine, IV (total of four treated sites = BCG-NP, IV-NP, BCG-HE, IV-HE). The vaccines were deployed via chemically marked-baits in selective feeders to target wild boar piglets to prevent infection acquisition. Each year summer, 18,000 marked baits were distributed among sites. TB prevalence was assessed by culture and presence of TB compatible lesions in hunter-harvested individuals.

Bait uptake, estimated by chemical marker detection in piglets, was 39 % and 77 % in the BCG- and IV- NP sites, and 94 % and 92 % in the BCG- and IV- HE sites. TB prevalence showed a 14 % increase in the control area and a 10 % in BCG-NP, a slight decrease in BCG-HE and IV-NP sites (11 and 12 % respectively) and a 74 % decrease in IV-HE site when comparing initial and final prevalences. The IV-HE site reached a 100 % reduction in the incidence rate among piglets and a progressive decrease in prevalence in older age classes during the study period. These results suggest that IV vaccination does not only protect piglets under field conditions, but can achieve an effect at the population scale. Nevertheless, protection seems to be context-dependent as results are different in the high and low initial prevalence setting. This could be due to different bait uptake rates or to

the different initial TB prevalence. Our results suggest that IV vaccination might be a useful single tool for TB control in wild boar. Further research will explore its combination with other tools within integrated disease management schemes.

## **Health outcomes and disease risk management in wildlife conservation translocations: a review of IUCN case studies**

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Conservation translocations, which include reintroductions, are part of the toolkit for recovering populations of endangered wildlife species. Although they have achieved some notable conservation successes, overall, conservation translocations appear to have had relatively low success rates to date. We aimed to explore health outcomes and the perceived impact of morbidity / mortality events in wildlife conservation translocation projects, as well as health management measures employed.

The IUCN's 'Global Re-introduction Perspectives' is a five-book series (Soorae 2008, 2010, 2011, 2013 & 2016) comprising nearly 300 case studies of plant, invertebrate, fish, amphibian, reptile, bird and mammal translocation. The case reports have standardised sub-headings, and authors rate the success of projects subjectively as 'Highly successful', 'Successful', 'Partially successful' or 'Failure'. We reviewed case reports of conservation translocations and described as 'notable' any episode of morbidity or mortality that was mentioned, or that was related to a management measure mentioned, under one or more of the following sub-headings: 'Major difficulties faced', 'Major lessons learned' or 'Reasons for failure'. We investigated the frequency with which 'notable' morbidity / mortality events were reported, their confirmed or suspected cause, and the relative frequency with which they were reported in different taxonomic groups. We also noted whether health management was reported as a component of project planning.

Over 240 case studies were included in the review of health outcomes, comprising projects across taxonomic groups, with mammalian translocations described most commonly. 'Notable' morbidity or mortality events were reported in over 70 % of projects; projects considered 'Highly successful' had the lowest reported level of 'notable' morbidity / mortality. Some form of health management planning was mentioned in over 50 % of case reports, although less than 3 % of reports mentioned that a disease risk analysis (DRA) or disease-related risk assessment had been conducted. We will describe our results in more detail and explore differences in reported health outcomes and health management practice between the major taxonomic groups.

Our findings provide evidence to help inform health management decisions for conservation translocations and suggest that DRAs will improve the chances of conservation translocations being successful. Health management should therefore be an integral part of translocation planning.

## **The use of assisted reproduction in Columbian sharp-tailed grouse (*Tympanuchus phasianellus columbianus*) as management tool in a species conservation project**

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The US population of Columbian sharp-tailed grouse (CSTG) has decreased extensively. Some conservation actions aim for the re-establishment of a self-sustaining population within the species' historic range in Nevada by translocations from larger source populations in Idaho. During a short period female CSTG visit leks to select mates and copulate before departing to nest alone. At the leks, capture of individuals is possible, but the prediction of temporal limits to female lek visitation is challenging. Females captured early during the mating period likely have not been naturally inseminated, increasing the risk of absent offspring upon release. However, it is logistically difficult to time the capture of females to occur late in the breeding period after they copulated, as they may not return to the leks.

Assisted reproduction was evaluated as a tool to facilitate timely capture and translocation of artificially inseminated CSTG, which then can lay fertile eggs after release. Using massage and a novel electro-stimulation technique, semen collection was attempted 51 times in 47 males and a complete semen analysis was performed. Seventeen females were artificially inseminated prior to release.

Semen collection was successful in all attempts. Semen volume ranged between 6 and 74  $\mu$ l. Median sperm motility was 43.3 %, median sperm viability 85.3 % and median sperm concentration 70,000/ $\mu$ l. In average 42.81 % of sperm were morphologically normal and 34.81 % had abnormal tails.

Paternity tests from eggshells are pending, but first tracking results demonstrate fertile clutches of artificially inseminated females and suggest assisted reproduction as useful tool in conservation projects.

## **The Sabah Rhino Breeding Programme: reproductive pathology of wild-caught Sumatran rhinoceroses and its implications in conservation**

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) was once found throughout Southeast Asia, but intensive hunting and habitat loss contributed to a precipitous decline in its numbers during the first half of the 20<sup>th</sup> century. In 1984 it was agreed that in addition to the protection of wild individuals it was time to establish a captive breeding programme. In the 32 years that followed, 46 animals were captured from the wild, resulting in only 2 breeding pairs and the death of 41 individuals. The Sumatran rhinoceros stands now on the verge of extinction, with only 9 captive animals and less than 100 free-roaming individuals scattered in three national parks in Sumatra. Since 2009 the Leibniz Institute for Zoo and Wildlife Research (IZW) has been collaborating with the Borneo Rhino Alliance (BORA) through the use of advanced imaging and assisted reproduction techniques on the 1.3 Bornean rhinoceroses (*Dicerorhinus sumatrensis harrissoni*) held at the Borneo Rhino Sanctuary, in Sabah. When captured from the wild, both young cycling females showed severe reproductive pathology that rendered them incapable of carrying a pregnancy, namely extensive cystic endometrial hyperplasia and a large number of uterine leiomyomas. Going back in time, several reports of reproductive pathology in Sumatran rhinoceroses suggest that at least 50 % of wild-caught females were affected by similar conditions, with variable consequences to their fertility. It becomes clear that low densities of a slow-breeding solitary species like the Sumatran rhinoceros drastically decrease the possibility of finding a reproductively active partner in the short frame of sexual receptivity (one day of oestrus in a 21 to 27-day cycle), thus leading to long non-reproductive periods in wild individuals. As described for other rhinoceros' species in captivity, the organs of non-breeding females are exposed to sex steroids for prolonged periods of time due to continuous ovarian cycle activity. This results in a phenomenon known as "asymmetric reproductive aging", which comprises the development of reproductive pathology, depletion of the follicular stock and premature senescence. The distressing discovery of this phenomenon occurring naturally in free-ranging Sumatran rhinoceroses clearly reflects the "Allee effect", which postulates that with low population size or density comes reduced individual fitness. The "Allee effect" and more specifically the phenomenon of "asymmetric reproductive aging" are now believed to play, both in the past and at present time, one of the most important roles in the fall of the Sumatran rhinoceros.

## **Devastating snowball formation on the radio-collar of a wild reindeer (*Rangifer tarandus tarandus*)**

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On the 14<sup>th</sup> of March 2015 by-passers called Norwegian Institute for Nature Research (NINA) to tell that an adult female reindeer was found walking alone in the mountains of Nordfjella in Southern Norway with a large ball of ice and snow attached to its radio collar. The animal seemed worn out, and walked and stood only for short periods before it had to pause, lower its head and kneel, so that the snowball rested on the ground. The day after the initial observation, the reindeer was found by a mountain ranger called out by NINA and a local guide. They considered the reindeer to be in poor condition and observed a large open wound under the collar, and chose to cull the animal to relieve it from suffering. The head and neck of the euthanised reindeer were cut off from the body. Together with the snowball and the collar they weighed 23 kg. At necropsy the snowball measured 25 cm in longitudinal, 30 cm in dorsoventral and 35 cm in mediolateral direction. After thawing away the snowball, the head and neck weighed 6.31 kg, while the collar itself weighed 1.0 kg, indicating that the snowball had weighed 15.69 kg while still on the animal. The point of origin of the snowball formation seemed to be the cotton break-away zone at the lower part of the collar, connecting the plastic collar with the battery box and partly protected by a flap of plastic. The rough surface and the protecting flap may have allowed snow to build up. The weather in the time before the reindeer was found was very volatile, with repeated periods of mild weather and precipitation both as rain, sleet and snow accompanied by strong wind, followed by very cold periods. These cycles of relatively high temperatures and precipitation followed by low temperatures may have facilitated repeated build-up and hardening of the snowball.

Based on our own experience, literature searches and informal surveys, we think this is something that occur very rarely. But – given that volatile weather is predicted to occur with increasing frequency, it is important to consider the danger of icing and optimise the construction of radio collars in order to avoid such incidents.

## **Transferring a structured 3R approach from laboratory animal sciences to the wildlife field**

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The principles of the 3Rs (replacement, reduction, and refinement) are well established within laboratory animal sciences, aiming at improving animal welfare and reducing and replacing animal use. The 3Rs as a concept has not yet gained the same attention in wildlife research, a vital part of wildlife conservation and management. Three R efforts are being made today in wildlife research, e.g., replacement with noninvasive research techniques, reduction with optimised experimental design, and refinement through improved methods of capture, anaesthesia, and handling. However, wildlife research would benefit from transferring a more structured 3R approach from laboratory research to the field. Experiences from a large Swedish toxicology unit show that organising the 3R work increases collaboration within and between research groups as well as the number of 3R activities, and decreases the animal use. This enhanced implementation and awareness of the 3Rs were the result of structured work with continuous improvements. The number of implemented refinement activities increased almost five-fold (from approximately five per year to 25 per year) and the proportion of collaboration between research groups increased from 10 % to 42 %. Prerequisites of structured 3R-work with continuous improvements are organised, designated project teams with a well defined purpose and tasks, and identified key problems and possibilities. The team can be arranged within or between research groups and should preferably consist of not more than eight team members. An active participation is required, with a team accountability where the team owns the problem and the solution. Regular meetings with a standard agenda including set-up and follow-up of action improvement plans between meetings are required. A key success factor is management

approval, support, and feedback. Structured work with the 3Rs increases the awareness among staff and stimulates the implementation of the 3Rs into research activities and development. We emphasise the importance of applying the 3Rs systematically throughout the research process. Despite differences between laboratory animal and wildlife research, transferring a structured 3R approach from laboratory animal sciences would enable further achievements in combining ethics, animal welfare, and high quality science in wildlife research.

## **Host movement constraints can critically influence pathogen virulence evolution**

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It is well documented that infections can change host behaviour, e.g. by causing lethargy and reduced movement. However, how such behavioural changes affect, in turn, epidemiological and evolutionary dynamics remains poorly understood. A well-known hypothesis proposed by Paul Ewald states that the influence of behavioural changes on virulence evolution depends on the transmission mode: vector- and water-borne transmission does not depend on host movement and related host contact, whereas direct transmission does. To compensate for reductions in host contact rates, directly-transmitted pathogens should therefore evolve lower virulence compared to vector- and water-transmitted pathogens.

While Ewald's hypothesis is consistent with empirical data on human diseases, its validity across different wildlife systems remains to be investigated. An implicit assumption in Ewald's argument is that water only influences the mode of transmission but not host behaviour per se. However, in dry ecosystems host movement behaviour can be strongly constrained by the distribution of water. Here we developed a mathematical model to investigate how limited water availability can influence host contact patterns and the evolution of pathogen virulence.

In absence of water limitations we recovered results that are consistent with Ewald's hypothesis. However, in cases of limited water availability our findings contradicted Ewald's hypothesis. Specifically, we found that infection-induced reductions in movement led to higher contact rates. This counter-intuitive result emerged because to reduce movement, infected individuals needed to stay in areas closer to water where encounter rates with others were highest. As a consequence of increased contact rates in our model, directly-transmitted pathogens became more virulent compared to water-transmitted pathogens.

Taken together, our results support the idea that infection-induced behavioural changes can critically influence virulence evolution. However, deducing the consequences of such behavioural changes is not always trivial. Our finding that movement constraints can critically influence virulence evolution questions the generality of Ewald's hypothesis and related management recommendations. Ewald predicted that for pathogens that use both transmission modes, the protection of water sources should reduce virulence. In contrast, our results show that under conditions where water-borne transmission is most efficient – i.e. when water availability is limited – protection of water sources might have the opposite effect of increasing virulence.

## **Spatial behaviour of wild boar does not predict spatio-temporal dynamics of the African Swine Fever in the wild boar population**

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The African Swine Fever (ASF) is a highly contagious hemorrhagic viral disease which affects wild and domestic suids, resulting in very high mortality among infected animals. The ASF is one of the most devastating infectious diseases of pigs and due to its severe socio-economic impact and transboundary character, it represents a major threat to pig production sector. Introduction of ASF into the Caucasus region in 2007, rapid spread across Eastern Europe, and entry to the European Union in 2014, has raised concerns about its further spread westwards. The ecology and behaviour of wild boar (*Sus scrofa*) – host of the ASF virus in the wild – may facilitate disease expansion due to high mobility and sociality of the species and its abundance in Europe. However, the role of wild boar behaviour in the epidemiology of the ASF is poorly understood. Here, we investigated the relationship between spatio-temporal patterns of the ASF spread in the wild boar population and seasonal changes in the wild boar spatial behaviour, hypothesising that increased movements and contact rates should result in elevated number of infections and expansion of the infected area. Using epidemiological data collected during 2-years period following first detection of the ASF in north-eastern Poland in February 2014, we calculated monthly prevalence, incidence rate, increments in the size of infected area, and the rate of spread towards west. We used radio-tracking data from 50 wild boar, originating from the population where the ASF virus is currently circulating, to estimate monthly range size of adult males, adult females, and dispersal distance of yearlings. During the study period, 140 infected animals were hunted or found dead and the mean prevalence was 5 %. Monthly prevalence and incidence rates were the highest in spring and the lowest in autumn. The variation in the size of monthly ranges of adult males and females did not influence ASF prevalence or incidence rate. Increased movements of yearlings tended to correlate with higher ASF prevalence, but not with the incidence rate. The epidemic frontline was advancing with an average speed of 1.5 km/month and the total size of the infected area reached 2,060 km<sup>2</sup>. The expansion rate of the infected area did not vary seasonally and was not affected by the spatial behaviour of wild boar. Our data suggests that spatial behaviour of wild boar plays a minor role in shaping patterns of ASF spread and persistence in the wild boar population.

## **Towards targeted removal of infected individuals: A novel approach for trap-side diagnosis of bTB in badgers**

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Population reduction by culling has been widely adopted for the control of infectious diseases in wildlife populations. An intuitively appealing and potentially more ethical approach would be to only remove infected individuals, although there are considerable practical challenges in doing so. The current strategy for achieving bTB free status in England includes badger culling within licensed, high bTB incidence areas. This study describes the development of a method for the safe restraint, blood sampling and identification of bTB antibody positive animals in the field.

We developed a specially designed cage to physically restrain and blood sample badgers without recourse to anaesthesia. The device was tested 48 times using badgers caught during routine trapping operations at Woodchester Park, Gloucestershire, UK, in 2014 and 2015. Capillary blood samples were collected and immediately applied to a serological cassette (ChemBio's DPP® VetTB) with a 20 minute run time. Post restraint we examined animals for injury under general anaesthesia, and used repeat capture rates during the following 12 months to assess longer term outcomes.

We were successful in obtaining a blood sample on 41 of 48 occasions. Blood volumes averaged  $7.44 \pm 3.04 \mu\text{l}$  (range 2.5 - 10, n = 41). All samples produced a valid test result. Animals were restrained for an average of  $3.38 \pm 1.32$  (SD) minutes (range 1.70 - 7.23, n = 41). Examination of restrained animals failed to detect injury over and above the 4mm lancet incision site. Contrary to expectation, animals that underwent restraint and conscious sampling had a higher rate of recapture during the following 12 months than animals which did not (IRR 2.09 95 % CI 1.50 -2.91). One possible explanation is that we unintentionally selected more compliant and potentially re-trappable animals for the trial.

The results of this study provide proof of concept for trap-side bTB testing in badgers and could present an opportunity for targeted bTB control interventions e.g. the removal of infected individuals or groups of badgers. Naturally, the potential success of such an approach is contingent on the availability of a sufficiently sensitive and specific diagnostic test and it is possible that the DPP® VetTB does not meet these criteria. However, novel tests may become available in the future. Finally, our methods may have more generic value in informing strategies for sampling wild animals for example in endangered host species where treatment might be important for affected individuals and for which anaesthetic risk might otherwise prohibit collection of diagnostic samples.

## **One decade of health monitoring in German free-ranging wolves (*Canis lupus*)**

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After a century of eradication, the Central European lowland wolf population has grown steadily in Germany and Poland within the last 16 years, and wolves are still a strictly protected species. Therefore, a health monitoring was established to identify potential health problems arising during resettlement and population expansion. The Leibniz Institute for Zoo and Wildlife Research (IZW) initiated this programme using post mortem investigations on underlying infectious diseases and causes of death in the German wolf population. It combines computed tomography, necropsy, histology, parasitology, bacteriology, virology and electron microscopy. Our results show that most wolves died due to traffic accidents and illegal killing. Only canine distemper virus infections were detected so far. Other tested viral diseases such as rabies, pseudo rabies, and infectious canine hepatitis were not confirmed. Additionally, quite a few cases of mange were detected. In conclusion, wolves from Germany play a minor role as vector of viral and parasitological infections of veterinary and zoonotic importance. Further investigations will identify potential wolf-driven transmission risks of infectious diseases to other wildlife and domestic animals during wolf population expansion. Intensifying the wolf health monitoring will help to create a reliable German and even European conservation management plan. Consequently, in cases of potentially arising health problems scientifically-based decisions and reactions can be implemented.

## **A local outbreak of tularemia in European hares (*Lepus europaeus*) in the Netherlands**

MAAS MIRIAM

on behalf of the Dutch project group on tularemia, including researchers of the National Institute for Public Health and the Environment (RIVM), Central Veterinary Institute of Wageningen UR, Dutch Wildlife Health Centre, Netherlands Food and Consumer Product Safety Authority, Public Health Services, Environmental Sciences Wageningen UR and GD Animal Health.

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Tularemia is a zoonotic disease, caused by the bacterium *Francisella tularensis*. Up to 2011, tularemia had only been reported in 1953 in the Netherlands. However, since 2011, six autochthonous human tularemia cases have been reported and *Francisella tularensis* was detected in 18 hares across the Netherlands. Humans and hares were all infected with subsp *holarctica*.

From February 2015, a sudden increase in tularemia-associated hare deaths was noted in a 50 km<sup>2</sup> area in the northern province of Friesland. This triggered a multidisciplinary and multi-institutional investigation in order to increase knowledge on tularemia ecology in the Netherlands and to improve risk management. In 2015 in the province of Friesland, several wildlife species were tested by histopathology and/or PCR. Arthropods and surface water were tested by PCR. Pro-active human case finding was performed by retrospective searches of laboratory records and by additional PCR testing of biopsies of patients with tularemia in the differential diagnosis. Questionnaires and interviews with local stakeholders were performed to gain more insight in the ecological processes preceding and during the outbreak.

Between February 2015 and May 2015, of the 40 hares that were tested in Friesland, eleven were found positive for tularemia. At two time points in April and May, surface water and/or sediment was tested on 34 locations, and 11 of these locations were positive for tularemia. At four locations, also sediment samples were positive. None of the 257 tested common voles were positive, neither were the mosquito larvae, adult mosquitoes, biting flies or ticks. No human cases were identified.

This is the first documented tularemia epidemic in Dutch hares. The detection of *F. tularensis* DNA in water and sediment specimens provides evidence for an aquatic cycle in the Netherlands, but questions about the relative contribution of different reservoirs and transmission routes remain. It does however emphasise the importance of sampling surface water and sediment, if available, in future outbreaks.

## **PLENARY TALK: Beluga whales of the St Lawrence River: life and death**

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The widespread distribution and poor spatial delineation of populations of marine mammals make it difficult to define the causes of mortality and the respective contributions of natural factors and human activities to these deaths. A possible exception is the beluga population that inhabits a stretch of the Saint Lawrence Estuary (SLE) about 500 km away of Montreal (Quebec, Canada). The SLE drains the Great Lakes, historically the most industrialised part of North America; for decades, the SLE has thus received a heavy input of industrial contaminants, municipal sewages and agricultural run offs. SLE beluga, the southernmost beluga worldwide, are unique by their accessibility to investigation and geographical isolation from the Arctic, which is the natural habitat of most other beluga populations. This isolation has been confirmed by genetic analysis and by the distinctly higher levels of industrial contaminants we and others have found in SLE beluga tissues. Beluga are similar to people in several ways: they are long lived mammals; similar to many Westerners, their body lipid content is about 40 % of their body weight; they occupy a top position in the food chain, and the durations of their lactation (20 - 24 mo.) and pregnancy (14 - 15 mo.) are comparable. Many pollutants and pathogens released on land are carried in marine or aquatic environments. Together, these factors make of SLE beluga suitable environmental sentinels to monitor the health of both marine and land ecosystems.

From 1983 to 2012, a total of 472 beluga were found stranded dead. Of these, 222 beluga (178 adults, 25 juveniles, and 19 newborn calves) were examined. Infectious diseases, the most prevalent cause of mortality, accounted for the death of one-third of all beluga (32 %). Verminous pneumonia caused the death of 13 juvenile beluga (52 % of juvenile beluga). A total of 39 cancers were observed in 35 beluga. Of those, 35 beluga, 31 were affected by cancers severe enough to have caused death (20 % of beluga > 19 years old). Environmental contaminants likely contributed to high rates of cancer and infectious diseases in this population. Dystocia and postpartum complications were the cause of death of 18 beluga (19 % of the females > 19 years old examined). The occurrence of parturition-associated complications and mortality of calves < 1 year old, have increased dramatically since 2010. Disturbance of pregnant mothers or mothers with calves by small crafts is suspected to have contributed to the latter events.

## **Life history correlates of immune profile in roe deer**

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Eco-immunology predicts that life history should be related to immune investment at the levels of species but also populations and individuals. Slow pace of life, limitation in resources or intense trade-off with other functions should result in qualitative shift towards more specific, antibody-mediated immunity and less innate and inflammatory responses.

We tested these predictions by characterising the immune profile of 378 roe deer captured between 2010 and 2015 in two populations. The two study sites markedly differed in the level of resources available. Using blood and faecal samples, we characterised the humoral (haptoglobin, hemagglutination and hemolysis capacities, alpha2 and gamma-globulins) and cellular (numeration and distribution of leucocytes, Neutrophil/Lymphocyte ratio) immunity of each individual. We also measured body condition (using body mass and several metabolic parameters) and the level of parasitism (using coproscopic examination). Each set of markers (immunity, body condition and parasitism) was analysed using principal component analyses (PCA). We then analysed whether each axis of the immunological profiles was related to population, age, sex, season, body condition and level of parasitism. PCA allowed us to define four main axes of immunity. These axes of variation corresponded to the intensity of cellular responses (PC1), the intensity of humoral response (PC2), the innate-adaptive gradient in cellular immunity (PC3) and the level of specific, mainly antibody-based, humoral response (PC4). As expected, roe deer living in the population with lowest resource availability had lower cellular responses but higher humoral and adaptive immunity than roe deer individuals benefiting from highest resources. The immune profile also varied with age, body condition and season. Adaptive, antibody-based immunity was strongest in old individuals, in roe deer having good condition and late in the season. On the contrary, no variation was detected with sex or depending on the level of parasitism. Our results confirm that immune parameters shape a multi-dimensional landscape that varies across populations, time periods and individual attributes. We discuss the interpretation and perspectives of such findings.

## **Mixed infectious disease outbreak in a herd of semi-domesticated reindeer in Sweden: alphaherpesvirus, parapoxvirus, and oral necrobacillosis**

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An outbreak of infectious diseases affected a herd of semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*) in Northern Sweden (March - April 2016). The herd had been fed ensilage since January, and was herded to the calving ground where they were corralled a few days prior to the outbreak. Five calves, two yearlings and three adult females were during one week affected by clinical symptoms typical of oral necrobacillosis. They stopped eating, had poor body condition, were drooling, and had discoloration of the hair around the mouth. Some individuals had large, black, necrotic lesions in the tongue and gingiva, or holes perforating the chin or tongue. One of these animals, and another eight reindeer in poor condition also had proliferative lesions in the oral mucosa and on the mucocutaneous junction around the mouth, characteristic of the zoonotic disease contagious ecthyma, caused by parapoxvirus. Out of eleven other inspected reindeer, two calves and one adult had keratoconjunctivitis, suggesting infection with cervid herpesvirus 2 (CvHV2). For diagnostic analysis, blood samples were obtained, and eye, nose and genitalia were swabbed for virus detection. From dead/euthanised animals, mucosal lesions were sampled. PCR amplicons of characteristic size for parapoxvirus (*GIF*, *B2L*) were generated from two animals; from a necrotised tongue and a proliferative oral lesion. Antibodies against CvHV2 were detected in 10 of 13 diseased animals and in 4 of 11 apparently healthy reindeer. *Fusobacterium necrophorum* is often regarded a secondary infection. Here, CvHV2 and parapoxvirus may have been the primary agents producing mucosal lesions, a port of entry for the bacteria. CvHV2 is endemic in Fennoscandian reindeer. In contrast, parapoxvirus infections, occurring almost annually in Finland since 1992, have been reported in Sweden only in 1973 and 2015, and in Norway in 1999 and 2000. Corralled semi-domesticated reindeer is associated to stress and increased animal density, contributing to disease outbreaks. This is the first documented case in reindeer where characteristic clinical symptoms of three well-known reindeer diseases were identified simultaneously.

## **What are the conservation and zoonotic implications of the wild meat trade in Malaysia?**

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The rise in commercial hunting of wildlife for food presents a major threat to biodiversity in tropical forests, whilst additionally posing zoonotic infection risks to humans from contact with wild animals. The study aimed to improve the understanding of the drivers of the wild meat trade, examine its impacts on wildlife populations in Malaysia and determine the potential zoonotic risks to humans. It utilised survey data from establishments selling wild meat across Malaysia (collected by TRAFFIC Southeast Asia in 2012) in order to identify commonly traded species and to provide data on wild meat availability for each species sold. A literature review explored the cultural, social and economic factors driving the trade of specific species. The impacts of this trade on these wildlife populations were assessed through an appraisal of the hunting threat (based on IUCN Red List data) and an analysis of survey and quantitative population data. The literature review also determined the potential zoonotic infection risks and transmission routes to humans involved in hunting, butchering and consumption of the wildlife taxa sold. The survey data highlighted the great diversity of wildlife traded for food (over 25 species) and the study identified that wild pig (81.1 %), deer (45.3 %) and reptile (16.6 %) species were the most commonly available taxa in Malaysia. Although the cultural traditions of indigenous people favour wildlife consumption, the majority of hunting is now for commercial rather than subsistence reasons. Whilst the results suggested that the wild meat trade may have significant impacts on the populations of several wild ungulate species (e.g. *Rusa unicolor* and *Sus barbatus*), its impacts may have been under-estimated for other species due to insufficient data. In addition, the study highlighted the great diversity of zoonotic pathogens (16 viruses, 19 bacteria and 16 parasites) potentially hosted by harvested wildlife and of risk to human health. It focused discussion on the most significant zoonotic infection risks from commonly sold taxa e.g. *Hepatitis E*, *Brucella*, *Trichinella* and *Escherichia coli* from wild pigs. Finally, a microbial food safety risk assessment of Shiga toxin-producing *E. coli* from the consumption of wild deer in Malaysia was proposed, which illustrated the importance of understanding foodborne infections from wild meat consumption. Overall, the intention of the study was to raise the profile of this wildlife exploitation issue and increase awareness about the possible human health risks, which are relevant for conservation and public health strategies in Malaysia.

## **Anthropogenic microbial marine pollution as a likely contaminant of coastal environments and source of infection for wild seals**

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Human pathogens, particularly bacterial pathogens such as *Salmonella* spp. and *Klebsiella* spp., have been implicated as causing infections in seals and sealions in Europe and several other parts of the world. It is likely that a proportion of these infections are probably acquired from human sewage discharged into the coastal environment, however this pathway at present cannot be proven. Serotyping and molecular characterisation of the pathogens from seals, compared with human isolates, together with analysis of antimicrobial sensitivity patterns, provide clear indications that some bacterial isolates from seals, are derived from man.

These early findings may have several implications. Firstly that bacterial pathogens causing severe and occasionally fatal disease in man are contaminating coastal waters and environments, probably through the sewage risk pathway. These pathogens are infecting seals, and possibly other marine wildlife. These pathogens sporadically cause disease in seals. The pathogens are not-infrequently antimicrobial resistant and therefore introduce antimicrobial resistance to the coastal natural environment. The findings, indicating that pathogens are present in coastal waters may present a health risk to humans bathing and utilising coastal waters for recreational activities and sporting pursuits. Finally, marine mammals may play a role as sentinels of health risks to humans associated with coastal waters.

In this presentation, the published work on human pathogens isolated from seals is reviewed, with examples from Europe, California and New Zealand. We describe investigations into deaths in three common seals (*Phoca vitulina*) from colonies on the East Coast of England, where we consider that each animal has been infected with bacteria of probable human origin.

## **Diagnosing drowning in seabirds: How the gross pathology differs from that in other causes of mortality**

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Seabird mortality due to entrapment in fishing gear is a global problem and is believed to contribute to population declines. The aim of this study was to characterise the gross pathology of drowned seabirds and compare the lesions with those observed in other causes of mortality. Necropsies were performed on 179 birds (103 guillemots (*Uria aalge*), 32 razorbills (*Alca torda*), 37 shags (*Phalacrocorax aristotelis*), 7 great northern divers (*Gavia immer*) that stranded or were retrieved on Cornwall's coast following incidents of: drowning in fishing nets (n = 12), oil pollution (n = 15), polyisobutylene (PIB) pollution (n = 1), adverse weather (n = 1), unknown causes (n = 8). Approximately 80 % of birds were examined whilst in fresh or slightly autolysed condition and the rest had been stored frozen. The drowned birds (40 guillemots, 28 razorbills, 28 shags, 5 divers) were, with few exceptions, in good to excellent nutritional condition. Approximately half had recently ingested fish in the upper alimentary tract. The major lesions did not vary between species. In freshly dead birds the air sacs, and in some cases the lower trachea, contained a variable amount of clear watery fluid; lungs were intensely congested, swollen and oedematous with white frothy fluid exuding when excised. In partly autolysed or thawed frozen birds the fluid in the air sacs or exuding from the lungs ranged from pale to deep red. The heart and major veins were grossly distended with dark blood: this extended to the dural venous sinuses. PIB-affected birds (n = 3) were in fat condition and had lesions consistent with drowning. By contrast, birds affected by oil (n = 53), adverse weather (n = 6) or unknown causes (n = 16) were mostly in poor or emaciated condition with minimal or no fat deposits, wasted muscles and regressed livers. Their body mass was almost half that of drowned birds and their alimentary tracts were mostly empty, often showing inflammation, haemorrhage and ulceration. None had fluid in their air sacs, distended veins or lung lesions as seen in drowning cases. Conclusion: The gross pathology of drowning in seabirds differs significantly from that in other common causes of mortality. Provided carcasses are examined in reasonably fresh condition, the lesions should be sufficiently distinctive to confirm a diagnosis. Where legislation aimed at protecting seabirds is introduced, e.g. European Commission ban on the use of drift nets in EU waters (Anon 2014), an awareness of these lesions will be of value for those investigating *stranding* incidents.

## **Flunixin meglumine is not better than diclofenac**

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The Griffon vulture (*Gyps fulvus*) is categorised as a vulnerable species in Israel, yet its populations are suffering an ongoing decline demonstrated by the sharp reduction in numbers of nests in the northern part of the country. Abuse of insecticides for intentional illegal poisoning of carnivores that might prey on livestock, has tremendously reduced populations of the vultures, being non-target scavengers. One of the tools that we employ to decrease the risk of exposure to poisoned baits is to use feeding stations that provide the vultures with carcasses from reliable sources. The dramatic extinction of the Indian vultures as a result of the consumption of cow carcasses that were treated with diclofenac, kept us alert to the risk of unintentional exposure of the vultures to flunixin meglumine, a widely used NSAID in grazing livestock. Despite our efforts, we found three dead Griffon vultures between December 2013 and January 2016 that showed typical pathological and histopathological signs of exposure to flunixin meglumine. These results were confirmed by detection of the drug in analytical tests (LC-MS-MS). A relatively new NSAID, meloxicam (which is the only NSAID that is safe for vulture consumption), can provide a reliable solution to this risk when its use is encouraged together with a ban on the use of the flunixin meglumine in pastures.

## **Second generation anticoagulant rodenticides in eagle owls, a non-target raptor species, in Norway**

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Eagle owl (*Bubo bubo*) is one of the largest owl species in the world and classified as an endangered species in Norway. These birds are mostly nocturnal predators, hunting for a range of different prey species, predominately small mammals like rodents, but also amphibians, birds, reptiles and fish. Eagle owls are predominantly found along the coastline in Norway, but also in remote mountain areas and coniferous forests.

Second generation anticoagulant rodenticides, with long half-lives, may pose a threat to the survival and welfare of non-target raptor animals, since these substances are persistent and may accumulate at higher trophic levels. The legitimacy of this concern has been confirmed by previous studies of a limited number of eagle owls in Denmark and Norway.

In this study, the concentrations of anticoagulants in the livers of 64 eagle owls were determined by LC-MS. These birds were found dead between 1998 and 2014 and submitted for necropsy, and subsequently anticoagulant determination, at the Norwegian Veterinary Institute.

Second generation anticoagulant rodenticides were detected and quantified in the livers of the majority of eagle owls examined in this study. Furthermore, a substantial proportion of these birds exceeded total anticoagulant concentration of 100 ng/g, a lower limit for potential mortality in eagle owls indicated in the literature. Our results confirm that that exposure to anticoagulant rodenticides is very common in eagle owls. Hence, efforts to ensure safe utilisation and limited use of anticoagulant rodenticides are important to ensure viable populations of eagle owls in Norway in the future.

## Poisoning of reintroduced red kites (*Milvus milvus*) in England

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Programmes to reintroduce predatory birds are resource intensive and expensive, yet there are few long-term studies on the health of these reintroduced birds post-release. In a study of reintroduced red kites (*Milvus milvus*) in England, detailed post mortem examinations were carried out on 165 individuals found dead or dying between 1989 and 2007. Toxicology was performed on one third of the 165 birds. Poisoning was diagnosed in 34 red kites. Nineteen had anticoagulant rodenticide poisoning; defined as internal haemorrhage without associated trauma and summed liver anticoagulant rodenticide concentrations of 100 ng/g or more. Nine birds had (non-rodenticide) pesticide poisoning and six lead poisoning. Summed anticoagulant rodenticide levels exceeding 100 ng/g were detected in a further eight red kites (with no visible haemorrhages post mortem, or haemorrhage being associated with trauma) suggesting exposure to high levels of rodenticide occurred in more birds. There was a significant peak in red kites (n = 17) poisoned by anticoagulant rodenticides between February and June, probably because of the high use of rodenticides for agriculture in winter. The anticoagulant rodenticides difenacoum and bromadiolone were the most common poisons involved during this period. Despite the success of the reintroduction programme, poisoning of red kites may be slowing their rate of population increase and range expansion in England. Simple modifications of human activity, such as best practice in rodent control campaigns, tackling the illegal use of pesticides and the use of non-toxic alternatives to lead ammunition, could reduce our impact on red kites and probably other populations of predatory and scavenging species. Although this long-term study finished in 2007, the Wildlife Incident Investigation Scheme and Predatory Bird Monitoring Scheme continue to monitor pesticide impacts on red kites and could demonstrate if modification of these human activities has been successful.

## Risks for red kites (*Milvus milvus*) going on winter holidays

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The decline of red kite (*Milvus milvus*) populations across Southern and Central Europe has been attributed mostly to pesticide and rodenticide exposure, changes in landuse, carrion, rubbish availability, direct persecution and pathogens. In this context, feeding sites for resident or wintering carrion eaters are of high value but do also create conflict, as the aggregation of individuals at these feeding sites and the exposure to dead livestock could promote pathogen transmission. Capture events of wild birds for tagging purposes are unique opportunities to carry out targeted disease and health surveillance.

We used samples from wintering red kites captured at the Binaced feeding station in three different migration stages (autumn, wintering and spring migration) during three wintering seasons: 2013 - 2014 (n = 52), 2014 - 2015 (n = 59) and 2015 - 2016 (n = 69) to explore exposure of these birds to different avian and shared pathogens. No antibodies against West Nile Virus were found until the 2015/2016 wintering season (89.6 %, 60 of 67). Meanwhile a decreasing prevalence of antibodies against Avian Influenza Virus (AIV) pattern has been observed in the last wintering season ( $\chi^2 = 6.904$ ,  $p = 0.032$ ,  $df = 2$ ; 2013 - 2014 = 10.4 %, 5 of 48; 2014 - 2015 = 8.8%, 5 of 57 and 2015 - 2016 = 0 %, 0 of 67). Five of 180 red kites carried *Salmonella* sp. in their intestinal flora. Higher prevalences of Gentamycin ( $\chi^2 = 14.868$ ,  $p = 0.001$ ,  $df = 2$ ) and Enrofloxacin ( $\chi^2 = 31.213$ ,  $p < 0.000$ ,  $df = 2$ ) resistant *E. coli*, including multi-resistances ( $\chi^2 = 17.371$ ,  $p < 0.000$ ,  $df = 2$ ), were found in the 2014 - 2015 wintering season. A higher prevalence of antibodies against WNV was observed in returning red kites in spring ( $\chi^2 = 20.198$ ,  $p < 0.000$ ,  $df = 2$ ; autumn migration = 29.3 %, 12 of 41; wintering = 28.2 %, 31 of 110 and spring migration = 77.3 %, 17 of 22). During wintering stage higher prevalence of resistant *E.coli* were observed when compared to migrating individuals namely 70 % (81 of 115) of *E. coli* showed a resistance against Enrofloxacin ( $\chi^2 = 17.949$ ,  $p < 0.000$ ,  $df = 2$ ) and 49.6 % (57 of 115) showed multi-resistances ( $\chi^2 = 11.05$ ,  $p = 0.004$ ,  $df = 2$ ).

The results show kites are exposed to avian pathogens with direct transmission routes, possibly through the food chain while they are exposed to the primarily vector transmitted Flaviviruses at destination. The potential acquisition of increased antimicrobial resistance phenotypes from food items at feeding stations should be further studied to determine the potential of red kites as sentinels for antimicrobial resistance spread and to detect any potential effect on the survival and migratory and reproductive performance of the kites.

## **Investigating the dynamics of lead exposure in an urban population of a wild New Zealand parrot**

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Lead is a highly toxic heavy metal with persistent effects on wildlife populations worldwide. Its extensive use in urban landscapes has led to widespread environmental contamination. This study investigates the prevalence, source and physiological effects of lead exposure in an endemic New Zealand parrot, the North Island kaka (*Nestor meridionalis septentrionalis*). Since Western settlement, kaka populations have declined due to habitat destruction, predation and competition for food from introduced pests. Kaka were re-introduced to the Zealandia sanctuary, a predator proofed reserve in urban Wellington, where their numbers have since increased dramatically. Increased food availability in combination with their inquisitive nature, has seen the kaka population disperse beyond the sanctuary fence and into the city's urban areas. Subsequently, this has led to an increased risk of exposure of the birds to anthropogenic sources of lead. We examined concentrations of lead in blood samples from adult and nestling kaka and analysed tissue concentrations of lead in deceased birds. Living nestling and adult kaka in the study were subjected to a veterinary clinical examination and brief neurological examination. Furthermore, lead levels in eggshells and yolk samples were investigated to identify the pathways of lead exposure in chicks. Preliminary results indicate a high prevalence of lead exposure amongst adult and nestling kaka. Plasma concentrations of lead ranged from 0.03 mg/dL to 1.01 mg/dL with no detectable neurological deficits identified in sampled birds. Early exposure of a proportion of chicks and concentrations of lead in eggshells suggests a maternal transfer of lead through the egg. However, the pattern of lead exposure in the majority of the chicks is indicative of direct parental feeding of lead following hatch. The significant concentrations of lead detected in this urban population of North Island kaka has the potential to contribute to the morbidity and mortality of this species. The lack of neurological and physiological deficits associated with lead exposure is suggestive of an innate tolerance to even high concentrations of lead in this species. However, the well-described subclinical and persistent effects of lead suggests a need for continued monitoring of this toxin and its effects on kaka populations, especially in regard to behaviour and neurodevelopment.

## **Immune function of the Egyptian goose (*Alopochen aegyptiacus*) – a highly successful neozootic waterbird species in Europe**

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Invasive species have dramatically expanded their range because of increasing globalisation. Parasites and host immune function have been considered to play an important role through mechanisms such as “enemy release”, “novel weapon” or “immunocompetence advantage”. According to the enemy release hypothesis, invasive animals lose their natural pathogens and parasites during establishment in new habitats, conferring them competitive advantage compared with local species. The novel weapon hypothesis assumes that invasive species carry pathogens, which are highly pathogenic to native species. The immunocompetence advantage hypothesis suggests that species which evolved strong immune defences are the prime candidates for successful invasion. These hypotheses have been mainly tested in plants, amphibians or passerines, despite the successful invasion of Europe by several water birds such as the Egyptian goose (*Alopochen aegyptiacus*). The Egyptian goose is one of the most successful neozootic bird species in Europe; just in Germany its population increased 40 fold over the last 26 years (from 250 to 300 breeding pairs in 1999 to more than 10.000 pairs in 2015). In frame of our project we study the endoparasites and immunity of native mallards (*Anas platyrhynchos*) and mute swan (*Cygnus olor*) and invasive Egyptian geese in Germany. We also collected samples from native Egyptian geese populations from Namibia. Parasites are determined using morphological identification keys and molecular tools, while the immune system, both innate and adaptive, is assessed by several assays developed for free-living wild birds. As far as we know there is no comparable study on the immune system and parasites of invasive waterfowl although water birds - in particular species of the order *Anatidae* - are highly successful invaders all around the globe. The results of this study will lead to a better understanding of the impact of water bird invasion on native ecosystems with focus on parasitic infections and immunity. Additionally, the study will provide data on the differences in immune functions between native and invasive Egyptian goose populations, and will test the different parasite-associated hypothesis of invasive species. As invasive water bird populations are rapidly increasing in Europe, the impact on native species, on their habitats and health status are of great research interest both from conservation and public health points of views.

## ***Yersinia* spp. in house sparrows along an urban gradient in Belgium**

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Only scarce information is available on the prevalence of pathogens in apparently healthy wildlife, and even less when considering an urban-rural gradient. However, this information is of great importance when suddenly confronted with wildlife mortality events, such as those observed in urban house sparrow populations over the past decades, whether or not related to human disturbance. Pathogens that have the potential to cause disease in passerines have been suggested as being jointly responsible for these declines. During this research, 36 house sparrow populations, which clustered pairwise in a polygon surrounding and including the cities of Ghent, Antwerp and Louvain, were sampled. The populations were chosen, based on the urbanisation level of their habitat, which was calculated using the Large-scale Reference Database (LRD) in ArcGIS v9.2. In addition, for each population the Normalized Difference Vegetation Index, as a measure for the greenness of the habitat was calculated. From September until December 2013 (fall) and from January until March 2014 (winter), 356 faecal samples were collected from apparently healthy house sparrows captured in 9 urban, 9 suburban and 18 rural populations, across Flanders, Belgium. All populations were at least sampled twice (once during the fall and once during the winter). All the faecal samples were investigated for the presence of potentially pathogenic *Yersinia* spp. by means of isolation methods. Biochemical tests and MALDI-TOF MS confirmed the presence of *Yersinia* spp. PCR on chromosomal (*ail*, *yst-A*, *yst-B*) and plasmid-borne (*inv*, *vir-F*) virulence genes was performed to confirm the pathogenic nature of the isolated *Yersinia* spp. Fifty-five percent of the house sparrow faeces harbored *Yersinia* spp. Four percent of these isolated *Yersinia* spp. belonged to the pathogenic *Y. pseudotuberculosis*, 53 % to *Y. enterocolitica*. Of the latter, the vast majority harbored the *yst-B*-gene and none of them possessed the *virF*-, *ail*- or *yst-A*-gene. Across an urbanisation gradient and in between seasons differences in *Yersinia*-prevalence were observed. No difference in scaled mass index was observed when comparing house sparrows from which *Yersinia* was isolated versus house sparrows without *Yersinia*. Combined, these results render a link with the ongoing population declines in the urban regions of Belgium unlikely.

## Living in the city: effects of urbanisation on house sparrow digestive yeasts

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The house sparrow (*Passer domesticus*) is one of the most widely distributed urban birds and has successfully expanded its range to all continents. Recent population declines of this and other garden bird species in central and Northern Europe have raised concern and are considered due to several factors, including pathogens such as the protozoan parasite *Trichomonas gallinae*.

To study the presence of this potential pathogen in Spanish sparrow populations we sampled 76 house sparrows in three areas with different degrees of urbanisation and during both wintering and the reproductive season. Swab samples from the oropharynx and oesophagus were cultured in trichomonas culture medium enriched with 10 % foetal calf serum and tested by direct microscopic observation and PCR for presence of *T. gallinae*. None of the sparrows was colonised by *T. gallinae* but two yeasts were detected, *Macrorhabdus ornitogaster* and *Candida* sp. in 29 % and 23.7 % of the cultures, respectively. *Candida albicans* was identified in most cases by culture in Sabouraud dextrose agar and biochemical tests while *Macrorhabdus ornitogaster* has characteristic microscopic features. *Macrorhabdus ornitogaster* was found more frequently in juvenile sparrows (38.89 %, 7 of 18) than adults (25.7 %, 15 of 58; GzLM,  $p = 0.049$ ,  $\beta = -1,304$ ,  $SE = 0.663$ ). *Macrorhabdus* prevalence also increases as degree of urbanisation raises (GzLM,  $p = 0.018$ ,  $\beta = 0.906$ ,  $SE = 0.383$ ). A co-infection effect with *Candida* sp. has been observed (GzLM,  $p = 0.034$ ,  $\beta = 0.043$ ,  $SE = 0.383$ ). *M. ornithogaster* is an ascomycetes yeast that is present at the junction of the proventriculos and gizzard of wild and domestic birds. It has been described in numerous captive and wild birds, but to our knowledge not previously in house sparrows. Although the sparrows were apparently healthy and *M. ornithogaster* is potentially a commensal, in some passerine, gallinaceous and psittacine species it has been identified as a significant pathogen. The degree of *M. ornithogaster* carriage in sparrows may be affected by environmental conditions and they may act as reservoirs for other more susceptible species. Urban birds have to cope with many stressors that could affect the presence of this commensal yeast. Finally, under stressful conditions fitness of infected individuals could be compromised and lead to clinical infections.

***Trichomonas gallinae* in northern goshawks (*Accipiter gentilis*) – does the habitat influence the risk to get infected?**

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Avian trichomonosis caused by the flagellate *Trichomonas gallinae* is described as one of the most important bird diseases. The parasite is located in the upper digestive and respiratory tract of the host. The effects of the infection vary from subclinical infections (trichomoniasis) to significant disease (trichomonosis) that leads to organ necrosis, caseation, tissue invasion and death of the host. The parasite is transmitted via a direct life cycle. Due to the crop milk feeding of pigeons, these species are described as the main host of the parasite. The northern goshawk (*Accipiter gentilis*) is found in urban and rural habitats all over Germany. The main prey of goshawks living in urban areas are feral pigeons (*Columba livia f. domestica*), whereas the prey spectrum is much more diverse in rural areas. Therefore, we assume that goshawks living in urban areas are facing a higher risk of parasite transmission that should lead to a higher infection rate and may also influence the causes of death significantly. To investigate the impact of *T. gallinae* to urban goshawk populations, goshawk nestlings in urban and rural areas were examined and the infection rate between these locations was compared. The parasite rate was determined by taking an oesophageal swab of the nestlings. Due to the occurrence of a high variation in the parasite strains from non-pathogenic, moderately pathogen to virulent the parasites were genetically classified. Sequences of the ITS region and Fe-hydrogenase were used to identify the different strains and their potential risk to the host. Dissections of urban and rural goshawks were conducted to investigate the influence of the disease to the goshawk populations. Preliminary results showed a clear difference in prevalence between urban and rural areas with a higher risk of infections at urban areas. Nevertheless, the rate of significant diseases of the infection in the nestlings was not higher at urban areas. Therefore, we expect a parasite-host-coevolution caused by the increased parasitic risk. The habitat due to environmental differences seems to have a strong influence on goshawk populations.

**Evidence for anthropogenic respiratory disease in wild great apes – simultaneous detection of human respiratory syncytial virus in wild western lowland gorillas (*Gorilla gorilla gorilla*) and humans**

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All great apes are listed as either endangered or critically endangered on the IUCN Red List. Infectious diseases have recently joined habitat loss and poaching as a major threat to great ape survival. For some disease outbreaks in great ape communities habituated to human presence, such as respiratory diseases, a human source of infection is suspected. Moreover, in certain cases, respiratory illnesses have led to high morbidity and considerable mortality in different great ape communities. Yet, thus far, little research has identified the causative agents – knowledge necessary for optimising preventive health management. In the few studies that do exist, either one of two common human paramyxoviruses, human respiratory syncytial virus (HRSV) or human metapneumovirus (HMPV), was identified as the causative agent. However, the viruses were never detected in humans at great ape field sites and assumptions of human origin are generally based on phylogenetic analyses that link the viruses found in apes to recent infections in humans. Here, we take the next step in providing further evidence for human-borne infections by simultaneously detecting HRSV in a habituated Western lowland gorilla (*Gorilla gorilla gorilla*) group and the local human population. Fifteen gorilla faecal samples and 80 throat swabs from humans were collected in the Central African Republic during a respiratory disease outbreak in a gorilla group in 2012. The samples were tested for common human respiratory viruses, including HRSV and HMPV. We were able to obtain identical sequences for HRSV A from four gorillas and four humans. Additionally, we detected the presence of HMPV and rhinovirus in humans who frequently enter great ape habitat. Our findings contribute to the growing body of evidence for interspecies transmission of respiratory pathogens from humans to endangered great apes. They also demonstrate the importance of continuous health monitoring of habituation project staff, the surrounding human populations, and, potentially, any visitors who intend to approach great apes. In settings where humans and great apes interface, it will be especially important to foster a One Health approach – an approach that also aims to reduce the disease burden in the local community. Such programmes would benefit people as well as the endangered great apes.

## **Tick-borne diseases in a one health perspective, host and pathogen interplays – the search for TBE virus and related pathogens in wild and production animals**

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TBEV (Tick-borne encephalitis virus) and *Borrelia burgdorferi* sensu lato are among the most important vector-borne diseases that are emerging. They are the main diseases transmitted by *Ixodes ricinus* ticks in Europe and present in several areas of Norway with TBE human cases every year. *I. ricinus* is known to transmit other pathogens like louping-ill virus, *Anaplasma phagocytophilum*, that may have an influence of the pathogenesis in humans and animals. Factors like climate change, human behaviour and migrating animals are involved in the distribution of diseases. The main route of disease transmission is through tick bites, but wild and production animals are also susceptible to infection and can serve as sentinels/early warning systems for transmission.

In total, 653 serum samples were collected in 15 of the 19 counties in mainland Norway from wild reindeer, moose, roe deer and red deer. Moreover, 3,240 nymph and 234 adult ticks were collected from six location sites of three counties in Norway, and 29 milk and serum samples were collected from bovine milk production farms in areas where TBE is known to be present.

TBE IgG seroprevalence results from cervids shows that 32 of 653 tested sera had antibodies. The positive TBE IgG sera were found in the counties of Vestfold, Østfold, Aust-Agder, Vest-Agder, Rogaland, Nord-Trøndelag and Nordland. Additional tests to discriminate between TBE and louping ill are currently under way (PCR and neutralisation assays). The estimated pooled prevalence (EPP) and the minimum infection rate (MIR) were calculated for nymphs and adults. TBEV were detected in most sites analysed with an overall EPP of 0.26 % in nymphs and 2.62 % in adults. The overall prevalence of TNA analysis of *B. burgdorferi* s.l. in adult ticks was 6.41 %, *A. phagocytophilum* in nymph was 8 % and in adult 19 %.

Of the total 29 analysed individual cow milk samples, results indicate prevalence of up to 25 %, and preliminary PCR results seem to confirm the presence of the viral RNA in individual milk samples.

These results seem to indicate that TBE (and likely other emerging agents) is circulating in wild species and production animals, for which further studies should be conducted to evaluate the importance of these animals in the maintenance and transmission dynamics of TBE in Norway or elsewhere.

## **Avian influenza virus in sympatric wintering aquatic birds: are landfills hotspots for surveillance?**

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Aquatic wild birds are the undebated reservoir for avian influenza virus (AIV) and are studied intensively in order to better understand their role in the maintenance and spread of AIV. To date, AIV surveillance focuses on natural aquatic environments where different bird species aggregate and viral survival is enhanced. Nevertheless, artificial habitats such as landfills also hold notorious aggregations of wild birds, including AIV reservoir species. The use of landfills as predictable food source has significantly influenced population size, migratory and feeding behaviour of white storks (*Ciconia ciconia*) and black headed gulls (*Chroicocephalus ridibundus*) among other species. During wintering, bird numbers at Spanish landfills increase as migrants and wintering individuals join local residents. Thus especially during the critical autumn migration and wintering period sampling of resting birds at landfills could be a useful surveillance tool in addition to surveillance carried out at wetlands. During the wintering season 2014 - 2015 we analysed the prevalence of AIV in sympatric avian species at two landfills in Ciudad Real and determined species related and temporal variation in AIV prevalence. We collected and tested 1,185 fresh faecal samples from white storks (n = 692), cattle egrets (*Bubulcus ibis*, n = 166) and mixed gull flocks (black headed gulls, lesser black backed gulls *Larus fuscus*, yellow-legged gulls *Larus michaelis*, n = 377) in addition to cloacal and oral swabs of five individuals that were found dead, by real time-RT PCR for AIV matrix gene amplification. We found an overall prevalence of AIV of 0.6 %, peaking in October (1.91 %), the time of arrival of migratory wild birds in Spanish territories. Gulls showed the highest AIV prevalence (1.31 %), followed by cattle egrets (0.86 %,) and white storks (0.14 %). Gulls carried H11 and H16 AIV subtypes, H16 was also detected in the cattle egret and H11 in a white stork, suggesting that, cross contamination may occur between wild birds that mix during foraging and resting at landfills. Our results indicate that AIV circulates in wild birds that forage in landfills, and at least in gulls, probably continuously during the wintering season. Fresh faeces collection at landfills offers a cost-effective sampling method for large-scale LPAIV surveillance in wild bird species that forage at landfills.

## **Highly pathogenic avian influenza surveillance in wild birds across the United States**

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A unique A(H5Nx) clade 2.3.4.4 highly pathogenic avian influenza virus (HPAIV) was detected in North America in late 2014. Motivated by both the alarming spread of new H5 reassortant viruses in Asia and Europe as well as by the detection of HPAIV in both domestic poultry in Canada and in wild and captive birds in Washington State, initial HPAIV surveillance was conducted among wild birds in the Pacific Flyway of the United States. This effort was later expanded to include the Central and Mississippi Flyways. Positive HPAI H5 findings from wild waterfowl samples suggested that while some of these species exhibit no detectable morbidity or mortality, clinical disease was documented for other wild bird species similarly infected. Also, losses in U.S. domestic poultry were unprecedented. In July 2015, state and federal agencies initiated a national surveillance effort to provide information to guide management actions to address some of the issues associated with HPAIVs in birds. This includes risks to commercial poultry, backyard poultry, game bird farms, wild birds, wild bird rehabilitation facilities, falconry birds, and captive bird collections in zoos/aviaries. Specific objectives of the plan are to: 1) determine the distribution of influenza viruses of interest in the U.S.; 2) detect spread of influenzas of interest to new areas of concern; and 3) provide a flexible surveillance framework that can be modified to monitor wild

waterfowl populations for avian influenza, detect reassortant avian influenza viruses, and estimate apparent prevalence of important influenzas once detected in an area of concern.

## **Widespread infection with leptospire and different serogroup profiles in wildlife in Switzerland suggest different multiple-host infection cycles**

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Leptospirosis is a widespread zoonotic bacterial disease emerging in Europe. In Switzerland, the number of reported dog cases has dramatically increased, cases are regularly observed in free-ranging beavers, and autochthonous human cases also occur. However, knowledge of the epidemiology of leptospirosis in Europe remains limited. Small rodents are known to act as reservoir but other wild and domestic animals may also maintain the bacteria. The aim of this study was to assess the prevalence of and risk factors for *Leptospira* infection in selected wildlife species.

We investigated archived (1998 - 2012) and newly collected (2013 - 2014) samples of wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*), Eurasian beaver (*Castor fiber*) and small rodents (several species). We tested serum (n = 2,360) and kidney (n = 1,005) samples by Microagglutination test (MAT) and PCR, respectively.

Seroprevalence was highest in beavers (58.5 %), followed by foxes (45.3 %), wild boar (22.4 %), and small rodents (5.0 %). PCR-prevalence was significantly lower (beaver: 23.7 %, fox: 5.8 %, wild boar: 1.6 %) than seroprevalence, except for small rodents (15.8 %), for which the opposite was true. Seropositive and PCR-positive animals were found in all considered regions but wild boar showed higher seroprevalence in areas north of the Alps compared to the south. The most frequent serogroup (sg.) varied among species: in wild boar and fox, sg. Australis prevailed, in beaver sg. Icterohaemorrhagiae, and in small rodents sg. Grippotyphosa. There was no difference in PCR- and seroprevalence between archived and new samples. Age class was a significant risk factor for all studied species, whereas sex was only significant for wild boar. Prevalence in small rodents was higher in summer and fall compared to spring and winter. Prevalence also varied among species of small rodents, but detailed analysis was difficult because of collinearity between risk factors.

In conclusion, infections with leptospires occur everywhere in Switzerland but with regional differences in prevalence. Wild boar are unlikely to play a reservoir role for leptospires in Switzerland while red fox appear to be a sentinel rather than a reservoir host. Beavers are susceptible hosts but their epidemiological role remains to be clarified. Small rodents are likely maintaining leptospires but the role of different rodent species needs to be assessed separately. However, serologic profiles of wildlife species, dogs and humans suggest the existence of several multi-host infection cycles. This points out the complexity of leptospirosis epidemiology, calling for a One Health approach in future studies.

## Survival of marine *Brucella* spp. in seawater

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The infection biology of marine brucellae is to a large extent unknown and unidentified hosts or reservoirs in the marine environment may exist. Recent information suggests that *Brucella pinnipedialis* infection is not sustained in adult hooded (*Cystophora cristata*) or harbor seals (*Phoca vitulina*) and an environmental source of infection is proposed. *Brucella ceti* and *B. pinnipedialis* have also been isolated from lungworms in cetaceans and pinnipeds, respectively, and under experimental conditions it has been shown that *B. pinnipedialis* sustain an asymptomatic infection in Atlantic cod (*Gadus morhua*). In addition, *Brucella microti* has been isolated from soil and potentially novel brucellae have been isolated from frogs, supporting a hitherto extended ecological niche for brucellae comprising ectotherms and possibly the environment. To investigate whether marine brucellae are able to survive or grow in the marine environment, *Brucella* spp. were cultured in a seawater model at two different temperatures (4 and 10°C) representative for the Arctic Ocean, based on present sea temperatures and future predicted climate scenarios. A seawater model was designed using Sera marine salt, and bacterial survival in seawater was compared to survival in Gerhardt's minimal medium (GMM) at neutral pH. The strains included were *B. ceti* reference strain 12891, *B. pinnipedialis* reference strain 12890, *B. pinnipedialis* hooded seal field isolate 17a-1, and *B. ceti* bottlenose dolphin (*Tursiops truncatus*) field isolate F5/99 of the zoonotic strain type (ST) 27. All strains survived for at least 6 weeks in the seawater and the GMM model. At 4°C the 12891, 12890 and ST27 strains survived significantly better in seawater than in GMM, while 17a-1 survived equally well in both models. At 10°C the survival of 12891, 12890, and 17a-1 was significantly lower compared to 4°C, except for ST27 where such increased temperature had no impact on the survival in seawater. Strain 17a-1 survived significantly better in seawater than in GMM at 10°C, while no differences were observed for the other strains. These results demonstrate that marine *Brucella* spp. can survive for extended periods of time outside of their main hosts. Furthermore, our findings suggest that the ecological range of marine brucellae include the environment and *Brucella* spp. may exist in a planktonic form in the ocean.

## **You are where you eat: high *Brucella* seroprevalence in coastal but not inland arctic foxes**

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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 recently demonstrated that high levels of mercury are present in coastal arctic foxes, but negligible amounts were found in inland populations. This study indicates that the two ecotypes are quite separate and distinct which may indicate that pathogen presence or prevalence between them may also radically differ. We tested serum samples from 39 arctic foxes for the presence of antibodies to *Francisella tularensis*, *Coxiella burnetti*, three *Leptospira* species and *Brucella abortus*. A seroprevalence of 51.2 % to *Brucella abortus* antigens was found, whereas no antibodies were detected against the other pathogens. There was no difference between gender and age groups in the exposure to *Brucella*, however coastal ecotype foxes had a significantly higher seroprevalence than inland ones (62.9 % and 25 %, respectively). Iceland is officially considered to be “brucellosis-free”, marine mammal species in the region, however, were found to be positive for this pathogen. Since coastal ecotype foxes had a significantly higher seroprevalence, it is likely that a spill-over from marine mammals to arctic foxes might take place. We are performing molecular analysis of tissue samples in order to better understand the bacterial species involved and the epidemiology of the brucellosis in Iceland.

## CONTRIBUTED POSTER PRESENTATIONS

### Improving myxomatosis outbreak mapping in Sweden (*Poster no. 24*)

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Myxomatosis is endemic in wild rabbits in Sweden after the probably deliberate introduction of the myxoma virus in the early 1960's. The conspicuous swollen eyelids are well-recognised lesions known by hunters and the public. That myxomatosis is a reportable disease is less well known. Therefore, outbreaks have been seriously underreported from Sweden as the authorities do not receive timely information. Wildlife disease surveillance is centralised in Sweden, being managed from the National Veterinary Institute (SVA). The institute reports all diagnosed cases of reportable diseases to the Board of Agriculture, where the Chief Veterinary Officer is responsible for the reporting to the OIE. A more active role by SVA was necessary to improve the reporting of myxomatosis. Improvements included using a new network of contact persons affiliated to the Swedish Association for Hunting and Wildlife Management in each of the 21 counties of Sweden. This became a more organised channel to send requests out for information, and samples of sick or fallen wildlife to the institute. For specific sampling efforts, project information was directed at local municipality authorities, and also posted on e.g. hunting magazine websites. To further increase inflow of reports of cases and outbreaks, a variety of communication paths are used. The development of a specific wildlife disease reporting app for smartphones is ongoing, in addition to telephone, email, and website reporting. The SVA website also contains information on commonly occurring wildlife diseases, links that are presented as first or second result on internet search engines, in this case when searching the words *myxomatosis* or *rabbit plague* in Swedish. SVA has media monitoring, so when reporters mention SVA, the resulting articles or broadcasts are retrieved and a possibility to follow up new outbreaks or new locations are given to the team working with wildlife disease surveillance. To disseminate information on ongoing outbreaks, gps-coordinates for each report or examined case are mapped and published on the SVA website, and are linked to from various SVA social media accounts. To improve diagnostics, a PCR-analysis for myxoma virus detection was set up at SVA, so that more cases could be verified, also when receiving only small biopsies from lesions from suspected cases. The number of reports and samples increased in the past year during a large outbreak of myxomatosis, and shows that the level of reporting is dependent on the effort put into promoting wildlife disease surveillance.

**Causes of mortality and morbidity in free-ranging mustelids in Switzerland: data from 50 years of general health surveillance** (*Poster no. 72*)

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Mustelids include a wide range of secretive species, partly protected and partly considered as pest. However, apart from bovine tuberculosis in European badgers, canine distemper and parasitic diseases in various species, little is known about diseases in free-ranging mustelids. In Switzerland, a programme for general wildlife health surveillance has been in place for nearly 50 years and mustelids have been regularly submitted for post mortem investigation. The aims of this study were to provide an overview of the causes of mortality and morbidity observed in mustelids since the beginning of the programme and to describe the pathological picture of the most frequent diseases.

All necropsy reports (553 carcasses and 13 organ samples) collected from 1958 to 2015 were analysed. The cases consisted mainly of stone martens (*Martes foina*, n = 262) and badgers (*Meles meles*, n = 249) but included also pine martens (*Martes martes*, n = 13), polecats (*Mustela putorius*, n = 27), stoats (*Mustela erminea*, n = 8), weasels (*Mustela nivalis*, n = 5) and otters (*Lutra lutra*, n = 2). There was a majority of adults (64 %) and sexes were nearly balanced (53 % of males). More than half of the animals had been killed because of disease signs (abnormal behaviour, skin lesions), other had been found dead, and few were apparently healthy animals culled for targeted investigations. Infectious diseases (n = 262), mostly of bacterial (n = 106) or viral (n = 60) origin, were more frequent than non-infectious conditions (n = 169), which included mainly trauma (n = 124) and metabolic diseases (n = 31). No definitive diagnosis was obtained in 117 animals. Some of the most frequent conditions were distemper (badgers, martens, polecats and a stoat), idiopathic amyloidosis (martens and a polecat), histoplasmosis (badgers) and bacterial infections (all mustelids: *Streptococcus* sp., *Staphylococcus* sp., *Pasteurella* sp., colibacilli). Ectoparasites (*Trichodectes* sp., *Ixodes* sp., *Sarcoptes* sp.) and endoparasites (*Toxoplasma* sp., *Isospora* sp., *Taenia* sp., *Atriotaenia* sp., *Uncinaria* sp., *Capillaria* sp., *Crenosoma* sp., *Filaroides* sp.) were frequently observed but considered clinically relevant only in few animals. Distemper was associated with pulmonary, central nervous and hepato-biliary inflammation, along with frequent inclusion bodies, foot-pad hyperkeratosis and splenomegaly. Bacterial infections affected mostly the respiratory tract and were associated with bronchopneumonia or pleuritis. Amyloidosis, often observed macroscopically as obvious splenomegaly, was confirmed histologically most frequently in spleen and kidney. This study shows that the disease pattern of mustelids in Switzerland is marked by a large proportion of infections due to a relatively narrow range of pathogens (mainly distemper virus and few bacteria). Additionally metabolic disorders are frequent in martens.

**Spatial characterisation of bovine tuberculosis in the Eurasian badger (*Meles meles*) in Northern Ireland** (Poster no. 38)

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The Eurasian badger in Britain and Ireland is an important wildlife reservoir for *Mycobacterium bovis*, the causative agent of bovine tuberculosis. Little is known about the relative importance of badgers as maintenance hosts and at which geographic scale this role is most prominent. Herein we describe the use of pathogen strain and badger population genetic data in a pilot study that makes initial approaches to addressing these questions.

In total, 279 *M. bovis* isolates from badgers killed in road traffic accidents (RTA) were collected across Northern Ireland (NI). All isolates were analysed by multi locus VNTR analysis (MLVA). Ten distinct MLVA types were observed. Data were plotted using ARCGIS. A spatial autocorrelation analysis on strain genetic distance vs strain geographic distance was carried out using a Mantel test. There was no relationship ( $p = 0.1497$ ).

For the most common strain, MLVA type 2, spatial distribution and mean nearest neighbour distance across the landscape of NI were determined. This distribution was skewed with a minority of badgers noted as over 20 km away from nearest neighbours. Using a 2.6 km mean annual dispersal distance determined from another Irish badger population, 7.8 km buffers, accounting for three years of potential movement, around each infected badger location were drawn to constitute a network of related strain clusters. Six distinct MLVA type 2 clusters were observed. The two largest clusters were found in the southwest and northeast of the country. The four remaining clusters were small geographic outliers.

A further 176 RTA badgers collected across NI in 2012 were genotyped using 14 microsatellites. Genotype data was analysed using the clustering algorithm GENELAND. Three sub-populations were observed and all locations plotted in ARCGIS. The two large clusters of strain type 2 were observed to be split across two neighbouring badger sub-populations.

Lack of spatial autocorrelation between strain genetic distance and geographic distance, at an NI wide scale, indicates that infection in badgers has not diffused across the landscape gradually in a manner consistent with badger dispersal. MLVA types exhibiting a dispersed distribution even after accounting for badger movement suggests that outside of major clusters, dispersal to distal new foci is unlikely to be caused by badger movement. Seeding of infection beyond strain

home ranges may be caused by cattle movement. That the two major clusters of strain type 2 aligned with badger sub-population spatial distributions may indicate that badgers are drivers of local persistence.

**Occurrence and molecular characterisation of *Bartonella* spp. and hemoplasmas in neotropical primates from Brazilian Amazon (Poster no. 48)**

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Little is known about the prevalence and genetic diversity of *Bartonella* spp. and hemoplasmas in nonhuman primates (NHP). The present study aimed to investigate the occurrence of and assess the phylogenetic position of *Bartonella* spp. and hemoplasma species infecting neotropical NHP from Brazilian Amazon. From 2009 to 2013, a total of 98 blood samples from NHP belonging to the Family Cebidae were collected in the island of São Luís, state of Maranhão, of which 87 NHP were from Wild Animal Screening Center (CETAS) in the municipality of São Luís, and 11 (9 *Sapajus* sp. and 2 *Saimiri sciureus*) were from NHP caught in the Sítio Aguahy Private Reserve. DNA samples were screened by previously described PCR protocols for amplifying *Bartonella* spp. and *Mycoplasma* spp. based on *nuoG*, *gltA* and 16S rRNA genes, respectively. Purified amplicons were submitted to sequencing and phylogenetic analysis. Bacteremia with one or more *Bartonella* spp. was not detected in NHP. Conversely, 35 NHP were PCR positive to *Mycoplasma* spp. The Blastn analysis of seven positive randomly selected sequencing products showed percentage of identity ranging from 95 to 99 % with other primates hemoplasmas. The Maximum Likelihood phylogenetic analysis based on a 1510 bp of 16S rRNA gene showed the presence of two distinct clusters, positioned within *Mycoplasma haemofelis* and *M. suis* groups. The phylogenetic assessment suggests the presence of a novel hemoplasma species in NHP from the Brazilian Amazon.

**Occurrence and molecular characterisation of *Bartonella* sp. in wild rodents in different Brazilian biomes** (Poster no. 47)

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*Bartonella* species comprises an ecologically successful group of microorganisms that infects erythrocytes of a wide range of mammals, including humans, with a long history of co-evolution with their hosts. Rodents are reservoirs of about two thirds of *Bartonella* species described to date, and some species have been implicated as the causative agents of human diseases. In our study, we performed the molecular and phylogenetic analyses of *Bartonella* infecting wild rodents from five different Brazilian biomes. *Bartonella* sp. was detected in 25.6 % (117/457) of rodent samples analysed. In addition, we highlighted that occurrence of *Bartonella* varies among different biomes. The phylogenetic relationship of *gltA* sequences performed by maximum likelihood showed that *Bartonella* spp. detected in rodents in Brazil were closely related to phylogenetic group A detected in other Cricetid rodents from North America, probably, constituting only one or a complex species. Lastly, the *Bartonella* sp. genogroup identified in the present study showed to be a monophyletic group, infecting seven different rodent species distributed in three distinct biomes, suggesting a probable dominance of this specie among wild rodents from Brazil.

## Micro-mammal reservoirs of *Trypanosoma cruzi* in semiarid Chile

(Poster no. 53)

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In the semiarid north-center of Chile, several wild mammals from the Orders Carnivora, Chiroptera, Didelphimorphia, Lagomorpha and Rodentia could be reservoirs of the protozoan *Trypanosoma cruzi*, the agent of Chagas disease. Rodents have a close relation with triatomine vectors, being part of their diet and sharing the same ecotopes. In this study we captured rodents using baited live traps in sylvatic, peridomestic and domestic sites of the semiarid area of Chile. The rodent species were identified, and after isoflurane anaesthesia, blood samples were obtained and processed by DNA extraction and conventional kDNA PCR using primers 121 and 122. The amplified samples were determined positive when a 330 bp band was observed by UV light transillumination of the agarose gel. We captured the native species *Abrocoma bennetti* (Abrocomidae); *Octodon degus* (Octodontidae); *Abrothrix olivaceus*, *Abro. longipilis*, *Oligoryzomys longicaudatus*, *Phyllotis darwini* (Muridae); and the synanthropic species *Mus musculus*, *Rattus norvegicus* and *R. rattus* (Muridae). From these, the natives' *A. bennetti*, *A. longipilis* and *O. longicaudatus* are reported positive for the first time; *A. olivaceus*, *O. degus* and *P. darwini* are confirmed infected in several sites (range 21.1 - 57.1 %). The synanthropic species *R. rattus* is confirmed positive in rural areas and *R. norvegicus* is reported infected for the first time in Chile. *Mus musculus* was not infected. *Octodon degus*, *P. darwini* and *A. olivaceus* were the most abundant native rodents in this area, and therefore their role as reservoirs of *T. cruzi* is relevant, considering their high frequency of infection (> 20 %) and their participation as triatomines' blood meal. These species have been detected in peridomestic areas, and even inside dwellings, so their potential capacity to disperse the parasite is high. The commensal species *Rattus* spp. are known to travel constantly between man-made constructions and their surroundings, even to wild areas; we detected them in rural environments with high frequency of infection (> 17 %). Given that they thrive easily in modified habitats, they can be the main rodent species inhabiting domestic and peridomestic areas. We conclude that rodents are important reservoirs for *T. cruzi* in these areas, without discarding that other hosts may be playing a role in the parasite's cycle. However, the maintenance of *T. cruzi* in semiarid Chile is ensured in the reservoirs' section of the cycle by the abundance and levels of infection of rodents.

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**Increased survival of adult wild boar under low hunting effort promotes tuberculosis-related mortality** (*Poster no. 92*)

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Understanding infection dynamics in different management scenarios is a key element of disease control. We therefore studied adult Eurasian wild boar (*Sus scrofa*) survival and the causes of their mortality in two tuberculosis (TB) endemic populations in Spain by comparing a mosaic of hunting estates (MT; 24 GPS-collared animals) with a National Park in which population control is restricted (DNP; 21 animals). Mean adult survival (overall 470 days) and annual mortality rate (overall 45 %) were significantly greater in the protected DNP ( $672 \pm 96$  days, 34 % mean annual mortality rate) than in the regularly hunted MT ( $297 \pm 41$  days, 56 %). Hunting/population control accounted for one half (53 %) of the total mortality in adults, whereas TB caused one third (30 %). Although annual mortality resulting from hunting differed between areas, 40.3 % and 8.4 % for MT and DNP, the TB-related mortality did not vary between sites: 12.4 and 14 %, respectively. Annual mortality rates for adults were within the ranges described for Europe, whereas our reported proportions of natural mortality (usually reported at 3 %), mainly due to TB, were higher. Since previous findings suggest that TB-induced mortality is relevant in piglets and subadults, our results are compatible with adult wild boar being able to cope chronically with TB (one would expect increased compensatory TB mortality as hunting pressure decreases if otherwise TB rapidly progressed in not hunted adults). This complex interplay between demographical, management and disease factors evidenced that currently applied hunting efforts (40 % for adults in the hunting estate area), are ineffective to reduce population (which still increase) and TB. These results have implications for TB control at the wildlife – livestock interface and as regards the role of hunters in wild boar management and TB control.

**Disease risk analysis for avian reintroduction projects** (*Poster no. 83*)

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Marked declines in biodiversity are leading to ambitious interventions for wildlife conservation, including translocations such as reintroduction. Excepting some notable successes, evidence suggests that conservation translocations have had relatively low levels of success to date: further research into their limitations, methods and management is needed in order to improve conservation outcomes. Disease hazards are one of the constraints on successful reintroduction. Guidelines for disease risk analysis (DRA) and health management for reintroduction of specific taxonomic groups would be beneficial, to build on existing overarching guidelines.

This PhD project (2014 – 2020) is using two UK reintroduction projects – for the Eurasian crane (*Grus grus*) and corncrake (*Crex crex*) – as case studies by which to review, and further develop, DRA and health management strategies for avian reintroduction. The project comprises:

1. A literature review, to identify the DRA methods employed for reintroduction projects to date and the types of disease encountered;
2. A retrospective critical review of the DRA conducted for Eurasian crane reintroduction, to determine whether, and how, DRA strategy might be improved;
3. Risk factor analyses, to determine whether husbandry and bird-related risk factors have affected a) the survival of corncrake chicks to the point of release, and b) the incidence of specific non-infectious disease conditions (in corncrakes and Eurasian cranes);
4. In light of the above results, the development of avian-specific DRA and health management guidelines for reintroductions.

Preliminary results show that both infectious and non-infectious disease conditions have been significant threats in both crane and corncrake reintroduction pathways. Husbandry modifications were required during the course of both projects to reduce the prevalence of non-infectious diseases. Strict biosecurity protocols and prophylactic medication successfully prevented infectious disease outbreaks during captive rearing for crane reintroduction. Disease threats (including those relating to husbandry) need to be factored into project planning at an early stage, in order to maximise animal health, welfare, and reintroduction success.

**Detection of *Batrachochytrium salamandrivorans* in amphibian skin samples**

(Poster no. 80)

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*Batrachochytrium salamandrivorans*, the causative agent of lethal chytridiomycosis in urodelans, is currently spreading across North-western Europe, where it is causing amphibian population declines. Alarmingly, *B. salamandrivorans* has been shown to have a broad host range, with infection of urodelan taxa resulting in different disease outcomes, ranging from species acting as asymptomatic carrier to species in which fatal disease occurs in all exposed individuals. Preventing introduction and spread of *B. salamandrivorans* into naïve area's and amphibian populations is of the utmost importance in order to limit the impact of this pathogen on amphibian diversity. Therefore, research focused on developing effective disease surveillance tools are essential.

In this presentation we will give an overview of current diagnostic assays available for *B. salamandrivorans* (histology, real-time PCR) including their advantages and disadvantages, and present a novel immunohistological assay for detection of *B. salamandrivorans* in amphibian skin samples.

Results show that the immunohistological assay can be used to detect *B. salamandrivorans* in amphibian skin samples, and thus diagnose chytridiomycosis, even in amphibian tissues fixated in formalin for over two years.

**Suspected osteochondrosis in a Swedish moose (*Alces alces*)** (Poster no. 29)

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A one and a half year old female moose (*Alces alces*) with a history of ataxia and difficulties to rise from recumbency, was shot in July 2009 in the county of Örebro in central Sweden and was sent for post mortem examination to the Swedish National Veterinary Institute (SVA). The main necropsy findings were severe bilateral and symmetrical lesions in the articular cartilage and subchondral bone of the elbow joints. Cartilage ulcerations with denuded subchondral bone and attached cartilage flaps were observed on the medial part of both humeral condyles. Moderate wear lines were evident in both the humeral and radial/ulnar articular cartilage. Ulceration was also noted on the ulnar articular surfaces. Proliferation of the synovial membrane and marked increase of synovial fluid was present in both elbow joints.

The ulcerated subchondral bone was depressed in a saucer-like form. The depression was filled with moderately cellular and vascular fibrous and fibrocartilaginous tissue, which contained foci of highly eosinophilic amorphous necrotic material. The fibrous tissue extended downward into the normal epiphyseal bone. Next to the denuded subchondral bone, the adjacent remaining articular cartilage had a transverse cleft which lifted the overlying cartilage forming an osseocartilaginous flap. In the superficial articular cartilage there were multifocal clusters of proliferating chondrocytes (chondrons) indicating osteoarthritic degeneration, while deeper areas showed marked necrosis. The elbow joint synovial membranes had a mild proliferation of synovial cells and a moderate subepithelial infiltration of plasma cells and lymphocytes. The lesions were bilaterally symmetrical and affected the medial aspect of the humeral condyle, which is a predilection site for OCD in several species. The location, together with the gross and histologic lesions are suggestive of osteochondritis dissecans (OCD). To the best of our knowledge, this is the first reported case of OCD-like lesions in a moose.

### **Chronic pyotraumatic dermatitis in Swedish moose (*Alces alces*) (Poster no. 28)**

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During the second half of 2015, an increased number of male moose (*Alces alces*) with severe and extensive skin lesions over the dorsal lumbar and sacral regions were reported and submitted to the National Veterinary Institute in Uppsala, Sweden. Sporadic similar cases had been reported earlier, but this apparent outbreak in moose was unprecedented. Lesions consisted of extensive, hairless areas of skin with dry crusts covering a deep, suppurative dermatitis. The lesions were located primarily on the sacral region, but extended along the entire dorsal trunk in some cases. Reports and samples from the field were solicited and a questionnaire was sent out to collect observations of lesions during the annual moose hunt in order to assess the incidence and distribution in the Swedish moose population. Between October 2015 and March 2016, 149 cases distributed throughout the southern half of Sweden were reported. The majority of cases (148/149) were adult males. Complete carcasses or skin samples from 58 cases were submitted for histopathological, parasitological and microbiological examination. Affected areas showed a deep, acute-chronic active dermatitis with complete ulceration of the epidermis and a mixed inflammatory response, including eosinophils, as well as marked thickening of the dermis due to fibrosis. The changes are comparable with chronic pyotraumatic dermatitis, typically seen in dogs due to self-trauma leading to an itch-scratch cycle, with secondary infection and epidermal ulceration. Deer keds (*Lipoptena cervi*) were observed in high numbers in most cases and *Chorioptes* sp. mites were seen in some cases. *Staphylococcus aureus* was isolated in 40/45 samples (89 %). Possible underlying factors include mass infestation with deer keds or other ectoparasites, in combination with climatic factors such as humid weather. Irritation from parasites could lead to intensive and prolonged scratching of dorsal regions within reach of the adult males' large antlers. In cases where the skin is traumatised, bacterial infection can ensue and cause further irritation. The observed lesions are in contrast to earlier cases of hairless moose attributable to deer ked infestation in Norway and Sweden where only a few animals had ulcerative lesions. Other possible etiologic agents or contributing factors therefore need to be investigated.

## **Approaches to the management of wildlife diseases: case studies from the musteloidea** (Poster no. 86)

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We recently reviewed a range of pathogens impacting musteloid species (weasel-like carnivorans: Mephitidae, Ailuridae, Procyonidae, and Mustelidae) worldwide (Newman & Byrne 2016 in *Biology and Conservation of Wild Musteloids*, Eds: Macdonald, Newman and Harrington, Oxford University Press). A diversity of pathogens and species were highlighted, some of which present significant challenges from conservation and disease management perspectives. Here we highlight two case-studies of wildlife diseases from the musteloidea and approaches taken in their management: 1. European badgers (*Meles meles*) and bovine tuberculosis (bTB; *Mycobacterium bovis*) in the UK and Ireland; 2. raccoons (*Procyon lotor*) and rabies in North America. Both pathogens are zoonotic, meaning there is a transmission risk to humans. In the case of badgers and bTB, the more significant risk is spill-over infection to cattle, which has serious economic impact on farming. A number of management approaches have been trialled as part of policies to control or eradicate disease. The basis of most approaches identified in the literature to reduce risk was: reducing density of hosts (culling/reproductive control), reducing density of susceptible hosts (vaccination), or keeping hosts apart (biosecurity). These broad classes of intervention impact disease dynamics by interrupting transmission, with the intention of reducing the number of secondary infections ( $R_0$ ) below one. While culling activities were found to have significant benefits to disease management, their effectiveness varied by species, location and intensity of culling, and their long-term sustainability was questioned in both cases. Large-scale vaccination trails have been successfully implemented for rabies in raccoons; however, vaccination (using Bacillus Calmette–Guérin) field trails are still ongoing for badgers. Biosecurity, the ability to reduce disease risk from wildlife to humans or domestic animals, has been investigated with badgers to understand how to reduce risk of transmission to cattle in farmsteads.

Overall, culling of animals was found to be challenging in both case-studies from animal welfare and efficacy grounds at large spatial-scales. This was particularly apparent in the case of badgers, which is considered a “flagship” animal species despite not being of conservation concern. Large-scale oral vaccination programmes are already in train in North America, and have been successful in rabies eradication schemes in red fox (*Vulpes vulpes*) populations in Europe. No oral bait vaccine is currently available for bTB in badgers. Mixed policy options (singular or combinations of culling, vaccination and biosecurity measures) are currently being implemented to varying degrees across the countries of the UK and Ireland.

**Operating protocol for urban wild boar incidences in the urban area of Barcelona** (*Poster no. 90*)

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Barcelona is one of the cities affected by wild boar (*Sus scrofa*) colonisation and it is increasingly facing conflictive situations caused by the presence of this species inside the urban area, which lead to the development of a specific operating protocol. This protocol was collaboratively developed by the Barcelona City Council and the Servei d'Ecopatologia de Fauna Salvatge (SEFaS) of the Universitat Autònoma de Barcelona (UAB), and has been active from May 2013 until date. The wild boars causing conflictive incidences within the urban area of Barcelona, and reported by citizens, are firstly assessed by the local police. If the situation is considered potentially dangerous but stable, the veterinary service is alerted and a veterinary is due to attend the incidence within an hour.

From May 2013 to date, 128 wild boars (and increasing) have been removed from the urban area of Barcelona. This includes 54 females (nine adults, 17 yearlings, 14 juveniles and 14 piglets), 70 males (nine adults, 30 yearlings, 21 juveniles and ten piglets), and four wild boars where sex and age could not be determined. The wild boars were anaesthetised with a blowgun using a combination of tiletamine/zolazepam (Zoletil®, 6 mg/kg) and xilazine (Xilagesic®, 3 mg/kg), according to the estimated weight of each individual. Once anaesthetised, blood samples and swabs were collected, T-61 (6 ml/50 Kg) was administrated for euthanasia, and the wild boars were carried to the Veterinary school for post mortem study and sampling. Location, date, sex and age were collected in order to understand the temporal and spatial pattern of wild boar apparition in the urban area of Barcelona. Mean real anaesthetic dose was 7.82 mg/kg for Zoletil® and 4.19 mg/kg for Xilagesic®. Wild boar incidences in the urban area of Barcelona occurred mainly from March to September, coinciding with the period of drought and food scarcity. The operating protocol is a model for collaboration between public administration and university, applying scientific knowledge in the management of animal-related public conflicts. The results obtained, as well as the interpretation of the information gathered in the collaboration between researchers and managers, will help to better comprehend the characteristics and patterns of wild boars entering the urban area and, therefore, to manage wild boar-human conflicts in Barcelona.

**Occurrence and genetic diversity of zoonotic *Campylobacter* species carried by wild boars from the metropolitan area of Barcelona** (Poster no. 40)

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Wild boar (*Sus scrofa*) can carry numerous pathogens and the risk of disease transmission to other wildlife species, domestic animals and humans has increased in recent decades due to its increasing abundance and adaptation to urban areas. This study aims at determining thermophilic *Campylobacter* spp. prevalence and genetic diversity in wild boars from the metropolitan area of Barcelona (NE Spain), where this species has colonised urban and peri-urban areas. Rectal swabs were obtained from 133 wild boars: 76 individuals hunter-harvested in the Collserola massif (a natural area adjacent to Barcelona city), 32 captured inside the urban area of Barcelona and 25 in the campus of the Autonomous University of Barcelona. *Campylobacter* isolation was performed by standard culture methods, by streaking the swabs onto selective agar (CCDA) and by subsequent PCR identification at species-level of the isolates. Genetic diversity of the isolates was determined by *flaA*-RFLP and PFGE. *Campylobacter* sp. overall prevalence was 59 % (78/133). *C. lanienae* was the most frequent species, isolated in 69 % (54/78) of the *Campylobacter*-positive wild boars, whereas *C. coli* appeared in 21 % (16/78) of the positive wild boars. Also, 6 % (5/78) of the positive wild boars presented mixed infections with *C. lanienae* and *C. coli*. The *Campylobacter* isolates could only be identified at genus level in 4 % (3/78) of the positive wild boars. The genetic diversity amongst the different strains is being analysed and the results will be presented and discussed. Close direct and indirect contact between wild boars and humans in the metropolitan area of Barcelona occurs due to hunting, wild boar penetration into the urban area and high human pressure in the Collserolla massif. Altogether, the results obtained highlight the risk of pathogen transmission and the precautions that should be taken in regions where wild boar is expanding, including urban areas.

## **Porcine respiratory syndrome causes significant mortality in wild boar piglets**

(Poster no. 6)

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Eurasian wild boar (*Sus scrofa*) populations are increasing throughout Europe. This causes concern regarding the maintenance and spread of emerging and re-emerging pathogens. Mortality has been studied mainly in adult wild boar, with contrasting results regarding the causes of death and the role of diseases in population regulation. Even less is known regarding piglet mortality.

This study was performed in a 6,000 ha large matrix of private-owned, intensely managed hunting estates, where high wild boar densities (> 10 per km<sup>2</sup>) are maintained by almost year-round supplementary feeding. Juvenile and adult wild boar, investigated during the hunting season, display high (> 65 %) infection prevalence of *Mycobacterium bovis*, the main causative agent of animal tuberculosis (TB). We hypothesised that in such a high-density/high-*M. bovis* exposure setting, TB-mediated piglet mortality would be important.

We combined fieldwork (sampling and camera-trapping) with laboratory analyses (pathology, microbiology and serology). Camera traps were set at all (n = 24) wild boar feeders for three consecutive nights in June, July, August and September 2014, respectively. Short (1 min) video footage was carefully analysed in order to generate indicators of abundance and on the piglet-to-adult ratio. Sixty fresh wild boar carcasses were necropsied and sampled for further analyses.

Field surveys and camera-trapping estimated a total summer piglet mortality rate of 70 %. Only twelve piglets (20 %) had TB-compatible lesions, and only three cases were generalised, potentially compromising piglet survival. By contrast, 48 (80 %) of the carcasses revealed pulmonary lesions compatible with the porcine respiratory syndrome. Bacteriology and pathology confirmed the diagnosis. Among these, lung consolidation varied from 1 to 84 % (mean 19 %). The presence/absence of lung consolidation was not linked to the presence/absence of TB-compatible lesions nor to the PCR detection of Porcine Circovirus type 2 and *Mycoplasma hyopneumoniae*.

In view of the results, disease-mediated piglet mortality is a significant driver of wild boar population dynamics, at least in semi-intensive management regimes. However, TB is irrelevant as a cause of death in this age class, while the porcine respiratory syndrome explains a significant proportion of the observed deaths.

**Preying brown hares (*Lepus europaeus*) is the sole method used by red fox (*Vulpes vulpes*) for causing their death?** (Poster no. 22)

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Generalist predators are at the top of the food chain and they can come in contact with many micro and macro parasites that occur in their prey species. Thus, they could be considered both potential carriers and sentinel of these infectious agents, even if they are not susceptible species, without developing both infection and clinical signs. This is the case of European brown hare syndrome (EBHS), a lagovirus endemic in Europe that causes severe disease in brown hares.

During the annual wildlife surveillance in force in Lombardy, we identified an outbreak of EBHS in a protect area by both serological examination (c-ELISA) on captured animals and virological analyses (sandwich ELISA and RT-PCR) on target organs of one dead hare. In addition, we examined by RT-PCR for EBHSV the liver, spleen, mesenteric lymph nodes and three intestinal tracks of four red foxes hunted in the same area. The intestinal content of one fox resulted positive, whereas its other organs and all the viscera of the remaining three foxes resulted negative. Moreover, among the food debris, present in the gastrointestinal contents of the positive fox, we found materials genetically identified as of hare origin. Then, we serologically tested the foxes for EBHSV antibodies by c-ELISA with negative results. The nucleotide identity of the EBHSV strains isolated in the hare and the fox was 99.9 %, and the amino acid identity was 100 %, indicating the presence of the same viral strain in the two species.

These results proved, for the first time in natural condition, the possible epidemiological role of carnivores as passive vectors of EBHSV. In particular, asymptomatic foxes that have consumed EBHS-infected hares can carry the virus and spread it with their droppings, maintaining active the viral circulation without the contribution of the defined reservoir species.

Predatory activity and EBHS are considered two of the main causes of the hare population decline in Europe. The evidence of the concrete possibility that red fox can be a "mechanical" carrier of EBHSV contributes to improve the comprehension of the epidemiology of EBHS and to specify that foxes can induce the death of hares not only preying them.

**Evaluation of pestivirus infection in the European hare (*Lepus europaeus*) and alpine marmot (*Marmota marmota*)** (Poster no. 21)

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The role of free-ranging sympatric wildlife is of special interest in Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (BDV) research. Those ruminant pestiviruses are worldwide distributed and the economic impact of their infections in livestock triggered the implementation of eradication plans in many countries. The description of high mortality outbreaks of Pyrenean chamois (*Rupicapra p. pyrenaica*) associated to BDV-4 extended the interest on the epidemiology and the livestock-wildlife interface of these viruses. In the present study, European hares (*Lepus europaeus*) and Alpine marmots (*Marmota marmota*) from Catalonia (NE-Spain) were sampled as a first approach on the potentiality of acting as wild reservoirs of ruminant pestiviruses. For this purpose the presence of antibodies against BDV and BVDV was evaluated by means of the Virus Neutralization Test (VNT) and the presence of viral RNA by Reverse transcription-polymerase chain reaction (RT-PCR) in sera samples. Ninety-four sera from wild hunted European hares were collected in two different areas: Pyrenees (alpine and subalpine ecosystems) versus Non Pyrenees (non alpine and subalpine ecosystems). Also, 49 marmots were captured and sampled in an area (Central Pyrenees) with high seroprevalence of antibodies against BDV in chamois. A total of 34 out of 94 European hares (36.2 %; CI<sub>95</sub> 0.26 - 0.46) presented neutralising antibodies against ruminant pestiviruses, and significant differences were found between BVDV1 and BDV4 titres in 3 and 4 samples respectively. Sera from alpine marmots had negative VNT results. PCR analyses to detect RNA virus in sera from both species were negative. No statistically significance was found when comparing seroprevalences of European hares between Pyrenean and Non-Pyrenean areas. To authors' knowledge, European hare is the third wild non-artiodactyl with reported antibodies against ruminant pestiviruses after the rabbit and Bennet's wallaby. The presented experience contributed with valuable information to BVDV and BDV epidemiology and could encourage more research in the role of sympatric free-ranging wildlife.

**Tackling lead poisoning: people, petitions, politics and painfully slow progress**  
(Poster no. 85)

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**Aim:** Are we any closer to reducing toxic risks from lead ammunition to humans, livestock, wildlife and the wider environment? The presentation will provide a brief review of recent developments, reflecting on the following:

**Evidence:** As awareness of the medical and veterinary risks of low level lead exposure has increased, further evidence of impacts of lead ammunition has emerged along with research into use of the non-toxic alternatives. Whilst valuable, evidence alone is insufficient to hasten transition to non-toxic ammunition.

**Policy:** The significant policy step of a 2014 UNEP-Convention on Migratory Species Resolution on phasing out lead ammunition by 2017, and a resultant Lead Task Force, have added pressure for transition. A current call for evidence by the European Chemicals Agency for restriction of lead ammunition under the REACH process offers a good prospect for eventual mandatory restriction on the use of lead ammunition.

**Politics:** In the UK, despite a 5 year government process to evaluate risks and suggest mitigation measures, at time of writing, political appetite for implementing the recommendations appears lacking. Pro-lead lobbying has been significant and a government debate on the issue highlighted numerous misrepresentations of the evidence. Related to this, the shooting media messaging promotes the idea that the evidence and its messengers are untrustworthy.

**People:** In the UK, petitions to both ban and keep lead ammunition have mobilised 10,000s of people to become involved in a debate that is often polarised and entangled with wider shooting, land-use, and ethical issues. Nonetheless, open and honest face-to-face discussions with shooters are invariably constructive and areas of consensus provide a starting point for approaching the issue. Indeed, some shooters and shooting commentators have ‘put their head above the parapet’ and spoken of the need to have a more sustainable approach to shooting.

**Conclusion:** As wildlife disease specialists our evidence is important but the mitigation of this long standing issue will be determined ultimately by politics and policies. We must work together as an expert community to call for evidence-based decision making rather than allowing industries to follow typical patterns in their approach to use of lead and perpetuate unsustainable and unhealthy practices.

**An ecosystem approach to health in wetlands** (*Poster no. 84*)

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Wetlands are vital for life and provide essential ecosystem services such as provision of food, water, and resources; water storage and flood reduction; carbon storage; cycling of nutrients; pollution control; and offer opportunities for learning, relaxation and wellbeing. This diverse range of habitats is, however, often subject to mismanagement at multiple scales from local to catchment level. Threats such as pollution, abstraction, salinisation following irrigation, canalisation, and drainage can ultimately result in a range of poor health outcomes for humans, domestic animals and wildlife. Taking an ecosystem approach to wetland use and management offers a means to promote health across the board yet this concept remains uncommon in current health policies.

An analysis of wetland-related diseases was undertaken to identify the 'upstream' causes. A range of both new and old anthropogenic activities were identified as being directly or indirectly causal of ill health in and around wetlands.

To guide and encourage countries to understand that working 'upstream' and taking an ecosystem approach in decisions about land management and policy, an important resolution (XI.12) with an associated human health report and animal disease manual, were adopted at the Ramsar Conference of Parties in 2012. This was intended to help promote health with a focus away from current areas of health expenditure on surveillance systems and treatment measures.

Measurement of the impact of the adoption of this particular resolution is complex and it is likely that a significant shift in health thinking and policies remains unrealised.

Whilst it is easy to theorise how ecosystem approaches to health in wetlands could be beneficial, some catchment or basin-scale demonstration projects, of this approach are required to help illustrate the cost benefits of this preventative and participatory approach. A growing body of literature plus the guidance texts produced by the Ramsar Convention offer practical solutions that ensure ecosystem services are maintained and health is promoted.

**Occurrence of *Giardia*, *Cryptosporidium* and *Entamoeba* in urban dwelling wild rhesus macaques (*Macaca mulatta*) in Chandigarh, India** (Poster no. 56)

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The transmission of zoonotic protozoans between human and nonhuman primates is facilitated by their close taxonomic relationship, posing a threat to public health and wildlife conservation. The aim of this study was to identify the occurrence of *Giardia*, *Cryptosporidium* and *Entamoeba* in urban dwelling wild rhesus macaques (*Macaca mulatta*) and use molecular characterisation to determine if they had a zoonotic or anthrozoonotic potential. Using direct immunofluorescence, *Giardia* cysts were identified in 31 % (53/170) and *Cryptosporidium* oocysts were identified in 0.6 % (1/170) of macaque faecal samples. PCR was performed on *Giardia* positive samples at the glutamate dehydrogenase, triose phosphate isomerase,  $\beta$ -giardin and SSU rRNA genes, and on the *Cryptosporidium* positive samples at the 18S rRNA locus. Molecular results will be presented at the conference. *Entamoeba* spp. were detected by genus specific PCR in 81 % (138/170) of samples. Using species-specific PCRs, *E. coli*, *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. nuttali* and *E. chattoni* were found in 48 %, 0 %, 0 %, 0 %, 0 %, 0 % and 0 % of samples respectively. Based on the results the potential for zoonotic/anthrozoonotic transmission of these three intestinal protozoan parasites is discussed.

**Lungworm disease in common seals of the Dutch North Sea: a study into the morphology and life cycle of seal lungworm** (*Poster no. 59*)

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Verminous pneumonia caused by the lungworm genera *Otostrongylus* and *Parafilaroides* is currently the primary cause of disease in common seals, *Phoca vitulina*, of the Dutch North Sea. A recent sharp increase in cases of lungworm infection in this location, and initial observations of extremely long *Parafilaroides* *sp.* adults, prompted us to investigate which prey species serve as intermediate hosts of these genera and whether the *Parafilaroides* *sp.* lungworms were, as expected, *Parafilaroides gymnuris*. Nematode larvae were isolated from wild-caught fish and shrimp by tissue digestion and the digests were screened by stereo-microscopy. DNA was extracted and PCR performed on all larvae collected. *Parafilaroides* *sp.* adults were retrieved from common seals and were characterised using both morphological and molecular methods (sequencing of the ITS-2, D3 expansion and COI regions of DNA). First-stage *P. gymnuris* larvae were found in hooknose (*Agonus cataphractus*), juvenile European plaice (*Pleuronectes platessa*) and juvenile common dab (*Limanda limanda*). *Otostrongylus* *sp.* larvae were not detected in any of the prey species examined. This is the first record of *P. gymnuris* in hooknose, but since these were first-stage larvae, experimental studies are required to determine whether the nematode moults to the infective stage in this species. We found no apparent morphological differences between *Parafilaroides* *sp.* from North Sea common seals and previous descriptions of *P. gymnuris*, with the exception of mature females averaging up to 3.59 times greater in length than previous mean values. Our ITS-2 data support parasite identification as *P. gymnuris*, in common with sequences reported previously from this seal population in German waters. D3 and COI sequences were not available for *P. gymnuris* on GenBank, but they were available for *P. decorus* (D3) and *P. normani* (COI). Our sequences did not match these species with high identity and provide new genetic data for *P. gymnuris*.

**A post mortem investigation on the 2015 mass stranding of juvenile Magellanic penguins (*Spheniscus magellanicus*) on the coast of São Paulo state, southeastern Brazil** (Poster no. 31)

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The Magellanic penguin (*Spheniscus magellanicus*), an inhabitant of the coast of Chile, Argentina and Falkland/Malvinas Islands, reaches the south-southeastern Brazilian continental shelf during their annual winter migration (March - September). Most of the beach-cast penguins are inexperienced and debilitated juveniles that while foraging in Brazilian waters may be impacted by anthropogenic activities, e.g., fishing interaction, pollution, ingestion of synthetic materials and oil spills.

We discuss the anatomopathological findings in Magellanic penguins stranded along the southeastern Brazilian coast from May to July, 2015. Beach-cast carcasses (n = 20), live birds deceased while in rehabilitation (n = 24), and birds drowned after becoming entangled in fishing nets (n = 6) were analysed. Due to freezing and advanced autolysis, only eight carcasses (16 %) were considered appropriate for histological evaluation.

Upon necropsy, all by-caught casualties were in excellent body condition, while all beach-cast and rehabilitation casualties were severely emaciated. Fragments of selected organs were fixed in 10 % buffered formalin and processed according to routine histopathology protocols. Sexing identified 41 females and nine males. Histopathological findings were observed in the following systems: cardiac (100 %), respiratory (100 %), hepatic (62.5 %), lymphoid (50 %), gastrointestinal (25 %), neurologic (25 %) and others (25 %). Fragments of inorganic (plastic, rubber, and ropes) and organic materials (wood and algae) were present in the gastric contents of 24 % of the animals. The anisakid nematode *Contracaecum pelagicum*, previously reported in Magellanic penguins was the only endoparasite morphologically identified in the upper gastrointestinal tract of 10 % of the

animals. The advanced stage of autolysis precluded further gastrointestinal evaluation.

Our results confirm previous studies indicating a higher mortality of juvenile females on our coast. The high number of foreign materials, poor body condition and pathological findings in beach-stranded and rehabilitation casualties indicate their difficulty in meeting their required daily nutritional needs, probably due to a combination of factors, e.g. pollution, anthropogenic activities (e.g. fishing, littering), low prey availability and the 2015 “El Niño” (one of the strongest on record). However, the excellent body condition of the by-caught birds suggests that some of these birds are finding successful feeding strategies, and while doing so, interacting with human fishing activities, thus increasing their chances of being injured and/or drowned. Further monitoring studies including post mortem examination are needed to clarify the number of annual casualties, their physical condition upon stranding and the threats faced by Magellanic penguins while on the Brazilian continental shelf, contributing to their conservation and our knowledge regarding their ecology and population dynamics.

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**From epizootic disease to asymptomatic *Mycoplasma conjunctivae* infections in the Iberian ibex (*Capra pyrenaica*)** (Poster no. 96)

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The susceptibility of the Iberian ibex (*Capra pyrenaica*) to the *Mycoplasma conjunctivae* ocular infection was studied in the context of an infectious keratoconjunctivitis (IKC) outbreak in a population maintained in captivity as a genetic reservoir stock, nearby the Sierra Nevada National Park, Southern Spain. Eye swabs and serum samples were collected from yearly routinely managements in November (2010, 2011, 2013 and 2014). In 2013, three managements from August to November were performed to follow-up an IKC outbreak. *Mycoplasma conjunctivae* was detected by qPCR in eye swabs in 2013 for the first time coinciding with the IKC outbreak. Clinical signs were severe as those reported for other outbreaks in wild Caprinae and several kids were necessary to therapeutically treat. Prevalence of *M. conjunctivae* had a decreasing trend in the consecutive months in 2013 (August 35.4 %; September 8.7 %; November 4.3 %) that was consistent with the clinical resolution. However, reinfections without IKC were observed in two ibexes on November 2013. Specific IgG production was assessed with an indirect “in-house” ELISA and was apparently not always triggered when *M. conjunctivae* infection occurred. Individual levels of IgG antibodies dropped steadily once the clearance of *M. conjunctivae* in the eye was effective and specific IgG did not seem to necessarily drive the resolution of *M. conjunctivae* infection. Surprisingly, high prevalence (75.3 %) of ocular asymptomatic infections was observed in 2014. Higher Ct-values from the qPCR were observed in asymptomatic infections 2014 than in clinical infections 2013 and age related differences of prevalence were only observed in 2013. Phylogenetic analyses of the *M. conjunctivae* strains based on the *lppS* sequence showed slight differences between 2013 and 2014 but within the same cluster. As similarly reported for the Alpine ibex (*Capra ibex*), both epidemic IKC and high prevalence of asymptomatic *M. conjunctivae* infections may occur in the same Iberian ibex population.

## **Syndromic surveillance of mange in wild boar from 1988 to 2015 in France**

(Poster no. 91)

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External parasites like *Sarcoptes scabiei* may cause mange in domestic and wild swine (*Sus scrofa*) under particular conditions (e.g., under-nutrition and extreme aggregation). While considered as an emerging phenomenon in some European areas, mange-like lesions in wild boar have been reported in France by the national mortality surveillance network (SAGIR) for more than 25 years. We studied the distribution of mange-like lesion reports performed by SAGIR from 1988 to 2015. Mange-like lesions were also retro-actively reported in live/hunted wild boars, using questionnaires to referent observers all over France. In total, 45 departments reported mange-like lesions, either by the SAGIR network and/or by local observers. In some departments the two reporting systems were in disagreement, which can be explained by an under-detection or under-notification of the disease in some areas. Parasites involved in the mange-like lesions were mainly *Sarcoptes scabiei* and *Demodex phylloides*, but one case was associated also to *Psoroptes* sp. and some acarid remained unidentified. This study is not representing the true distribution of parasites within the wild boar population since clinical cases have been recorded in an opportunistic and participative way, but it gives a first sight of mange-like distribution, probably at an advanced/notifiable stage. An active surveillance of wild boar hunting areas and the establishment of a systematic data collection process would improve our understanding of the parasites distribution and of the conditions of mange-like expression.

**Molecular detection of *Plasmodium malariae* in captive non-human primates of Costa Rica** (Poster no. 51)

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Between 2012 and 2013, human cases caused by *Plasmodium malariae* were diagnosed for the first time since 1959 in Costa Rica, although *Plasmodium brasilianum* was reported in blood smears of wild howler monkeys (*Alouatta palliata*) since 2006. South American monkeys are naturally infected with *P. brasilianum*, and the high genetic identity found between *P. malariae* and *P. brasilianum* suggests the occurrence of recent transfers between hosts. In order to establish whether non-human primates from Costa Rica could act as potential reservoirs, presence of *Plasmodium* parasites were determined in autochthonous (*Saimiri oerstedii*, *Ateles geoffroyi*, *Cebus imitator*, *Alouatta palliata*) and nonnative (*Callithrix jacchus*) monkeys living in captivity in our country.

A total of 158 blood samples were collected from non-human primates from thirteen rescue centers and analysed using thick blood smear and semi-nested multiplex Polymerase Chain Reaction (SnM-PCR) for species differentiation. The parasite *P. malariae* was identified in two samples using thick blood smear, these samples yielded also positive results in the SnM-PCR. A total of five (3.3 %) samples were found positive to *P. malariae* in the SnM-PCR. Sequence analysis of one sample confirmed the results, the sequence resulted identical to the sequences of the three human *P. malariae* cases reported recently in Costa Rica. Finding *P. malariae* / *P. brasilianum* in different non-human primates living in captivity suggest, that latter may act as a reservoir of *P. malariae*.

**Mycoplasma infections in ibex (*Capra ibex*) and chamois (*Rupicapra r. rupicapra*): not only *Mycoplasma conjunctivae*! (Poster no. 45)**

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Despite the wide knowledge on *Mycoplasma conjunctivae* in wild ungulates, less information are available on other Mycoplasma infections in free-ranging ruminants although they may affect these wild species. Mycoplasma, acting alone or in association with other infecting agents, can indeed cause severe pneumonia for which particularly free-ranging ibex (*Capra ibex*) and chamois (*Rupicapra r. rupicapra*) may be very susceptible till having effects on populations' dynamics.

Here, we report Mycoplasma infections recorded in free-ranging ibex and chamois from Italian Central Alps describing gross pathology lesions and the diagnostic trial we used to successfully detect Mycoplasma species.

Five found-dead ungulates (three ibexes and two chamois) and lungs of an hunted chamois were analysed. Animals were necropsied and a standardised diagnostic trial was performed. For Mycoplasma analyses, lung samples were inoculated into both pleuropneumonia-like organism (PPLO) agar and broth. Positive samples were confirmed by PCR and identified by partial sequencing of the 16S rRNA gene.

A septicaemia of *M. mycoides* subsp. *capri* with generalised lymph-nodes hyperplasia, bronchopneumonia and spleen hyperplasia was recorded in a young ibex. The same Mycoplasma was detected in the lung of a hunted young chamois with pleuritis and bronchopneumonia localised in apical lobes of both lungs. In both the infections, Mycoplasma acted in association with a virus, parapoxvirus in ibex and gamma-herpesvirus in chamois. *M. agalactiae* and *M. ovipneumoniae* were detected in two adults, an ibex and a chamois, respectively. In both cases Mycoplasma were in association with *Pasteurella sp.* and, only in the ibex, also with herpesvirus (BHV4). In other two cases, an ibex and a chamois, Mycoplasma were detected by PCR but the identification was ambiguous.

Mycoplasma recorded in ibexes and chamois are among the most spread and pathogenic for small ruminants. As during summer pastures these wild species can have a spatial overlap with flocks, sheep and goats may have a role in the spread of these infections crossing the interface wild-domestic animals. However the prevention of these transmissions could be hard to put into practice, considering the difficulties of diagnosis and control of the mycoplasmosis in domestic animals, the presence of asymptomatic carriers and the management of grazing livestock.

**Absence of Rift Valley Fever Virus in wild and domestic ruminants from Spain** (*Poster no. 10*)

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In recent years, there have been growing concerns that Rift Valley Fever virus (RVFV) may reach the north of Africa, in which case, the risk to Spain would increase significantly, mainly because of the short distance to Africa and the possibility of infected vectors being transported on the wind. In the event of introduction of RVFV into Spain, conditions (temperatures and presence of competent vectors and hosts) are likely to be favorable for transmission. Therefore, a serosurveillance study was carried out on wild ruminant species (red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and mouflon (*Ovis aries musimon*) and livestock (cattle, sheep and goats) from Andalusia (Southern Spain), the region closest to North Africa, to determine whether RVFV had been circulating between 2009 and 2015. Antibodies to RVFV were not detected in any of the 1,016 wild ruminants and 977 domestic ruminants analysed using a RVFV specific blocking ELISA. Our results provide evidence to support the absence of RVFV circulation in Andalusia between 2009 and 2015.

**Bats and their endoparasites: analysing infections and toll-like receptor gene polymorphisms** (Poster no. 64)

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Bats (Chiroptera) are one of the most successful and diverse of mammalian orders, with an estimated 1100 species worldwide. They are host to a range of infectious agents including rabies, SARS and ebola viruses, and they also harbour endoparasites. However, studies of bat endoparasites are relatively limited when compared with that of other mammalian orders such as the Rodentia. As such, there are many fundamental questions concerning parasites of bats that remain unanswered. To this end, we initiated a parasitological analysis of a population of pipistrelle bats (*Pipistrellus pipistrellus* and *P. pygmaeus*) obtained from the South Lancashire/Greater Manchester area of the United Kingdom. These specimens were either dead upon acquisition, or, were euthanised due to extent of injury. Our published findings show that pipistrelles are commonly infected with trematodes and that interestingly, there was a statistically significant difference in the distribution and abundance of these parasites between male and female bats. We have also confirmed that bats may act as a reservoir host for the protozoan parasite *Toxoplasma gondii*. We have further utilised molecular screening to detect the presence of trypanosomes, *Babesia vesperuginis* and coccidian parasites. We are now proceeding to address how the bat immune system interacts with this plethora of endoparasites. To this end, we have initiated an analysis of pipistrelle toll-like receptor (TLR) genes. Currently, we are screening pipistrelle TLR4 and TLR2 genes with a view to characterising the genetic variation in these toll-like receptors and whether, or not, particular haplotypes correlate with the observed parasite infection profiles. Our data confirms that genetic variants of both the aforementioned pipistrelle TLR genes occur. Interestingly, correlating the bat infection profile with the TLR gene sequences offers the intriguing possibility that specific polymorphisms may be associated with resistance, or susceptibility to endoparasite infection.

**Microscopic and morphological study of pangolin scales using Scanning Electron Microscope-EDAX - a case study** (Poster no. 76)

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Under the Indian Wildlife Protection Act of 1972, poaching incidents of endangered species of pangolin; *Manis crassicaudata* (thick-tailed pangolin / scaly anteater), in local language commonly known as “Khawalya Manjar”, are increasing. This mammal covered in hard keratinous scales is trafficked in large volumes and a very real possibility exists that it could go extinct before most people realise its existence. Identification by means of a forensic investigation could help in identifying the species.

We received scales which were identified by DNA sequencing as that of Pangolin. Current work undertaken in forensic science laboratories deals with the possibility of utilising SEM-EDAX technology in studying morphological and microscopic characteristics. In doing so, a biometric database can be accumulated as reference. The morphological and microscopic observation of individuals and their body parts, such as pangolin scales, sourced from a pangolin poached scene can be studied.

Technology such as the use of a Scanning Electron Microscope-EDAX (SEM) could contribute significantly towards assessing and characterising a variety of other morphological characteristics such as elemental profile of scale, striations on the scales etc. for identification. The elemental profile of the pangolin scale by making use SEM coupled with the use of energy dispersive spectrum analysis will provide further insight into the structure of certain pangolin scales.

## **Findings of *Salmonella enterica* in red foxes (*Vulpes vulpes*) in Tyrol, Austria** (Poster no. 37)

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Based on a statistical sample plan liver and intestine of 434 red foxes hunted in the nine districts of the federal province of Tyrol were investigated for the presence of *Salmonella* using standard microbiological procedures. Genotyping of the isolates was done by pulsed field gel electrophoresis. Fox faecal samples collected on a mountain pasture during an outbreak of salmonellosis in chamois were also included in the investigation.

Of 434 foxes nine animals (2.1 %) were positive for *Salmonella* spp. In post mortem no specific macroscopic lesions could be detected in the infected animals. Serotyping revealed five (1.2 %) *S. Dublin*-positive foxes originating from three districts of Tyrol. Furthermore single strains of *S. Abony*, *S. Typhimurium*, *S. enterica* subsp. *diarizonae* and a monophasic strain of the group B could be isolated. The faecal samples collected on the mountain pasture also tested positive for *S. Dublin*.

The present results show that different *Salmonella* strains exist in the Tyrolean fox population. The majority of our detected *Salmonella* strains belong to the serovar *S. Dublin* which is a highly host-adapted serotype mostly restricted to cattle. It is not surprising that four out of the five detected *S. Dublin*-isolates in our study originated from two districts where this serotype is known to be present in cattle herds. The source of infection of these foxes is unknown but we strongly believe that the positive animals were primarily acquired through ingestion of *S. Dublin*-infected cattle material e.g. abortion tissue. The occurrence of *S. Dublin* in the fox faeces found near skeletonised chamois is not unexpected as these chamois were killed by an outbreak of *S. Dublin* caused by diseased cattle's which were shedding the bacteria in single water pools on the alpine pasture during the summer period.

Our results highlight the role of red foxes as an indicator animal for *Salmonellae* infection and emphasise the importance of wildlife monitoring in Austria. The occurrence of *S. Dublin* in the Tyrolean fox population demonstrates an existing interface between domestic animals and wildlife.

## Host clustering of *Coxiella burnetii* genotypes in coexisting wild mammals

(Poster no. 43)

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Evidences of the implication of wildlife in the ecology of *Coxiella burnetii* - the causal agent of Q fever in ruminants, pets and humans - are increasing. This suggests that wildlife may pose a threat to domestic animal and human health in interaction scenarios. Any potential attempt to link Q fever outbreaks in domestic animals and humans with wildlife would need from wildlife *C. burnetii* genotyping studies.

This study aimed, on one hand, to genotype *C. burnetii* strains circulating in wildlife to assess for any link with strains isolated in livestock and humans and, on the other, to assess for inter-species transmission in coexisting wild mammal species. Multiple-locus variable number tandem repeat (MLVA-6-marker) and/or PCR followed by reverse line blot (RLB) hybridisation were performed over *C. burnetii* PCR-positive samples from red deer (*Cervus elaphus*), Eurasian wild boar (*Sus scrofa*), racoon (*Procyon lotor*), European wild rabbit (*Oryctolagus cuniculus*), black rat (*Rattus rattus*), wood mouse (*Apodemus sylvaticus*), common vole (*Microtus arvalis*) and house mouse (*Mus musculus*). Goat and sheep *C. burnetii* PCR-positive samples were included for comparison. MLVA typing was performed by using six variable loci in *C. burnetii*: Ms23, Ms24, Ms27, Ms28, Ms33 and Ms34. The *C. burnetii* cooperative database from MLVABank 5.0 was employed to compare MLVA genotypes found in this study with 344 isolates of diverse origin. PCR-RLB results were compared with available information in wildlife, domestics and humans.

We identified 22 MLVA-6-marker genotypes from wildlife and 2 from domestic goats. By PCR-RLB, genomic groups I, II, VI and VII were found in wildlife and groups I, II, III and IV in sheep and goats. Some MLVA and PCR-RLB genotypes of wildlife clustered with isolates from human Q fever cases, livestock and ticks. Most PCR-RLB genotypes identified in sheep and goats in this study clustered with genotypes reported in livestock. Interestingly, MLVA and PCR-RLB genotypes in coexisting red deer and wild rabbits clustered around its host of origin. Very few

genotypes were shared even though deer and rabbits coexist in time and space. Our findings support the idea that wildlife constitutes a source of *C. burnetii* for domestics and humans. However, our results point to host specificity of *C. burnetii* strains in complex ecological contexts that merits further research. Generally speaking, our findings provide important insights to understand the epidemiology of *C. burnetii* at the wildlife-livestock-human interface.

**Could vaccination reduce *Coxiella burnetii* infection and shedding prevalence in red deer (*Cervus elaphus*)?** (Poster no. 42)

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*Coxiella burnetii* is a gram-negative zoonotic bacterium that uses several animal species as hosts. Wild animals may constitute an important source of *C. burnetii* infection for humans, pets and livestock in wildlife-livestock-human interaction scenarios. The red deer (*Cervus elaphus*) may be among the most relevant wild reservoirs of *C. burnetii* in Europe because of its increasing demographic and geographic trends, its high appreciation as game species, its gregariousness and its ability to replicate and shed *C. burnetii* to the environment. Therefore, it is of paramount relevance to design accurate control measures of *C. burnetii* in red deer to control inter-species transmission and prevent wildlife-related Q fever outbreaks in domestics and humans.

Vaccination is one of the most effective *C. burnetii* control measures in domestic ruminants and it could also be a control tool in red deer populations. As a first stage to test the efficiency of vaccination in controlling *C. burnetii* infection in red deer, we selected a *C. burnetii* endemic semi-extensively bred red deer population and a commercial phase I inactivated *C. burnetii* vaccine (Coxevac, Ceva Santé Animale, France) as models. Around the 75 % (n = 517) of females within the study population (n = 699) were vaccinated and revaccinated along a 3-year period whereas around the 25 % of them (n = 182) remained as control group. Vaccine efficiency was evaluated in terms of seroconversion (ELISA) and reduction of *C. burnetii* shedding prevalence and burden (semi-quantitative PCR) in vaginal secretions, milk and faeces along the study period.

Vaccination induced high seroconversion rates and long-lasting antibody levels. There was no reduction in *C. burnetii* shedding prevalence and burden in vaginal secretions and milk in any of the groups. Nonetheless, the prevalence of *C. burnetii* shedders in faeces significantly decreased in vaccinated animals and coexisting non-vaccinated mates along the study period. The reduction in faecal shedding along the vaccination experiment coincided with a reduction in the annual incidence of infection by *C. burnetii* in coexisting non-vaccinated yearling females. Since faeces are considered the main source for environmental pollution with *C. burnetii* and the bacterium is transmitted predominantly by infected aerosols, long-time vaccination may be a promising tool to reduce the risk of *C. burnetii* transmission from red deer in scenarios of interaction with domestics and humans.

***Staphylococcus* diseases and associated lesions in European hedgehogs (*Erinaceus europaeus*) admitted to wildlife rescue centres in North-West France** (Poster no. 66)

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Wildlife rescue centres have been in operation in France for decades. Even if most admitted animals are wild birds, the European hedgehog (*Erinaceus europaeus*) is the more frequent mammal admitted to these centres. The main diagnosed reasons for live casualty admissions are trauma, infectious diseases and orphanage. Skin lesions are frequent and result most often from gardening activities, predation, parasitic or fungal diseases.

In spring and early summer 2015, hedgehogs showing unusual clinical skin lesions were admitted in two wildlife rescue centres in North-West France. Similar cases were observed in spring 2016. Lesions were prominent purulent crusts with underlying deep ulcers, affecting mostly skin areas free of spine (limbs and head). Some individuals were in very bad condition and spontaneously died or were euthanised for welfare reasons.

To investigate these cases, diagnostic tests were performed on live or dead animals. Swabbing of skin lesions was done for bacteriology and antibiotic resistance testing on live and dead casualties. Histopathology was performed on skin lesions and on any other post mortem examination findings.

Affected animals were adults as well as juveniles. Adults were all males whereas the same ratio of sexes were recorded in juveniles. Various amounts of ectoparasites (fleas, ticks and/or mites) was noticed on all individuals. Two species of *Staphylococcus* were mainly identified: *S. aureus* or *S. xylosus*, often with resistance to beta-lactam antibiotics. Histopathological findings on skin lesions consisted of diffuse hyperplastic, hyperkeratotic and multifocal ulcerative and suppurative, deep and chronic dermatitis, with extension to the muscle and periosteal tissue. In several cases, pronounced parakeratosis, intracellular edema of middle layer epidermal keratinocytes and marked hyperplasia of basal keratinocytes reminiscent of superficial necrolytic dermatitis and generic food dermatosis observed in dogs was noted.

To the best of our knowledge, this is the first report of *Staphylococcus* skin disease in European hedgehogs in France. Further studies are needed to precisely characterise this apparent emergent skin disease and to identify the associated risk factors. Similarly, the observed “generic food dermatosis-like” lesions are a new finding in this wild mammal species and need more investigation to identify their origin. This report will be useful to inform the public and the medical staff of wildlife rescue centres on zoonotic hazards when handling a hedgehog especially with skin lesions. Finally, this work demonstrates once again that French wildlife rescue centres can contribute to epidemiological surveillance of wildlife diseases.

**Experimental infection of bustard houbara (*Chlamydotis undulata*) with an avipoxvirus (Poster no. 101)**

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Avian pox is a viral disease due to viruses belonging to the Avipoxvirus genus. It is regularly observed in Houbara bustard conservation project, despite a systematic vaccination programme. Because the impact and the physiopathology of the disease is not well known in houbara bustard (*Chlamydotis* spp.), we set up an experimental model of avian pox disease in these birds, trying to reproduce a natural infection by stinging insects. Then nine 12 months old captive bred houbara bustards were infected with a subclade B2 canarypox-like virus strain (CNPV/*Chlamydotis undulata*/MA/007/2011) extracted from a cutaneous lesion, using the intradermal route. The proximo-lateral part of the tarsometatarsus of both legs were infected with a 50 µL drop containing the virus by 10 pricks on each leg. We observed a morbidity rate of 78 % with strict cutaneous lesions on 12 legs (50 %), exclusively localised at the inoculation site. Among these lesions, ten reached the papule stage, and only two the scabby mass stage, which is the last evolution of the cutaneous form of the avian pox disease. The average incubation time was higher than in the literature, at 39 days post-infection, with a minimum at 20 days and a maximum at 63 days. The infection had a significant negative effect on several parameters such as the weight and the food consumption. Moreover, basophils were significantly positively influenced by the infection, whereas the alpha-2 globulins were negatively influenced. Then, histopathological analysis performed on the sampled lesions all showed massive secondary infection but only few cytopathic lesions with characteristic intracytoplasmic eosinophilic inclusions (Bollinger bodies). The presence of the virus DNA was detected by performing qPCR on the sampled lesions. No control birds (n = 3) showed any sign of the disease. The model developed in this study allowed to reproduce a cutaneous form of the avian pox disease in the houbara bustard. Even if few lesions reached the final stage of the disease, some haematological and physiological changes showed that even sub-clinical infections can be deleterious for houbara bustards, which is to be taken into account in a conservative field.

### **Cetacean morbillivirus in Brazil** (Poster no. 16)

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Morbillivirus is an emergent pathogen associated with severe epizootic outbreaks causing high number of deaths among pinnipeds and cetaceans, mainly in the northern hemisphere. Two species of the genus *Morbillivirus* (family Paramyxoviridae, order Mononegavirales) are known to affect aquatic animals: *Phocine distemper virus* (PDV) and *Cetacean morbillivirus* (CeMV). PDV has been isolated from pinnipeds, and four strains of CeMV (porpoise morbillivirus-PMV, dolphin morbillivirus, pilot whale morbillivirus and beaked whale morbillivirus) have been isolated from dolphins and whales. Little is known about the morbilliviruses in southern hemisphere marine mammals. On November 30<sup>th</sup>, 2010, a female Guiana dolphin calf, with 108 cm in total body length, severely emaciated, was found stranded dead in Guriri (18°44'S; 39°44'W), northeastern Brazil. Grossly the animal presented multifocal ulcers with necrosis on the oral and genital mucosa; and pulmonary and leptomeningeal congestion and oedema. Selected tissue samples were collected, fixed in buffered formalin and processed according to routine histopathological methods. The most significant microscopic lesions included: linfoplasmacytic and neutrophilic meningoencephalitis, optical nerve perineuritis and hypophysitis; linfoplasmacytic and neutrophilic interstitial pneumonia; multicentric lymphoid depletion; and multifocal necrotising hepatitis. Immunohistochemistry analysis was performed using a monoclonal antibody against the nucleoprotein antigen of Canine Distemper Virus (CDV-NP MAb, VMRD Inc). Viral antigen was detected in the brain, lung, liver, lymph nodes, stomach, intestines and genital slit. RNA was extracted from frozen lung samples and RT-PCR amplified an expected 374 bp conserved fragment of the viral phosphoprotein (P) gene. Considering the sequences available in GenBank, the fragment presented the highest similarity with PMV, DMV and PWMV, with nucleotide identities ranging from 78.7 % to 79.8 % and amino acid identities ranging from 62.1 % to 65.7 %. The sequence data and phylogenetic analysis suggested that the virus from this Guiana dolphin might be a novel strain or represents a new lineage of the cetacean morbillivirus species. These findings constitute the first evidence that morbillivirus is extant in the Brazilian waters and

that Guiana dolphins are susceptible to infection, raising concern on the pathology and epidemiology of morbillivirus in cetaceans in Brazil.

***Francisella tularensis* subsp *holarctica* in meat from tularemic hares**

(Poster no. 41)

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Tularemia caused by the bacterium *Francisella tularensis* may cause disease in a large number of animal species including humans. Common gross lesions in the mountain hare (*Lepus timidus*) and European brown hare (*Lepus europaeus*) who die of acute infection are enlarged liver and spleen, and necroses in the liver, spleen and bone marrow. Necropsies at SVA have shown that it is not unusual that gross lesions are lacking or easily overseen, increasing the risk that a hunter dressing a hare does not realise that the hare is infected. There are descriptions of people contracting tularemia after handling and consuming the meat from infected hares. Here we investigated to what extent hares diagnosed with tularemia have *Francisella tularensis* in the muscle (meat), being a possible source of infection for humans. Muscle samples from 43 hares diagnosed with tularemia, either found dead or euthanised, were investigated at SVA. Seven of these hares had no obvious gross changes. The muscle tissues were stored in -20°C in sealed plastic bags for a period varying between six months and eight years. Real time (RT) PCR was used to detect *F. tularensis* subsp *holarctica*. Histopathology was performed to characterise lesions, and immunohistochemistry (IHC) to detect and locate the bacteria in the tissue using a mouse primary antibody towards *F. tularensis* sp. (FB11, BioSite). For RT-PCR, thirty-nine samples were positive and four were negative. IHC detected small amounts of *F. tularensis* sp. in 14 muscle samples, most frequently in a perivascular and perimysial location. The bacteria were commonly found intracellularly in macrophages. In one sample, focal necrosis was associated to the bacteria. To evaluate the viability of the bacteria after variable length of storage in the freezer, culture for *F. tularensis* was performed on 14 of the samples that were positive for RT-PCR. In two of the samples, both with a storage period of less than one year, *F. tularensis* was detected. Since *F. tularensis* sp. was detected in the muscle samples, and gross lesions may not be obvious, there is a risk for humans to contract disease from the meat of infected hares.

**The Koala Health Hub: koala health, conservation and welfare through collaborative, evidence-based management** (*Poster no. 87*)

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Koalas are part of Australia's national identity but koala numbers have been in severe decline for many years and in most states the species' status is listed as "vulnerable", based on a dramatic reduction in koala numbers over the past 5 to 10 years, over most of its range. Threats to koalas include habitat loss, disease, domestic dog attacks, motor vehicle collisions and climatic extremes.

The Koala Health Hub (KHH) is based at the University of Sydney but brings together research and clinical expertise from across the country to benefit koalas directly, by connecting researchers in pathology, pharmacology, microbiology and genetics with ecologists and those on the coalface of koala management; in koala hospitals and in the wild. In doing so, the Hub is leveraging existing physical and intellectual capacity to bring together a diverse and innovative source of support and expertise for koala health management in Australia.

Through provision of field and laboratory support and disease expertise to koala care groups, research groups and zoos nationally, the KHH has engaged a diverse range of stakeholders enabling us to now make key contributions to the conservation of threatened populations by delivering research outcomes to koala care, state government and non-government collaborators. By supporting introduction of health and genetics studies into a range of koala ecology projects, we have not only provided an evidence base for koala management decisions, but also a common link between diverse koala management projects across the koala's range. The resulting knowledge has been instrumental in protecting the genetic integrity and reproductive health of threatened populations by informing translocation planning and management.

The KHH also hosts annual national workshops for key koala care groups, universities, state and local government bodies and non-government organisations. These have achieved consensus, among diverse stakeholders, on key issues for the management of hospitalised, captive and free-ranging koalas including: evidence base for koala care and management, risk assessment for translocation and re-release, and facilitation of research through optimal communication and standardisation of sampling and secure storage. Outcomes include sample collection workshops for care groups and researchers; necropsy workshops for veterinary and animal science students; provision of fact sheets and other resources; cross-validation of laboratory tests among research groups; and development of position statements to inform government policy.

**Disentangling the link between supplemental feeding, population density, and prevalence of pathogens with different transmission modes in urban stray cats**

(Poster no. 94)

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Supplemental feeding in free-roaming animals can alter host-pathogen interactions through mechanisms such as increased host population density and/or aggregated host distribution. In this study, we investigated the potential link between pathogen prevalence and host population density in urban stray cats, which are subject to varying intensities of cat caretaker activity involving supplemental feeding. We selected six districts as our study sites in Seoul, Korea, three with high and three low intensity areas of cat caretaker activity (CCA). Population density of stray cats was estimated in residential areas of each district. Blood samples of stray cats (n = 302) were collected through local animal hospitals participating in a trap-neuter-release (TNR) programme and tested for eight different pathogens (feline immunodeficiency virus, feline leukemia virus, feline panleukopenia virus, feline calicivirus, feline herpesvirus-1, *Bartonella henselae*, hemoplasma, *Toxoplasma gondii*). We analysed the prevalence of tested pathogens in relation to stray cat population density and intensity of CCA. There was no significant association between stray cat population density and CCA. Neither *B. henselae* nor feline hemoplasma infection was associated with CCA or host population density. However, prevalence of feline leukemia virus was significantly higher in areas with high CCA (P = 0.03), whereas that of feline parvovirus was higher in areas with low CCA (P < 0.001). We suggest that supplemental feeding may influence the prevalence of feline leukemia virus and feline parvovirus in urban stray cats by changing cat demographics, foraging patterns, and aggregation behaviour. Results from our study may have further implications for other urban-adapted wildlife using anthropogenic food sources.

**Importance aspects in the coexistence between human and wildlife (*Procyon lotor* and *Nasua narica* situation in Costa Rica)** (Poster no. 93)

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Costa Rica has great forests and protected areas but a lot of housing and touristic projects that mix the coexistence between wildlife and humans causing consequences for both. The programme Control y Prevalencia de Enfermedades en Animales Silvestres from the Escuela de Veterinaria de la Universidad Nacional Costa Rica provides services in wildlife aspects and diseases related. Government entities (Sistema Nacional de Áreas de Conservacion) contact the programme to evaluate different situations related to raccoons (*Procyon lotor*) and coatis (*Nasua narica*) principally, but other species such as wild felines, monkeys, iguanas, birds etc. were also treated. We focus on these ones because problems related to them in urban and touristic areas were higher.

Evaluation in infrastructure and animal health was carried out. In total, 180 raccoons and coatis were evaluated. All animals have an overall objective examination, parasitic test (sheather method), speed test for distemper, parvovirus, coronavirus and blood test. Relevant results: 87 % of the animals had overweight, 71 % were positive to parasites in faeces, all were negative to speed testes and there was no significant results in blood test.

The main parasites found were Ancylostomidae (72 %), *Strongyloides spp.* (35 %), coccidian oocyst (67 %), Oxyuridae (23 %), Acantocephala (17 %). Parasites were found in 85 raccoons (47,2 %) and in 43 coatis (23,8 %).

People in urban areas and tourism places coexisting with wild animals have troubles managing waste (including food). Some people also believe feeding wildlife animals helps animal welfare and also tourist like to feed animals to get closer to take pictures. This allows wildlife animals to have more and easy access to food and They learn to come and look for food in houses, hotels and areas habituated by humans instead searching their natural habitat. So they eat in these areas and then return to the forest. They start making their burrows closer to humans because they use human infrastructure. Reproduction rates growth more with easy access to food and infrastructure.

There are deficiencies in education for tourism and national population. This lack of education is principally refers to building houses and human infrastructure, as well as waste management. Education, evaluation of infrastructure, waste manage, animal health control are factors that need to be in constant vigilance to avoid vulnerabilities in both wildlife and humans that can lead to an antroponosis and zoonosis.

**Genetic diversity and antimicrobial resistance of *Campylobacter* and *Salmonella* strains isolated from decoys and raptors in Andalusia**

(Poster no. 33)

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Infections caused by thermotolerant *Campylobacter* spp. and *Salmonella* spp. are the leading causes of human gastroenteritis worldwide. Wild birds can act as reservoirs of both pathogens. A survey was carried out to determine the prevalence, genetic diversity and drug resistance of thermotolerant *Campylobacter* and *Salmonella* in waterfowl used as decoys and wild raptors in Andalusia (Southern Spain). The overall prevalence detected for *Campylobacter* was 5.9 % (18/306; IC<sub>95</sub> %: 3.25 - 8.52) in decoys and 2.3 % (9/387; IC<sub>95</sub> %: 0.82 - 3.83) in wild raptors. Isolates were identified as *C. jejuni*, *C. coli* and *C. lari* in both bird groups. *Salmonella* was isolated in 3.3 % (10/306; IC<sub>95</sub> %: 2.3 - 4.3) and 4.6 % (18/394; IC<sub>95</sub> %: 3.5 - 5.6) of the decoys and raptors, respectively. *Salmonella* Enteritidis and Typhimurium were the most frequently identified serovars, although *Salmonella* serovars Anatum, Mikawasima, London and Bredeney were also isolated. Pulsed-field gel electrophoresis analysis of isolates showed higher genetic diversity within *Campylobacter* species compared to *Salmonella* serovars. *Campylobacter* isolates showed resistance to gentamicin, ciprofloxacin and tetracycline, while resistance to erythromycin and tetracycline was found in *Salmonella* isolates. The results indicate that both decoys and raptors can act as natural carriers of *Campylobacter* and *Salmonella* in Spain, which may have important implications for public and animal health.

**Exploring primate diseases in the context of natural ecosystems: when are we allowed to talk about a reservoir for human infection?** (Poster no. 81)

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Central to the ‘One Health’ approach and any disease eradication programme is the question, whether or not a pathogen has a nonhuman reservoir. This has been shown for the successful eradication of small pox as well as the ongoing eradication campaigns in the Guinea worm disease (*Dranunculiasis*) or human yaws, which is caused by *Treponema pallidum* subsp. *pertenue*. The acceptance or rejection of a nonhuman reservoir for a disease, however, is often controversial.

We think that a critical discussion about the definition and use of the term “natural reservoir for human infection” is necessary. As infectious disease research is a multidisciplinary field, a common understanding of terminology is inevitable to avoid misunderstandings and wrong classifications. Based on a meta-analysis of current literature in combination with a critical review of findings from our nonhuman primate (NHP) treponematoses and malaria research we point to some essential problems in the field.

For instance, it is not sufficient for a true nonhuman reservoir that wild NHPs harbour human pathogenic *Plasmodium* spp., if no mosquito species with a host preference for NHPs and humans occurs in the same area. Exploring infectious diseases within the context of natural ecosystems is therefore important to evaluate the presence of a nonhuman host species as a true reservoir for human infection.

One of the three criteria of Henle-Koch’s postulates requires that an experimentally

transmitted pathogen causes a specific disease in a host, which for ethical reasons, is not applicable in humans. As a result, the causative relationship between simian *T. pallidum* strains and human yaws causing strains cannot be tested by inoculation experiments in humans. Therefore, NHPs are not regarded as a reservoir for human yaws infection until natural infection with the same strain has been found. However, given the high genetic similarity of treponemes isolated from NHPs and humans, the question arises, which degree of genetic similarity qualifies a pathogen to be regarded as the same? Most likely, the question here is not only about genetic similarity of the genome, but where exactly the similarities and differences are located and what impact they have on the biology of the microbe and its pathogenicity in humans. Such questions may provide a basis for a discussion on a common understanding on the acceptance of a nonhuman reservoir and a critical review in multidisciplinary infectious disease research.

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## **Endemic and emerging biotypes of *Salmonella* Enteritidis ST183 infection in the western European hedgehog (*Erinaceus europaeus*) in Great Britain**

(Poster no. 65)

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The western European hedgehog (*Erinaceus europaeus*) is a nocturnal insectivore which has undergone sharp decline in recent decades in Great Britain (GB), where it is now considered threatened. Whilst a multifactorial causation has been proposed, including diet limitation and habitat degradation, the extent to which disease is contributing to the population collapse requires further investigation. Hedgehogs are the most frequently admitted mammalian casualties to wildlife centres in GB. Opportunity therefore exists for close contact between hedgehogs and humans with potential for zoonotic and anthroponotic pathogen transmission. *Salmonella enterica* serovar Enteritidis is one of the major causes of human and animal *Salmonella* infections worldwide. Historically, *S. Enteritidis* provisional phage type (PT)11 has been reported in hedgehogs from GB and continental Europe and this infection may be endemic in this species; however, there is a paucity of data to inform wildlife and public health implications. We conducted post mortem examinations (PMEs) on a convenience sample of 171 hedgehogs submitted from across GB, August 2012 - December 2015. A standardised protocol supported by parasitological and histopathological examinations was employed. Microbiological examination was performed on liver and small intestinal contents, plus macroscopic lesions. Phage typing and whole genome sequencing (WGS) by Illumina HiSeq was conducted on all *Salmonella* sp. isolates. *Salmonella* Enteritidis multi-locus sequence type (ST)183 was isolated from 46/171 hedgehogs which comprised 28 *S. Enteritidis* PT11 and 18 of a novel PT66 biotype. PMEs demonstrated severe enlargement and/or abscessation of the mesenteric lymph nodes in all of the hedgehogs with PT66 infection; the bacterium was consistently recovered from these lesions. In contrast, PME revealed a more diverse range of macroscopic and microscopic findings in the hedgehogs with PT11 infection. WGS single nucleotide polymorphism phylogenetic analysis of the hedgehog isolates and of these biotypes available from humans in GB found that PT11 and PT66 form two distinct, clonal and divergent clades within ST183. PT66 was isolated from a localised cluster of hedgehogs in southern Scotland only whilst PT11 was recovered from cases across England and Scotland. Hedgehog isolates were

interspersed throughout the human isolates in the phylogeny. There was a strong geographical signal in the phylogenetic analysis with clustering of spatially proximate isolates. These results are consistent with the hedgehog acting as a local source of infection for humans with these ST183 biotypes. PT11 is considered likely to be an endemic infection of the British hedgehog population whilst PT66 is a recently identified, and potentially emerging, biotype.

**Molecular detection of Avipoxvirus associated to cutaneous lesions in a stone curlew in Sardinia (Italy)** (*Poster no. 26*)

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Avipoxvirus (APV) infections have been observed in a wide variety of wild, captive and domestic avian hosts, recently including a range of island endemic and endangered species, yet not enough is known about APVs genome diversity and phylogenetic relationships, as well as their host-range specificity. A wild stone curlew (*Burhinus oedicanus*) was recovered in Sardinia (Italy), showing large wart-like lesions and nodules on both legs and toes, which resulted positive to poxvirus by PCR. Histopathological examination of the lesions showed ballooning degeneration and large intracytoplasmic inclusion bodies consistent with an Avipoxvirus infection. A multiple gene sequencing approach was applied to highlight the phylogenetic relationships of this virus with a panel of selected APVs at the clade and subclade levels. This novel isolate was characterised by sequencing partial P4b, P35 (locus fpv140) and DNA polymerase genes and phylogenetic analyses assigned it to clade A, (Fowlpox virus, FWPV), subclade A2. Conservation implications of avian pox presence in Sardinian stone curlews and possibly in other island bird species are discussed.

**White-tailed eagle mortality causes from Estonia: shared health risks to raptors nesting and wintering in Estonia** (*Poster no. 100*)

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Historically our raptor populations have suffered from pollution related decline and so called “raptor war” where in soviet time birds of prey were sectioned as nuisance species and killed actively. To date most of raptor species populations have grown and reached stable status. At the same time, we are experiencing annually more sick, injured or dead raptors.

Until lately there has not been any monitoring programme to diagnose the causes of morbidity and mortality of protected raptor species in Estonia. Since 2013 we have been collecting dead eagles with the aim to preserve them for diagnosing the causes of mortality. To date, we have collected 46 dead white-tailed eagles (WTEA) (*Haliaeetus albicilla*) as well other eagle and raptor species. Additionally we have data of treated and released eagles and raptors in Estonia.

In current monitoring for cause of death, we perform physical examination, radiography and necropsy of the bird with collection of diagnostic samples (liver, kidneys, part of humerus, pectoral muscles, body fat, feathers, part of intestines, stomach contents and brain), what are preserved. Additional diagnostic need is decided on the basis of findings, but all birds are screened for most common contaminants.

Currently we can present the results of 29 birds, which were analysed in 2014 and 2015. We diagnosed the cause of death through lead poisoning in 52 % of studied WTEA. Other poisonings were found only in 7 % of birds. Traumas of different causes were diagnosed in 37 % of birds (projectile 17 %, transportation 10 %, wind power 2 % and unknown cause trauma 7 %). We could not diagnose the cause of death in 3 % studied WTEA's.

Based on the findings on studied birds, we can see high risk of lead poisoning for scavenging birds. Illegal shooting (killed by projectile) of WTEA shows that still so called “raptor wars” should be considered as an important risk for raptors in post-soviet countries. Therefore we see benefits of setting in national monitoring scheme what includes more species and is broad specter to detect causes of morbidity and mortality, including illegal hunting of protected species.

## **Multi-drug resistant *Pseudomonas aeruginosa* isolated from wildlife**

(Poster no. 34)

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*Pseudomonas aeruginosa* (PAO) is an opportunistic pathogen for humans and a major cause of nosocomial infections mostly in immunocompromised patients. PAO is intrinsically resistant to many classes of antibiotics. Due to its adaptability, the pathogen is difficult to control in the hospital environment resulting in frequent therapeutic failures. It also causes numerous diseases in various domestic animal species. Information about PAO from wildlife is scarce. The aim of this study was to investigate the presence of PAO in wildlife (i.e. mammals and birds) from Austria and Germany. In total, 366 free living wild animals belonging to 14 distinct species were necropsied between January 2014 and April 2016 to determine the cause of death. Five PAO isolates could be detected. Susceptibility testing was performed by agar disk diffusion for the following antibiotics: piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, colistin, polymyxin B, gentamicin, tobramycin, amikacin, and ciprofloxacin. Three isolates were susceptible to all tested antibiotics, while the remaining two were resistant to several antipseudomonal antibiotics. Both isolates originated from European hares (*Lepus europaeus*). Strains were recovered from the lung of a hare suffering from pneumonia and from liver of a hare suffering from hepatic necrosis. One isolate (AC 933) was resistant to all tested antibiotics except polymyxins. The second isolate (AC 328) was resistant to piperacillin, piperacillin-tazobactam, ceftazidime, aztreonam, and ciprofloxacin. Currently, both PAO isolates are further characterised using a multiphasic approach [DNA microarray analysis, Multi-Locus-Sequence-Typing (MLST), and multiple-locus variable-number of tandem repeat analyses (MLVA)]. Even though the presence of PAO was low, the detection of MDR isolates in wildlife is a new finding. Whether this represents a potential public health risk remains to be answered. However, more systematic studies are needed to determine the role of wild animals as reservoirs for antibiotic-resistant PAO.

## High prevalence of tula virus in common voles in the Netherlands

(Poster no. 11)

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In Europe, five different rodent-borne hantavirus species have been detected: Dobrava virus (DOBV), Saaremaa virus (SAAV or DOBV-Aa), Seoul virus (SEOV), Puumala virus (PUUV) and Tula virus (TULV), all having their own main reservoir rodent species. PUUV, SEOV, SAAV and DOBV are known to cause hemorrhagic fever with renal syndrome (HFRS) in humans. Though zoonotic transmission of TULV has been demonstrated, only few clinical patients have been described in literature thus far. Knowledge about TULV circulation and prevalence in rodents in the Netherlands is scarce. The aim of this study was to determine the prevalence and genetically characterise TULV in rodents at different locations in the Netherlands.

In 2014 and 2015, surveys on TULV were performed in different geographic locations in the south of the Netherlands (province of Limburg) and the north of the Netherlands (province of Friesland). Common voles (*Microtus arvalis*) were trapped and tested for the presence of virus specific antibodies and/or the presence of TULV RNA in tissues by RT-PCR. Multiple sequence alignment of the partial S-segment sequence was performed.

Almost 300 common voles were trapped and tested. TULV RNA was detected in 38 % of the animals, but the prevalence varied by geographic region from 12 to 45 %. Phylogenetic analysis indicated that Dutch TULV strains are closely related to strains from Germany and Belgium. However, even within the Netherlands, genetically distinct groups of TULV were identified, corresponding with their geographic origin.

Interestingly, while TULV was present at high prevalences in two geographically distinct locations, no human cases of TULV have been reported in the Netherlands. Human diagnostics should include a TULV specific assay to assess the pathogenic ability of TULV.

## **Surveillance and first detection of European bat lyssavirus type 2 (EBLV-2) in Norway** (*Poster no. 63*)

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Rabies has not been diagnosed in mainland Norway for two centuries, however European bat lyssavirus 2 (EBLV-2) has been detected in bat species in other Scandinavian countries, raising the possibility that it can also be present in Norwegian bat populations.

During 2014 and 2015 sixteen Daubenton bats (*Myotis daubentonii*) were sampled during routine checks performed by the Norwegian Zoological Society's Bat Care Centre. Swabs were collected and tested by PCR at the Norwegian Veterinary Institute for both classical rabies and European bat lyssavirus. Simultaneously some of these samples were submitted to pyrosequencing for metagenomic analysis. All PCRs were negative and pyrosequencing failed to identify any relevant viral sequences.

However in October 2015 a bat was found at 800 meters above sea level in the county Oppland, southern Norway, and brought to a care centre. After a short time, however, it displayed problems with moving and swallowing and eventually died two days after arrival.

The necropsy performed at the Norwegian Veterinary Institute stated that the bat was in poor condition with no specific gross findings, while histopathological examination revealed aspiration pneumonia. The rabies diagnosis was based on repeated RT-PCR analyses of brain tissues and partial sequencing of the nucleoprotein gene. A BLAST search against the GenBank database showed 96 % homology with EBLV-2. The fluorescent antibody test on brain impressions was negative.

This represents the first identification of EBLV-2 in a bat from Norway. Given this first positive result approximately 100 dead bats collected and stored at the Norwegian Bat Care Centre are being analysed to further understand the prevalence of this disease in Norway.

**Is spatial behaviour of Alpine ibex (*Capra ibex*) driving brucellosis transmission?** (Poster no. 95)

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France is faced with the management of an unexpected brucellosis outbreak in Alpine ibex (*Capra ibex*), that was discovered in the Alps after two human cases, were reported and associated to a single brucellosis event in cattle. This outbreak was monitored by capturing and testing ibex revealing a very high seroprevalence (> 40 %) associated with a frequent infection by *Brucella melitensis* biovar 3. This outbreak seems to concern only the ibex population of the Bargy massif, which is unfortunately in the heart of a raw milk cheese area. This ibex population has been monitored using different approaches including pedestrian census for calculation of abundance index, hormone dosages, serological examination and GPS collaring. GPS data and serological tests were more particularly explored here in order to investigate the potential link between ibex space use and their exposure to brucellosis. GPS data were explored using the spatial overlap between GPS-collared individuals as a measure of distance between them. We highlighted a strong clustering in female ibex, with individuals living in five distinct units with very low contacts between each others. On the contrary, no clustering was detected in male ibex, each male having potential contacts with other males and females all over the massif, especially during the rut. Each captured individual was then attributed to a spatial cluster according to its capture. We found that females were more infected than males, that seroprevalence increased with age until 8-10 years of age, and then decreased slightly, and that seroprevalence varied strongly among the spatial units identified from the females structured space use. We discuss how these results improved our understanding of the transmission routes of brucellosis and of spatial spreading of the disease.

## **Social status mediates the life-history consequences of canine distemper virus infection in an abundant yet overlooked keystone group-living carnivore**

(Poster no. 19)

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For any pathogen infecting multiple host species, understanding the role of each host species to disease dynamics is essential to predict the resilience of populations to future epidemics. Here, we focused on canine distemper virus (CDV), a highly infectious multi-host virus of aquatic and terrestrial carnivores. In the Serengeti ecosystem in Tanzania, the outcome of CDV infection varied considerably in different host species during several epidemics that occurred in the past few decades. Our main objective was to quantify the impact of CDV infection in a keystone and abundant yet often overlooked social carnivore, the spotted hyena (*Crocuta crocuta*). We assessed the effect of CDV infection on survival and explored how social rank, sex and age shaped the probability of infection. The novelty of our approach was to combine detailed longitudinal data from a population of individually known spotted hyenas (n = 625 females, n = 816 males) monitored continuously and non-invasively for more than two decades with a robust statistical method to account for uncertainty in the detectability and assignment of individual disease states. Using a hidden Markov process in multi-event capture-mark-recapture models allowed us to considerably improve the accuracy and precision of survival and infection probability estimates. We found that CDV infection in spotted hyenas is akin to a ‘childhood disease’, with cubs (< 1 year) having a significantly higher chance of developing clinical disease and hence of spreading the virus than older animals. In this highly social species where an individual’s rank within a linear dominance hierarchy determines access to food, health, allostatic load (‘stress’) and Darwinian fitness, cubs of high-ranking mothers were less likely to become infected and more likely to survive infection than those of low-ranked ones, demonstrating the importance of social status effects on susceptibility to disease in this species. Our results indicate that detailed and robust knowledge about the impact of pathogens in wildlife populations can be gained by combining non-invasive individual disease data with such a modeling approach. They also highlight the importance of long-term research and disease monitoring programmes in wildlife.

**Classification of macroscopical lesions compatible with avian trichomonosis – a common framework for clinical evaluation** (*Poster no. 55*)

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Avian trichomonosis is a fatal parasitic disease of wild birds that affects mainly columbiforms and raptors. The latest outbreaks among passeriform species in Europe and Canada has lead to consider it as an emergent disease. Here, we propose a clinical classification of macroscopical lesions compatible with avian trichomonosis in order to help wildlife professionals with the initial evaluation of the birds. This system will enable a better understanding and exchange of the information following a consistent methodology in the evaluation of macroscopical lesions. The first approach to the evaluation of the bird, relying in the visual inspection and palpation, without histopathological data, provides an easy to use classification system that could be used by anyone. For this purpose, we have evaluated 40 cases of avian trichomonosis in wild birds with lesions at the oropharyngeal cavity. Columbiforms (n = 6) as well as birds of prey, diurnal (n = 23) and nocturnal (n = 11), were included. The clinical cases were obtained from animals examined as part of a project that sampled more than 2,500 birds from wildlife recovery centres, scientific ringing campaigns and captive raptor breeding centres. The procedure must be initiated with the visual evaluation of the animal, opening of the beak to inspect the oropharyngeal cavity and palpation of the crop and breastbone. Presence, extension and location of macroscopical lesions compatible with avian trichomonosis, as well as body condition, must be evaluated. In that respect, we have established grade 1, 2 and 3 as levels of severity. Therefore, grade 1 is applied to small ulcers of less than 0.5 cm in length in the mucosae of the oropharyngeal cavity with a white surface, light tissue inflammation, no signs of regurgitation at the feathers surrounding the beak and

good body condition. Grade 2 corresponds with lesions less than 1 cm in length, mild tissue inflammation and white or yellowish colouration of the lesion. Finally, grade 3 is applied to granulomatous lesions of more than 2 cm in length, yellowish colour of the surface of the granuloma, severe tissue inflammation, wet feathers surrounding the bill due to regurgitation or sialorrhea and poor body condition with noticeable weight loss and, in extreme cases, cachexia. Nevertheless, in lesions of grade 1 and 2 proper differential diagnosis should be taken into account. In all cases from nocturnal raptors, lesions were located at the upper part of the oropharyngeal cavity, extending to the choanal papillae and craneal sinuses.

***Crassicauda* sp. in an Indo-Pacific humpback dolphin (*Sousa chinensis*) in Hong Kong** (Poster no. 58)

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*Crassicauda* is a genus of spiruridan nematodes which have odontocete and mysticete cetaceans as hosts. A review of the parasites from Indo-Pacific humpback dolphins (*Sousa chinensis*) does not report the presence of specimens of *Crassicauda* in this species. A 2.63 m long female Indo-Pacific humpback dolphin was found drifting in Hong Kong waters on 4<sup>th</sup> March 2013. On necropsy, the animal was gravid with a late term male foetus, measuring 0.84 m. During the post mortem examination, the mother's mammary glands were examined and a single nematode, with a diameter of 0.1 cm was observed in the left mammary gland. The material was recognised as a species of *Crassicauda* based on site, size, and morphology. The probable cause of death for the adult animal was a multi-systemic bacterial infection and was related to the foetal death. Histological examination of the mammary tissue showed that there was mild multifocal chronic mastitis. "Verminous mastitis" due to the presence of specimens of *Crassicauda* has been described in a group of Atlantic white-sided dolphins. To our knowledge, this is the first record of a species of *Crassicauda* occurring in an Indo-Pacific humpback dolphin, and also represents a new geographical record of this genus of nematodes for the South China Sea. It is not known whether *Sousa chinensis* is a new host for *Crassicauda* or whether it has remained undiagnosed due to the fact that the mammary glands are not always examined during cetacean post mortem examination. This case report highlights the fact that mammary glands, as well as cranial sinuses and mammary secretions should be examined systematically across the geographical range of this species in order to diagnose and determine the prevalence of this parasite. This will assist in evaluating its possible effects on mammary tissue, lactation and calf rearing.

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**Capturing Persian leopards (*Panthera pardus saxicolor*) using foot snares and anaesthetised with Ketamine, Medetomidine to apply iridium GPS collars in NE Iran (Poster no. 102)**

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The present investigation has been launched in Tandoureh National Park, near Turkmenistan border in NE Iran on Persian leopards (*Panthera pardus saxicolor*). Lotek Iridium GPS collars supplemented with a drop-off buckle with timer only option (working after 52 weeks since deployment) were used. Collars weighed 640 g; equivalent to less than 1 % of body mass, well below the 3 % limit recommended. Foot-snares were deployed and fitted with numerous modifications to minimise the possibility of injury. As leopards are known to respond fairly to baits, killed boars were utilised to improve trapping. Each trap was composed of two sets of snares to increase capture chances. Remote monitoring of snares achieved by fitting a Telonics TBT-500 trap site transmitter (Telonics Inc., Mesa, USA), to pinpoint signals and also notify triggering of traps which were checked by radio-receiver at short intervals. Typically, after fitting the collar, blood and tissue were sampled and morphological measurements taken. The animal's age was estimated based on dental features. During first attempt, eight nights of bait trapping at two sites, there were two successful captures of two adult males. The second attempt, twenty one nights of bait and road trapping at three sites, there were no successful capture. The third attempt, fourteen nights of bait trapping at three sites and there were one successful capture. The fourth attempt, fourteen nights of bait trapping at three sites and there were one successful capture of a sub adult male. The last attempt, eight nights of bait and road trapping at three sites and there were one successful captures of an adult female. All the leopards captured with fore paw except the first one which captured by hind paw. Anaesthesia was safely achieved using a combination of Ketamin 10 % (Alfasan, Nederland BV) 3 mg/kg and Medetomidine HCL 20 mg/ml (Kyron Laboratories (Pety) Ltd., Johannesburg, South Africa) 30 µg/kg administered IM in the same 1.5 ml dart by Daninject darting gun. The darting sites were biceps femoris except one witch was triceps brachii and capture-related injuries included minor cuts and abrasions to the feet.

**Detection of IgM and IgG antibodies against Canine Distemper Virus in wild canids of North-East provinces of Iran employing enzyme-linked immunosorbent assay (ELISA) (Poster no. 18)**

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Canine distemper virus (CDV) has a wide host spectrum and induces a highly contagious disease. This virus can affect canids, marine mammals, felids and many other wild species. There have been many reports that indicate a dramatic decline in wild felids population such as African lions and Siberian tigers, caused by CDV. There are two critically endangered feline species in Iran and they share their habitat with free ranging and stray dogs as well as herd dogs and the wild canid species like foxes, jackals and wolves. In such habitats which Asiatic cheetahs and Persian leopards are living, there are some reports of wild canids being hunted by Persian leopards and also sharing the same bait with Asiatic cheetahs. Regarding this, investigating the prevalence of IgM and IgG antibodies against CDV among the wild canids in Tandooreh national park, Khar Touran national park and Golestan National park which are the prime Cheetah and Leopard habitats in Iran, took place. An enzyme-linked immunosorbent assay (ELISA) for the detection of IgG and IgM antibodies against canine distemper virus (CDV) was employed, using BioPronix ELISA kits (Agrolab, Italy). The titers of IgM and IgG antibodies against CDV were also measured. In this study, prevalence of CDV-specific antibodies in 88 animals including common foxes (*Vulpes vulpes*), Grey wolves (*Canis lupus*) and Golden jackals (*Canis aureus*) sampled in north eastern parts of Iran within the period of four years, was investigated. Seventy-one out of these 88 canids were positive for IgM antibodies against CDV and 87 out of 88 were positive for IgG. Thus a sizeable proportion of them have been exposed to CDV in the wild. This can be a serious threat for Asiatic cheetahs and Persian leopards, which are respectively critically endangered and endangered due to IUCN's red list of threatened species. Therefore, that's necessary to do more studies on free ranging dogs and also the feline species themselves to find the best way for protecting Asiatic cheetah and Persian leopard against this disaster.

## **Are seabirds in Western Cape (South Africa) a source of zoonotic bacteria?**

(Poster no. 35)

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Thermophilic *Campylobacter* and *Salmonella* spp. are the leading causes of food-borne human gastroenteritis worldwide, being of economic and public health concern. Wild birds can be carriers of zoonotic bacteria due to their scavenging feeding habits. Little is known about the importance of seabirds from the African continent as a reservoir of these pathogens. This study reports the occurrence and genetic diversity of *Campylobacter* and *Salmonella* spp. from seabirds along the Western Cape coast of South Africa. A total of 229 seabird chicks (129 kelp gulls *Larus dominicanus* and 100 swift terns *Thalasseus bergii*) from five colonies from the Western Cape were sampled during summer 2013/2014. Bacteria were isolated using standard culture methods and identified to species level by PCR (*Campylobacter*) or serotyped (*Salmonella*). Genetic diversity was analysed by ERIC-PCR, *flaA*-RFLP and PFGE. The frequency of *Campylobacter* spp. was 14 % (12 % in kelp gulls, 16 % in swift terns); *C. jejuni* was the most frequently isolated species. A high genetic diversity was observed among *C. jejuni* isolates, with 19 different PFGE profiles. However, two kelp gulls from different colonies carried the same strain, and another strain was detected in the two seabird species. *Salmonella* was more prevalent in kelp gulls (43 %) than in swift terns (7 %). Genotyping of the isolates belonging to 16 different serotypes of *S. enterica* subsp. *enterica* showed 26 different PFGE profiles. Some kelp gulls from different colonies carried the same strain of the most frequently isolated serotypes (Anatum, Enteritidis and Hadar). The same strain of serotype Hadar was isolated in both species of seabirds. The high occurrence of these zoonotic agents in the studied seabirds highlights the importance of these wild birds as reservoirs of *Campylobacter* and *Salmonella* and their potential role in the maintenance and transmission of these bacteria in the environment. The diversity of isolates suggests that there are different sources of infection, as well as a transfer of these pathogens across different gull colonies and bird species.

***Brucella* infections in phocids versus otariids in Alaska** (Poster no. 44)

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Alaskan waters accommodate several pinniped species, both phocids (true seals); Pacific harbour seals (HS, *Phoca vitulina richardsii*), spotted seals (SS, *Phoca larga*), ribbon seals (RBS, *Histiophoca fasciata*), ringed seals (RNS, *Pusa hispida*) and bearded seals (BS, *Erignathus barbatus*) and otariids (eared seals); Steller sea lions (*Eumetopias jubatus*), eastern and western distinct population segments (ESSL and WSSL, respectively), and Northern fur seals (NFS, *Callorhinus ursinus*). *Brucella pinnipedialis* was first isolated from harbor seals (*Phoca vitulina*) in Scotland in 1994. Few pathological findings have been associated with infection in phocids, however, reproductive pathology and abortions, and isolation of zoonotic strain type 27, have been documented in otariids. Serum samples from Alaskan phocids (HS;n = 1122, SS;n = 85, RBS;n = 55, RNS;n = 150, BS;n = 124) and otariids (WSSL;n = 76, ESSL;n = 48, NFS;n = 107) were analysed for anti-*Brucella* antibodies. The seroprevalences were higher in phocids than in otariids (HS;25 %, SS;20 %, RBS;16 %, RNS;14 %, BS;13 % vs. WSSL;1 %, ESSL;2 %, NFS;0 %). Seropositive seals were detected in all locations investigated and throughout the sampling period (1975-2011). There was no difference by sex, however, phocid pups (< 1yr) had the lowest seroprevalence, juveniles (1-3yrs) had the highest, and adults (> 3yrs) were in between (HS;7 %, SS;8 %, RNS;14 %, BS;0 % vs. HS;45 %, SS;32 %, RBS;19 %, RNS;56 %, BS;10 % vs. HS;19 %, SS;11 %, RBS;14 %, RNS;4 %, BS;14 %). This pattern is in accordance with reports on *B. pinnipedialis* infections in phocids and suggests environmental exposure to *B. pinnipedialis* at the juvenile stage, with a later clearance of infection. This is in line with our work on *B. pinnipedialis* in phocid cell models and experimental infection in Atlantic Cod (*Gadus morhua*). The low seroprevalences in otariids suggest a low level of exposure or innate resistance to infection. Considering the reports of pathology in otariids, morbidity/mortality due to infection is a possibility. Further studies are needed in order to determine whether the infection is a threat to otariid populations, some of which are listed as “Endangered” (WSSL) and “Depleted” (NFS, Pribilof Island stock) under the the Marine Mammal Protection Act.

## Identification of *Sarcocystis* in native rodents and treeshrews from Borneo

(Poster no. 69)

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Rodent species are well known reservoirs for many pathogens, some of which may have serious implications for veterinary or public health for their zoonotic potential. The health status of rodent populations in tropical forests, however, are poorly studied and it is often not clear how forest conversion and fragmentation results in an increased infection of native wildlife with pathogens also found in invasive species. To better understand the possible exchange of pathogens between wildlife species and/or invasive species it is crucial to study the health status of native rodent populations. The tropical rainforests on Borneo are one of the most important biodiversity hotspots of the world with high levels of endemism. We assessed the presence of subclinical histopathological lesions in small mammals from Borneo. For this purpose, 117 individuals of native Rodentia from 8 different genera (*Callosciurus*, *Maxomys*, *Niviventer*, *Sundamys*, *Sundasciurus* and *Leopoldamys*) and 15 individuals of 3 treeshrew species (*Tupaia* sp.) were collected in forest and rural landscapes in Sabah, Borneo between 2012 and 2013. Histopathological examination was carried out on tissue samples of striated muscle, lung, heart, liver, kidney, intestine and stomach. Main findings showed that the lung was the organ most often affected by inflammation (105 rodents, 14 treeshrews) followed by the liver (81 rodents, 9 treeshrews), heart (54 rodents, 1 treeshrew) and kidney (46 rodents). In the gastro-intestinal tract tissue lesions associated with helminths and/or coccidia were observed in stomach (38 rodents, 5 treeshrews), small intestine (37 rodents, 8 treeshrews), large intestine (30 rodents, 4 treeshrews).

We were able to detect specific parasitisation in the skeletal muscle by the Apicomplexa protozoa *Sarcocystis* sp. in 47 rodents and 10 treeshrews. To identify different *Sarcocystis* species we used PCR and amplified a partial region of the 28S rRNA suitable for phylogenetic comparison. Genetic analysis revealed that rodents and treeshrews are mainly infected by *Sarcocystis singaporensis* and *Sarcocystis zamani*, which have Asian snakes, e.g. python species, as their definitive host and invasive rats (*Rattus* spp.) are commonly reported as their intermediate hosts. Our study provides evidence that *S. singaporensis* and *S. zamani* use an even broader range of intermediate host species than anticipated. The infection of native small mammal species in high prevalence ensures the continuation of the parasite's lifecycle irrespective of the predators' habitat choice as pythons are known to roam in urban areas as well as deep into the tropical forests.

## **Tracheobronchial tree and lungs in hedgehog: morphological study**

(Poster no. 67)

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Hedgehog is one of the omnivorous mammals of the wildlife that it had not been studied well, anatomically. Whereas recently this animal is considered as the pets of the human community, and according to the importance of the respiratory system in animals, the anatomical study of this system had discussed with emphasis on morphology.

In this study, five healthy male adults long-eared hedgehog (*Hemiechinus auritus*) in various ages were applied. After euthanised, the airway had separated (from the larynx to lungs) and in this path trachea and lungs have studied anatomically. The area of trachea bifurcation in the thorax, the length of the trachea, the diameter of the trachea and the number of its rings and also the volume of lungs have measured, and the results have compared according to available reports about this animal and some other small mammals. In this study, we have used a casting technique for determination bronchial tree with its branching. Trachea bifurcated into right and left principal bronchi in the region between 2<sup>nd</sup> and 3<sup>rd</sup> rib. The right principal bronchus was branched into four lobar bronchi (cranial, caudal, middle, accessory). The left main bronchus had not any lobar branches and was branched directly into several tertiary bronchioles. Then, the right lung has four lobes, and the left lung has only one lobe. Results of this study may be beneficial for the diagnosis and treatment of respiratory diseases and disorders in hedgehog.

## **Three year follow-up of an outbreak of rabbit haemorrhagic disease due to RHDV2 in wild rabbits (*Oryctolagus cuniculus*) in North-Eastern Italy**

(Poster no. 23)

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Rabbit haemorrhagic disease is a fatal disease of European rabbits (*Oryctolagus cuniculus*) caused by a lagovirus (RHDV). Since 2010, RHDV2, a new strain with a specific antigenic profile, is spreading worldwide in domestic and wild rabbits. We describe the first outbreak of RHDV2 in Italy in wild rabbits living in a natural preserved area close to Trento (North-Eastern Italy), and the results of a virological and serological follow-up (2012 - 2014). In January 2012, due to unusual mortality among wild rabbits, we planned passive and active surveillance. During the three-year period, 296 carcasses (19 found dead and 277 hunted) were submitted to the lab. At necropsy, organ samples were collected from 292 individuals, including when possible, sera from cardiac clots. Specific sandwich MABs ELISAs and RT-PCRs for the different RHDV strains were performed on liver/spleen samples. Out of 292, 28 samples (9.6 %) were RHDV positive: 26 for RHDV2 and 2 for RHDVa. All positives were detected on early 2012 but in November 2015 two more rabbits found dead in the same area resulted positive for RHDV2. 224 sera, 121 in 2012 (P1), 41 in 2013 (P2) and 62 in 2014 (P3), were tested by specific cELISAs and isotype ELISAs for RHDV and RHDV2 (P3 only) antibodies. The seroprevalences differed along the time, being: 1) quite low in P1 (18.2 % positives and 27.3 % not conclusive (N.C), close to the threshold value < 1/10) in sera collected contemporarily to the outbreak; 2) sharply increasing in P2 (51.2 % positives and 19.5 % N.C) with higher titres (1:160-1:320), showing an active spread of the infection among the population; 3) decreasing in P3 (38.7 % positive and 21 % N.C), indicating a reduction of viral dissemination. The titres found in P3 were 4-8 times higher for RHDV2 than for RHDV. The detection of specific IgM in P1-P2 and IgA in P2-P3 confirmed the active circulation of RHDV2 in the area. We could conclude that: (a) the epidemic event caused low mortality rates and ended within 2/3 months (P1); (b) the infection quickly became endemic during P2-P3 with high prevalence and without evident mortality; (c) different RHDV strains were circulating at the same time; (d) the re-occurrence of the disease after 3 - 4 years is likely due to the rabbit population's cyclic renewal and to the RHDV easy transmission.

**The potential role of wild pigs in the spread and maintenance of African swine fever at the wildlife-livestock interface in Uganda** (Poster no. 5)

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In Uganda, African swine fever (ASF) is endemic in domestic pigs and causes large economic losses for rural communities. Although warthogs (*Phacochoerus africanus*), in association with soft ticks are considered the main wild vertebrate host of the virus in the endemic African setting, they are not the only wild African suids with a potential role in ASF epidemiology. The sylvatic cycle also involves the bushpig (*Potamochoerus larvatus*) but to date, little is known about the epidemiological role of this species in the maintenance and spread of ASF, although it is likely to share habitats and resources with both, warthogs and free ranging domestic pigs. A previous pilot study carried out in Uganda showed that bushpigs can be naturally infected with ASF virus and can seroconvert. Furthermore, spatial overlap between bushpig and domestic pig home ranges was observed, based on GPS tracking data from three bushpigs. The aim of this ongoing study is to increase the sample size of captured and sampled bushpigs 1) to be able to estimate ASF prevalence and seroprevalence and 2) to better evaluate their temporal and spatial interaction pattern with domestic pigs.

As capturing this elusive and nocturnal species is challenging, we try different capture methods including a drop net and drop door metallic cages monitored by camera traps. The captured bushpigs are sedated with a mixture of Azaperone, Tiletamine-Zolazepam and Medetomidine. Blood is sampled and tested for ASF virus genetic material and antibody detection. Finally, we deploy GPS harnesses with an objective of collaring four bushpigs.

The expected results should highlight the role of the bushpig in the sylvatic cycle of ASF and the way it could be connected with the domestic cycle. Further research will then be needed to investigate if this species is able to maintain the virus and how the transmission can occur at the interface with domestic pigs.

## **Organ tropism of *Trypanosoma cruzi* in the naturally infected reservoir *Octodon degus* (Poster no. 71)**

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Most studies about *Trypanosoma cruzi* infection within its mammal hosts involve laboratory animals undergoing acute infections that were experimentally induced using massive amounts of parasites. Here, nine naturally infected rodents captured in the field (and kept in captivity for two years) were used to assess the natural localisation of the parasite in the organs of the host. After euthanasia, we obtained 14 organs by necropsy: spleen, liver, skin, kidney, lungs, brain, muscle, uterus or penis, heart, gonads, esophagus, stomach, intestines and diaphragm. The tissues were preserved in alcohol 95 % until processing. Genomic DNA was extracted using a commercial kit. The eluted samples were frozen at -20 °C until used to perform PCR, amplifying the hypervariable region of *T. cruzi* minicircles' kDNA. The samples were considered positive when a 330 bp band was visualised in a stained agarose gel under UV light. We detected *T. cruzi* in the organs of the nine analysed rodents. We classified the organs from frequent to infrequently positive: brain, gonads (5/9); muscle, esophagus, intestines (4/9); spleen, skin, lung, heart, stomach (3/9); liver, kidney (2/9); uterus (1/9). Diaphragm was not detected positive in any of the rodents. There were some *O. degus* that had many positive organs (9/14, n = 1; 8/14, n = 1; 7/14, n = 2; 5/14, n = ), while others had only a few positive (2/14, n = 2; 1/14, n = 2). These results show that the chronic infection of a naturally infected reservoir involves organs that are not usually considered in the pathology of Chagas disease, which might indicate that organ tropism in *O. degus* differs from what was described in human and laboratory animals, since brain and gonads were the most frequently positive organs of *O. degus*. The positive reproductive tract may indicate that congenital transmission in this species could occur, but it needs to be experimentally proved. The sampled *O. degus* had a variety of organs infected; this might be related to their immune status, since other pathologies may have contributed in debilitating some of the specimens. Overall, this study shows that *T. cruzi* is probably hiding in several organ tissues in the chronic phase of the infection.

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**Sudden death associated with hyperacute catarrhal bronchitis and brain oedema in a roe deer buck immediately after capture – a previously undescribed form of capture myopathy? (Poster no. 103)**

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Capture myopathy (CM) or exertional myopathy is a syndrome of major concern in wild animals undergoing immobilisation or restraint, particularly in birds and ungulates including roe deer (*Capreolus capreolus capreolus*). CM is a complex and multifactorial disease with a not completely understood pathogenesis that is considered to involve overwhelming stimulation of the sympathetic nervous system and of the chromaffin cells of the adrenal gland primed by perception of fear. This stimulation coupled with extensive muscle activity is believed to lead to myofiber damage. In ungulates different forms of CM are described either according to the clinical course (hyperacute, acute, subacute or chronic) or to the clinical signs (capture shock syndrome, ataxic and myoglobinuric syndrome, ruptured muscle syndrome and delayed peracute syndrome). Here we report the macro- and histopathological findings in a young roe deer buck dying minutes after driving net capture and acepromazine administration. Gross findings included marked brain and lung oedema along with lung hyperemia and petechial bleeding in the skeletal and cardiac muscles. Histological examination revealed severe catarrhal bronchiolitis and bronchitis with multifocal massive deposition of mucus in the airways' lumina and severe degranulation of globular leukocytes with oedema. Muscle changes included hyperacute myofibers degeneration with ruptures and haemorrhages. The muscle lesions and the vascular-associated lung changes were consistent with those documented in acute exertional myopathies. However to the best of our knowledge the brain oedema and the severe bronchiolitis and bronchitis have never been documented in CM cases. We presume that a capture shock syndrome associated with an obvious vasogenic component partially resembling an anaphylactic reaction occurred in this roe deer, although an adverse drug reaction cannot be ruled out. The case documented here contributes to uncover the complexity and severity of capture-associated fatalities in wild ungulates.

**First record of *Mycoplasma citelli*-like organisms in the European ground squirrel (*Spermophilus citellus*)** (Poster no. 46)

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Two European ground squirrel road kills originating from the Province of Lower Austria were submitted for post mortem examination in 2015. Both were male and classified as adult. Apart from haemorrhages presumably caused by traffic, both animals showed pathological alterations in the lungs. The lungs of the first animal appeared multifocally dysteletic. In pathohistology there was moderate multifocal perivascular cuffing, minimal hyperplasia of pneumocytes type 2, and thickening of the interstitia due to an inflammatory infiltrate (mostly lymphocytes). Furthermore there was hyperplasia of the peribronchial lymphoid tissue and a histiocytic inflammatory infiltrate in the alveoli. The left lobe of the lung of the second animal adhered to the thoracic wall and was dysteletic. In pathohistology a moderate perivascular cuffing and multifocal moderate inflammatory infiltrate consisting mostly of histiocytes with fewer lymphocytes and plasma cells was noted, as well as moderate hyperplasia of peribronchial tissue.

A first bacteriological analysis of the second lung yielded moderate amounts of  $\alpha$ -haemolysing streptococci and *Staphylococcus aureus* as well as abundant growth of mycoplasma. Due to these findings a bacteriological examination of frozen lung tissue of the first animal was performed yielding moderate growth of mycoplasma as well. 16S rDNA sequence analysis demonstrated that both mycoplasma isolates are closely related exhibiting identical 16S rDNA sequences and are members of the genus *Mycoplasma*. Comparison with 16S rDNA sequences available in the NCBI GenBank revealed a highest similarity value of 97.1 with *Mycoplasma citelli* RG-2C<sup>T</sup>, a validly described mycoplasma species previously isolated from Richardson's ground squirrel (*Urocitellus richardsonii*), indicating a close relatedness but as yet uncertain species affiliation of both mycoplasma isolates to *Mycoplasma citelli*.

To the authors' knowledge this is the first report of *Mycoplasma citelli*-like organisms in the European ground squirrel. As the alterations in the lungs solely were moderate the impact on the animal is still questionable, although the inflammatory reaction could have led to a decreased body condition. Nonetheless it is of high importance knowing which bacteria circulate in wild populations of European ground squirrels, as this species is ranking as highly endangered within its range in the European Union. The impact of mycoplasma on the ground squirrel population is therefore still under evaluation and will be monitored over the next years.

**Spatial ecology of Eurasian badgers (*Meles meles*) in a high bovine tuberculosis incidence area from Asturias (Northern Spain) (Poster no. 39)**

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Bovine tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium bovis*. In Asturias (Northern Spain), TB in cattle declined from a herd incidence of 0.32 % in 2002 to 0.18 % in 2015, although geographical pockets remained where the incidence was up 5.38 % in 2013. Recent studies suggest that badgers may be a potential reservoir of *M. bovis* in these areas. The objective of this study was to investigate the spatial ecology of badgers around infected farms in order to extract information of relevance to understand this complex epidemiological system. The study area is located in Parres, Asturias, northern of Spain (43° 23'N; 5° 14'W), and extended over 17 km<sup>2</sup>. Forty five farms are settled in the area, of which eleven had tested positive for TB at the official single intradermal cervical tuberculin test (SID) between 2013 and 2015. Approximately 60 % of the territory is farmed grasslands and tillage, while 30 % of land cover is forested. A total of 19 setts were found in the area (1.12 setts/km<sup>2</sup>), and the badger population density estimated on n° of main setts and captured was 6 badgers/km<sup>2</sup>. Nine badgers were captured between 2013 and 2015. Trapped badgers were anaesthetised and their location, sex, age and body condition were scored. Each badger was implanted with a CRG250G collar transmitter. In total 6,736 locations events were recorded during the study period. Plots of positive farms tended to be closer to a setts the negative holdings. The frequency of badger locations in the prairies of positive farms was significantly higher than those in the negative farms. No differences in the size of seasonal home range, daily movements and seasonal maximum dispersal between sexes neither among seasons were found. One relevant finding was the presence of one long-distance dispersal male (13,652.32 m of maximum seasonal dispersal distance). Excluding this rare individual, the average seasonal home range was (Kernel 95 %) 128.26 ha (21.65 - 203.42), daily movements of 1,842 m (803.40 - 2861.28) and dispersal distance of 2,060.64 m (633.39 - 6100.60). The implications of these results on badger's spatial ecology for designing TB intervention strategies in the study area are potentially significant.

**West Nile Virus circulation in Emilia-Romagna region, Italy: serological survey in Eurasian magpies (2013 - 2014)** (*Poster no. 9*)

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The West Nile virus (WNV) is nowadays the most widespread arbovirus in the world, being present in all continents exception made for the Antarctica. Migratory and resident wild birds are highly implicated in spreading and maintaining WNV in a territory. Eurasian magpie (*Pica pica*), well known reservoir for WNV, is strongly preferred by *Culex pipiens* and may play a crucial role in WNV seasonal spill-over events to humans and horses in most European countries.

The objective of this study was the titration of WNV neutralising antibodies in fledgling magpies (less than one year old), using serum samples collected in Emilia-Romagna region (Italy), during two consecutive vectorial seasons (2013 and 2014). Samples were collected from 135 animals (46 in 2013 and 89 in 2014) and then analysed using a serum neutralisation (SN) test. The SN assay was set up according to the OIE recommended protocol, using a WNV field strain isolated during the 2014 season.

The 46 samples from 2013 were collected between the 5<sup>th</sup> of June and the 20<sup>th</sup> of September. Out of them 16 (34.8 %) were negative whereas 30 (65.2 %) presented WNV-neutralising antibodies with a neutralisation titre ranging from 1:10 and 1:320. Such a result was interesting taking into account that the surveillance programme in this area did not evidence any WNV circulation during the 2012.

The 89 samples from 2014 were collected from the 20<sup>th</sup> of May to the 18<sup>th</sup> of June. Forty-three (48.3 %) of the 89 sera analysed were negative and 46 (51.7 %) were positive with a neutralisation titre between 1:10 and 1:640.

Since 2014 WNV has become clearly endemic in some Italian areas (as Emilia-Romagna, Lombardy, Friuli-Venezia-Giulia, Veneto, Sicily and Sardinia regions), but there are no-data available about the WNV seroprevalence in the avian reservoirs, although the viral circulation was well demonstrated. This study reports the detection of WNV neutralising antibodies in Eurasian magpie, adducing data about the seroprevalence of this virus in one of its wild birds reservoirs, giving also an idea of the degree of WNV endemisation in Italy.

## **Serological survey of selected viral pathogens in Egyptian geese (*Alopochen aegyptiacus*) from Germany (Poster no. 13)**

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The Egyptian goose (*Alopochen aegyptiacus*) is one of the most successful neozootic bird species in Europe. In Germany the population increased 40 fold over the last 26 years from 250 - 300 breeding pairs in 1999 to more than 10,000 pairs in 2015. Similar to other waterfowl species, Egyptian geese are in close contact to humans and domestic animals, and can play an important role as reservoir and/or carrier for different pathogens with public health or economic interest. In order to understand the epidemiological role of expanding Egyptian geese in Germany, serum samples from > 100 birds were collected and were examined using commercial enzyme linked immunosorbent assays (ELISAs) for antibodies (Ab) against avian influenza viruses (AIV), Newcastle disease virus (NDV) and West Nile virus (WNV). In addition, the hemagglutination inhibition (HAI) test used in domestic poultry was applied for Ab detection against the Egg Drop Syndrome 76 (EDS) virus. Of 113 serum samples tested, 12 animals were Ab positive and 8 animals inconclusive against AIV. The prevalence of NDV Ab positive animals was low (0.88 %, n = 114), while all geese were negative for Ab against WNV. From 39 serum samples that were investigated for EDS, 6 geese (15.38 %) were Ab positive. Virological studies including genetic analyses from oral and cloacal swabs are in progress and will be used for comparison of serological results. By comparing our results with previous studies on migrating Arctic geese species, especially bean geese (*Anser fabalis*) and white-fronted geese (*Anser albifrons*), and on another invasive, resident geese species, the Canada geese (*Branta canadensis*), it is obvious that differences exist between native migrating geese and introduced resident species in the seroprevalence of different viruses. While all four species show comparable seroprevalence for AIV, native migratory species are more exposed to NDV showing a seroprevalence of 45 % compared to 0.47 % in introduced species. In contrast, invasive, resident species have a higher seroprevalence for EDS (i.e., 17.8 % in introduced and 8.11 % in native species). Seroprevalence differences in geese species might reflect their epidemiological roles as carriers of avian viruses with contrasting movement ecology for important poultry pathogens.

## **Influenza-A Virus in the American mink (*Neovison vison*) from freshwater ecosystems in Spain (Poster no. 73)**

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Influenza-A viruses (IAVs) are RNA viruses of the *Orthomyxoviridae* family that cause acute respiratory disease in a wide range of birds and mammals, including human beings. Waterfowl are considered as the main natural reservoir for IAV, from which transmission to other species occasionally occurs. Susceptibility of several species within the family Mustelidae to IAVs has been reported as a result of natural and experimental infections. The aim of this study was to assess the infection of IAV in free ranging American mink populations from Northern Spain and to assess its epidemiological role in IAVs. Sera collected between 2011 and 2014 from 689 American minks from Northern Spain were tested for the presence of antibodies against IAV using a commercial competition cELISA assay. Sera positive to the cELISA assay were also analysed with a hemagglutination inhibition (HI) assay for Influenza. Prevalence of IAV antibodies by cELISA was 2.2 % (15/689, 1.3-3.6 CI<sub>95</sub>%) and seropositive American minks were detected throughout the study period. No significant differences were observed between year of capture, province, river basin, sex and age of the animals. None of the positive-cELISA sera reacted against the IAV strains used in the HI assay. These results suggest that the most common strains circulating in swine, the most common avian subtypes (H5 and H7) and the H10N4 subtype isolated in minks have not been circulating in this wild mustelid in Spain. In conclusion, the free-range American mink from Northern Spain do not seem to have an important role in the IAV epidemiology.

## Evaluation of three enzyme-linked immunosorbent assays for sarcoptic mange diagnosis and assessment in Iberian ibex (Poster no. 62)

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Sarcoptic mange is a contagious skin disease caused by the mite *Sarcoptes scabiei*, affecting different mammalian species worldwide, including Iberian ibex (*Capra pyrenaica*). There is not an efficient diagnosis method for this disease, particularly when there are low mite numbers and no or mild clinical signs.

The objective of this study was to evaluate three different enzyme-linked immunosorbent assays (ELISA), developed for dog (ELISA A), Cantabrian chamois (*Rupicapra pyrenaica parva*) (ELISA B) and Alpine chamois (*Rupicapra rupicapra*) (ELISA C), for the detection of specific antibodies (IgG) against *Sarcoptes scabiei* in Iberian ibex.

Serum samples from 131 Iberian ibexes (86 healthy and 45 scabietic) were collected from 2005 to 2012 in the Sierra Nevada Natural Space (southern Spain). Based on visual inspection, ibexes were classified into one of three categories, namely healthy (without scabietic compatible lesions), mildly (less than 50 % of the body surface affected) and severely (with more than 50 % of the body surface affected). The optimal cut-off point, specificity, sensitivity and the area under the curve (AUC) were calculated, and the agreement between tests was also determined by the Cohen's Kappa coefficient and Bland-Altman plots. Differences in optical density (OD) related to scabies severity were also evaluated for ELISA C, which showed the best performance. ELISA C reached the highest sensitivity

(93.5 %) and specificity (93.9 %) as compared to ELISA A and B against visual inspection diagnosis, chosen as the gold standard method. Differences in OD were detected among sarcoptic mange categories with ELISA C.

Based on the results obtained, ELISA C was an optimal test to diagnose sarcoptic mange in Iberian ibex. However, further studies characterising the immune response against sarcoptic mange in Iberian ibex throughout the evolution of the disease should follow this first step.

**Identification of pangolin from confiscated scales by DNA sequencing – forensic aid and application: a case study** (Poster no. 75)

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Wildlife forensics has gained more and more attention due to the awareness programmes being carried out by Indian government to protect wildlife as wildlife poaching has become a serious crime under the Wildlife Protection Act of 1972. As such, the positive identification of an animal to its species level has great importance in judiciary aspects.

Identification of closely related species is often very difficult in poaching cases where the biological samples are often processed and decomposed. Anatomical, biochemical and phenotypic tests had been the methods of choice for the detection and identification of plants and animal cases received in forensic laboratories for investigations. Often, the positive identification is also dependent on the physiological condition of the sample. With the introduction of molecular methods for identification of plants and animals these limitations are reduced to a greater extent resulting in making identification more of science than art.

In the present paper, we have focused on DNA techniques to identify animal (pangolin) from the scales confiscated. Pangolins are unique mammals as they possess scales that serve as a protective shield. Illegal trade of these scales is frequent and difficult to investigate or even to prosecute. DNA was extracted from these confiscated scales and identified by using DNA sequencing as belonging to family Manidae, genera *Manis crassicaudata* (thick-tailed pangolin/scaly anteater), in local language commonly known as “Khawalya Manjar”.

Furthermore, these technologies could provide the scientific aid to the forest officials for establishing the crime scene and in incriminating criminals involved in the illicit trade of wild pangolins and also in assisting law enforcement authorities in collecting additional appropriate evidence that may lead to prosecution.

**First record of the nasal mite *Halarachne halichoeri* in a grey seal from the German Wadden Sea** (Poster no. 57)

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The nasal mite *Halarachne halichoeri* (Acari: Halarachnidae) is a phocid seal ectoparasite that has been described to affect grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) in the North Atlantic Ocean, causing different levels of upper respiratory diseases and rhinitis. Typical clinical symptoms include sneezing, coughing and nasal discharge, possibly leading to an impairment of respiration. The earliest descriptions of this arthropod species date as far back as 1847. It is a regular finding in stranded seals along the US East Coast and has been thoroughly described in England and the North-Western Spanish coast. In July 2015 an adult female grey seal died on the German island of Sylt in the North Sea. Through the German stranding network and in the frame of the consecutive wild seal population monitoring projects in Schleswig-Holstein waters, the animal was transported to the Institute for Terrestrial and Aquatic Wildlife Research and directly necropsied in a fresh state. Examination of the external nose and the nasal cavity showed a mild presence of live nasal mites, later identified as *H. halichoeri*, including adult and larval stages. The seal was in poor nutritional status and the pathological examination revealed severe diffuse catarrhal purulent bronchopneumonia and moderate gastritis. Further parasitic findings included a severe infection of anisakid stomach nematodes and a severe infection with *Corynosoma* spp. (acanthocephala) in the small intestine. To our knowledge, this case report represents the first finding of *H. halichoeri* in grey seals in the German North Sea. It is suggested that the prevalence of *H. halichoeri* in seals from German waters may be higher than previously thought and that examinations of the nasal cavity during post mortem investigations have to be conducted systematically to gain sufficient data on the prevalence of this potentially emerging parasite mite infecting seals in the North Sea. Further studies on the pathogenicity of *H. halichoeri* and its effects on the host will be commenced.

## **Discovery of porcine atypical pestiviruses in Austria** (Poster no. 20)

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Pestiviruses, within the family *Flaviviridae*, are important pathogens of cloven hooved animals. Until 2007, classical swine fever virus was regarded as the sole pestivirus having pigs as its main host. This was changed with the discovery of Bungowannah virus in an Australian pig farm experiencing a marked increase in stillborn piglets due to non-suppurative myocarditis. However, until now, Bungowannah virus has never been isolated again.

In 2015, a novel pestivirus, tentatively termed atypical porcine pestivirus or porcine pestivirus 1, has been discovered in pigs in the US. Typically, piglets are born viremic and display congenital tremor of different strength. To the current knowledge, this tremor subsides with age, and unless the tremor interferes with suckling, the piglets survive. Until now, very little about the epidemiology of this virus is known. When we started to screen piglets displaying signs of congenital tremor by a self-developed panpestivirus RT-PCR targeting the NS5B coding region, sequences of APPV could be detected in samples derived from seven farms. All Austrian isolates are more closely related to each other (> 99 % sequence identity, apart from one isolates with > 93 % sequence identity) than to the sequences reported from the US (< 91 % sequence identity). Preliminary serological investigations revealed a seroconversion rate of about 25 % of sows in an affected pig farm. A surprising finding was the isolation of yet another novel pestivirus from piglets displaying signs of congenital tremor that is equidistantly related (sequence identity < 70 %) to Bungowannah virus, CSFV and BVDV. This provides clear evidence that novel or so far undetected pestiviruses circulate in domestic pigs of Europe and the US while the classical have disappeared in many countries due to eradication programmes.

On the other hand, the unique detection of Bungowannah virus and our remotely related isolate indicate that there are pestiviruses that get introduced in one pig farm and are, after clinical symptoms subside, not detected again. This clearly points to other reservoir species of pestiviruses, which as of yet still have to be identified. Also, the presence / impact of these atypical pestiviruses in / on wildlife still has to be determined.

## **Ranid herpesvirus skin disease in common frogs (*Rana temporaria*) in Great Britain** (Poster no. 79)

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Amphibians are the most endangered taxonomic group on the planet, with > 70% of the world's amphibian species in decline. Several causes are believed to be involved, including infectious disease, habitat loss, over-exploitation, pollution, pesticide use and introduced species. However, the drivers of many amphibian declines are still poorly understood. In Great Britain (GB), frog mortalities associated with ranaviruses have been known to occur since the early 1990s. *Batrachochytrium dendrobatidis* was first described from GB in 2005, since when it has been reported from many sites across the country, but the extent and impacts of chytridiomycosis on amphibian populations in GB remain unknown. Little is known about other infectious diseases that can potentially affect anurans in GB. Ranid herpesvirus skin disease is known to affect the common frog (*Rana temporaria*). Skin lesions are characteristic, having the appearance of grey-white 'candle wax'. There is still a paucity of knowledge about this disease and the threat that it might pose to British amphibians even though it was first identified in GB in 1997. The disease appears in early spring, usually during the breeding season, regressing over the late spring and summer. In 2015, we received reports of 17 common frogs during the months of February and March with photographic evidence of lesions from 10 sites across Scotland, England and Wales indicating a widespread distribution of this disease. In three of these sites two or more individuals were affected. A common frog carcass was submitted for post mortem examination from a site in Greater London and was found to have macroscopic lesions consistent with ranid herpesvirus skin disease. Approximately 40 % of the body surface was affected by multifocal to coalescing dark-blue to grey, shiny, well demarcated areas of thickened skin with an irregular and smooth surface. The dorsal and inguinal regions were more severely affected. Histopathological examination of the dermal lesions revealed epidermal and dermal hyperplasia with mild, multifocal granulocytic infiltrates. Additionally, there was multifocal and moderate granulocytic nephritis with small areas of necrosis, the cause of which could not be determined. Electron microscopy of skin lesions revealed multiple viral particles with morphology consistent with a member of the *Herpesviridae*. A modified PCR targeting highly conserved regions of the herpesvirus DNA polymerase gene was performed on the skin lesions and no herpesvirus DNA could be detected. Further investigation is required to understand the diversity, impact and epidemiology of this viral infection.

**Pandora: a quick and practical first-line risk screening tool for parasitic and pathogenic micro-organisms** (*Poster no. 7*)

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Before prevention or early eradication measures on potentially invasive species (macroscopic or microscopic) can take place, it is essential to first identify those species that pose the highest risks. Given the huge and still-increasing number of macroscopic species that become transported that may co-transport emerging infectious diseases, such a prioritisation must allow for a high number of species to be assessed in a relatively short time, using risk assessment tools. Pandora is a new first-line risk screening tool for parasitic and pathogenic micro-organisms in terms of their potential (re)emergence and impacts. Impacts are relative to a given target area and may refer to health impacts (on domestic or wild animals and plants, and humans), trade and public perception.

Pandora is a semi-quantitative risk assessment protocol, based on general epidemiological principles from existing schemes (OIE, EPPO, WHO). After having defined the pathogen, area and targets at hand, the user is asked to score the likelihood of pathogen release, transmission and exposure, and to score the likelihoods and effect sizes for consequences towards targets. Pandora provides ample guidance, suggested data sources and examples to assist the user in performing the analysis. The answers are ordinally scaled, and this allows to put these risks into scores, and thus, to classify pathogens accordingly. This may support policy makers in the prioritisation of preventive control measures, and also point to caveats in our knowledge on particular pathogens. This protocol draws on the same

concepts as the Harmonia+ procedure for alien animals and plants . It was realised by a consortium of eight Belgian scientific institutes, each providing their expertise on components of the protocol. Experts that tested the protocol overall granted the Pandora protocol medium to high scores for clarity, consistency, completeness, novelty & usefulness. The Pandora procedure can be consulted at <http://ias.biodiversity.be/harmoniaplus>.

Pandora was used for a quick overall screening of the risk posed by ASFV to Belgium early 2014. A set of integrated risk scores was calculated within the framework. Experts scored the questions and uncertainty levels in the Pandora modules individually, after which the calculations were performed and averaged scores were used within pre-defined risk scales to define and visualise the ASF risk to Belgium. Emergence risk was considered low (score 0.29), while disease consequences were deemed high (0.93); the resulting multiplicative overall risk of ASFV for Belgium was low (0.27). The Belgian experts tended to give lower risk scores than the European experts, especially for entry risk and trade/public opinion consequences. These risk scores are further interpreted with a due consideration of the qualitative data in the expert remarks and of other ASF risk assessments. The results are similar to more extensive and elaborate risk assessment models/procedures which may require more time and resources. The Pandora tool allows sequential updates to monitor (rates of) increasing risk and provides information for risk managers to organise targeted control.

**TBEV seroprevalence and test accuracy in Flemish wild boar** (*Poster no. 8*)

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The risk of TBEV-introduction into Belgium remains high and the presence of seroconverted wildlife and domestic animals in Belgium has already been demonstrated in multiple studies. In the frame of a Flemish wildlife surveillance in 2013, a serological screening was performed on sera from Flemish wild boar (n = 238) in order to detect TBEV-specific antibodies. These sera were taken in 2013 throughout the whole Flemish wild boar population range.

All samples were subjected to gold standard TBEV seroneutralisation (SNT). Seven wild boar were seropositive and showed moderate to high SNT-titers -three had borderline results. Seroprevalence as estimated around 4.20 % (95 %CI: 1.65 - 6.75 %). Other Flaviviridae (Classical Swine Fever, West Nile Fever, Louping Ill) were ruled out and thirteen available tonsils tested negative in TBEV RT-PCR. The test characteristics of a commercially available TBEV-ELISA were assessed against the gold standard results.

Using the manufacturer's cut-offs and an alternately positive/negative interpretation of SNT-borderline results, the IgG protocol of this ELISA showed low diagnostic sensitivity and good diagnostic specificity (DSe: 40 - 57 % and DSp: 91 - 92 %). ELISA agreement with the SNT was judged "slight to fair".

ROC-analysis showed that for early detection screening purposes (with SNT follow-up), the ELISA cut-off might be placed as low as low as 35 Vienna-units: this would result in improved DSe (70 - 71 %) at the cost of DSp (64.04 - 69.74 %). This study showed the presence of TBEV-specific antibodies in wild boar and potential TBEV-foci in Flanders. Ongoing wild boar surveillance could serve as sentinel warning system for public/human health prevention. Additional active surveillance and direct testing are now recommended to attempt virus detection and to further determine the characteristics of endemic foci, while continued passive medical and veterinary surveillance is indicated to monitor the potential risk for Belgian public health.

**Amino acid polymorphisms of PRNP in Korean water deer (*Hydropotes inermis argyropus*)** (Poster no. 3)

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) or prion disease, fatal neurological disorder, that occurs in free ranging cervids, including white-tailed deer, mule deer, elk, and moose. CWD has occurred five times up to now since it was first reported in 2001 in Republic of Korea (ROK). Korean water deer (*Hydropotes inermis argyropus*) is the dominant species of the wild deer in ROK. Polymorphisms in the PRNP gene can lead to different conformation of prion protein that modulates individual susceptibility to and progression of TSE infection. The aim of this study was to identify the PRNP polymorphism related to CWD in a large number of free-ranging Korean water deer.

PRNP genes have been sequenced in one hundred forty four Korean water deer. Four amino acid changes were identified within the 256 amino acid open reading frame in the third exon of the PRNP gene. Four Polymorphisms in Korean water deer were G96D (1.4 %), N100S (23.6 %), D170G (0.7 %), A187V (100 %) when compared to the reference gene (GenBank, DQ358969.1). The PrP amino acid sequence of the Korean water deer showed 98.4 ~ 99.2 %, 97.9 ~ 98.8 % and 98.8 ~ 99.6 % homology with those of American elk, mule deer and white-tailed deer, respectively.

We found four novel polymorphism of PRNP gene for Korean water deer comparing our results with published date. Newly discovered PRNP polymorphisms have not been confirmed whether there is a relationship between CWD infectivity and polymorphism. The PrP allele for CWD susceptibility of the Korean water deer is not yet determined. However, the amino acid sequence of Korean water deer was shown to high genetic similarities to those of the elk, white-tailed deer, and mule deer. Furthermore, we focused on identifying known polymorphisms in the PRNP gene, related potential risk for natural CWD infection of the Korean water deer. Future research is required to identify the allele for CWD susceptibility of the Korean water deer.

## **Monitoring for Chronic Wasting Disease in Korean water deer in the Republic of Korea (Poster no. 1)**

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of captive and free-ranging white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*) and Rocky Mountain elk (*Cervus elaphus*) in several US states and Canadian provinces. Korean CWD was introduced by elk imported from Canada in 1997. CWD outbreaks in farmed animals have been reported five times through 2001, 2004, 2005, 2010 and 2016 in Republic of Korea. CWD surveillance of wild Korean water deer (*Hydropotes inermis argyropus*) that lived in the country has not been performed. Here, we described preliminary data of CWD monitoring in Korean water deer for last two years.

All tissue samples of Korean water deer were obtained from Korean Association for Wild Animal Protection in the eight provinces and National Institute of Environmental Research. For all animals (n = 462), obex, retropharyngeal lymph node, tonsil and rectal mucosa were tested for PrP<sup>CWD</sup> using TeSeE<sup>TM</sup> SAP Combi Kit (Bio-Rad).

Our sample sizes were adequate to detect CWD even at a prevalence of 1 % for inhabited Korean water deer at normal risk with 95 % confidence.

Korean water deer that is estimated to be about 610,000 is inhabited in Korea. Based on statistical significance, we investigated CWD from 2014 to 2015. By province, in Kangwon (229), Gyeonggi (43), Gyeongnam (56), Gyeongbuk (16), Jeonnam(43), Jeonbuk (11), Chungnam (30), Chungbuk (34), we have collected a total of 462 samples of Korean water deer. Most of those being run over have been struck and killed by car. They were selected for the only weight 14 kg or more objects. The obex, retropharyngeal lymph node, tonsil and rectal mucosa of these objects were examined for CWD. All the Korean water deer were negative for PrP<sup>CWD</sup>.

Monitoring for CWD in Korean water deer was carried out from 2014, and has been investigated for about two years. To date, there has not been confirmed for CWD infected Korean water deer. However, in consideration of the incubation period of CWD, CWD investigation of Korean water deer should be carried out five or more years. Furthermore, although the format population of Korean roe deer is small, it must also promote research of those populations.

***Trypanosoma cruzi* lineages fluctuate in the naturally infected rodent *Octodon degus*** (Poster no. 70)

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*Trypanosoma cruzi*, a flagellated protozoan, infects several mammal species in Chile. The rodent *Octodon degus* (Octodontidae) has been described as one of its reservoirs. We evaluated *T. cruzi*-infection in six wild *O. degus* by means of PCR on blood and xenodiagnosis coupled with PCR (XD-PCR), using the endemic triatomine vector *Mepraia spinolai* grown under laboratory conditions. This insect vector inhabits the same environment of *O. degus*. Xenodiagnosis was performed three times, each one separated by a period of six months, using six triatomines in each opportunity. The infection in triatomines' faeces was assessed by XD-PCR at 40, 80 and 120 days post xenodiagnosis, by means of re-feeding with uninfected *Mus musculus* to promote vector defecation. The temporal variation of *T. cruzi* lineages was evaluated using DNA blot and minicircle hybridisation with lineage-specific probes in the amplified products, to detect TcI, TcII, TcV and TcVI DTUs. All six individuals presented mixed infections in blood. By XD-PCR we found temporal variations in parasite detection, and differential presence of lineages, changing from four to one, and finally to two individuals presenting mixed infections in the successive xenodiagnosis.

These variations could be caused by elimination of *T. cruzi* infection by this rodent species, or by differential tropism for *O. degus*' organs. TcV and TcII were frequently detected, which may indicate that when sylvatic triatomines feed from these rodents, they would be more likely to become infected with these lineages

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**Advances on current challenges posed by wild boar to African swine fever control in Europe: a networking approach** (*Poster no. 4*)

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African swine fever (ASF) is a devastating haemorrhagic disease of suids endemic to Africa. Outbreaks of ASF out of the African continent occurred in Europe and in the Caribbean on the second half of the 20<sup>th</sup> Century. The disease was eradicated from those territories and until 2007 the only non-African ASF endemic territory was Sardinia (Italy). After the introduction of ASF into Georgia in 2007, the disease spread in the Caucasus and Eastern Eurasia causing severe damages to the pig industry, pig trade and rural economies in Georgia, Armenia, Azerbaijan, Russia, Ukraine and Belarus. ASF spread from domestic pigs to Eurasian wild boar (*Sus scrofa*). Since then, wild boar have been implicated in the spread of ASF in Europe. The continuous and dense wild boar meta-population in Europe, estimated to approximately 3.5 million individuals, is favouring ASF spread. The lack of vaccines and other potential control tools prompts to develop efficient ASF control approaches focused on wild boar management. The spread of ASF in the EU, with its 149 million pigs and an annual export of over 2.17 billion Euro, could be devastating.

This presentation aims to: i) show an update the status of ASF in Europe; and ii) present a recently started initiative, ASF-STOP. The first objective is approached through a compilation of published data on ASF in Europe and epidemiological reports by the OIE and the EU-Animal Disease Notification System.

The first cases of ASF in EU member states (MS) were detected in 2014 in Lithuania and afterwards in Poland, Latvia and Estonia; the index case in all these MS was a dead wild boar. European wild boar populations are still increasing in distribution and density. This is probably favouring the spread of ASF in Eastern EU MS. Epidemiological data suggest local but continuous spread of ASF in wild boar in affected countries.

ASF-STOP is a 4-year networking Action funded by the EU COST Programme. ASF-STOP gathers scientists with outstanding expertise from 27 European countries. Its main aim is to achieve a better understanding and combating of ASF in Europe. ASF-STOP involves many topics and disciplines related to ASF, including virology, vaccinology, immunology, diagnostics, pathology, epidemiology - and its hosts - both wild and domestic suids. ASF-STOP is structured in five working groups and has a strong component of wildlife work and expertise, for example wild boar ecology, biology, management and ASF epidemiology and pathobiology.

**First molecular characterisation of cetacean poxvirus in odontocetes from South America: implications on cetacean poxvirus taxonomy** (*Poster no. 25*)

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The *Poxviridae* family consists of large, linear double-stranded DNA viruses that replicate within the cytoplasm of their host, and are comprised of two subfamilies: *Entomopoxvirinae* (invertebrate-infecting) and *Chordopoxvirinae* (vertebrate-infecting). The poxviruses identified in cetaceans are currently tentatively named cetacean poxviruses (CePVs) and are associated to skin lesions, known as “tattoo” or “ring” lesions. CePVs are considered emerging pathogens in cetaceans.

We evaluated a male bottlenose dolphin (*Tursiops truncatus*) found stranded dead in the Laguna estuary (28.46° S - 48.79° W), on the coast of Santa Catarina, southern Brazil. Upon necropsy, the animal presented multifocal oval skin alterations, with a pale light grey stippled interior, and well-defined dark gray irregular edges, ranging from 3 to 4 cm in diameter, consistent with tattoo lesions and suggestive of poxvirus infection. Hematoxylin-eosin stain was used in formalin fixed skin samples to characterise the lesions histologically, while frozen skin samples were screened by PCR using primers for DNA polymerase and DNA topoisomerase I gene fragments in order to detect the agent.

Microscopic findings consisted of epidermal ballooning degeneration, and keratinocytes containing small, spherical or irregular, homogenous, pale eosinophilic or amphophilic intracytoplasmic inclusions, characteristic of poxvirus. Our molecular findings are the first report of poxvirus amplification in odontocetes from South America and are in agreement with recent studies that identified a possibly new poxvirus genus affecting cetaceans. The DNA polymerase gene presented a 95.7 % nucleotide identity to the poxvirus sequence of an Indo-Pacific bottlenose dolphin (*T. aduncus*) from Hong Kong, and 92.6 % identity to a *T. truncatus* from the USA for DNA topoisomerase I gene, while the deduced

amino acid sequence presented high identity of DNA polymerase (98.9 %) and DNA topoisomerase I (97.3 %) genes with the ones observed in a rough-toothed dolphin (*Steno bredanensis*) from the USA. Our data confirm a relative stability between the studied CePV sequences obtained from *Delphinidae*, despite the geographic distance. This study also identified specific amino acid motifs for all cetacean poxviruses, further reinforcing the suggestion of a new genus. Additional research, including complete genome of selected members of this tentatively new genus employing next generation technology, would greatly contribute to clarify the taxonomy and evolutionary history of CePV.

**Disease risk analysis for conservation translocations** (*Poster no. 82*)

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There are risks from disease when translocating wild animals, stemming from changes in parasite encounters, the stressors placed on the translocated animals and from non-infectious agents, such as toxins, at the destination. Disease risk analyses (DRAs) have been completed for 11 translocations of native UK species for conservation purposes by the Disease Risk Analysis and Health Surveillance (DRAHS) project, a collaboration between Natural England and the Zoological Society of London. A method has been developed specific to conservation translocations which (i) identifies hazards on the basis of novelty to the destination environment alone, (ii) denotes translocations which cross ecological and geographical barriers as being of higher risk, (iii) categorises hazards according to their origin in the translocation pathway and (iv) includes non-infectious agents, such as toxins, as hazards. Risk assessment is carried out using a qualitative, transparent method developed by the World Organization for Animal Health. This is used to provide recommendations for disease risk management which are communicated to all stakeholders. A recommendation to proceed with translocation was made in all 11 cases provided that disease risk management recommendations were followed. In general, projects involving complex translocation pathways, and which cross ecological and geographic barriers, carry a higher risk from disease and require more effort in disease risk management. Experience gained from these DRAs will help translocation practitioners plan future interventions.

**Pulmonary histopathological findings in odontocetes (Cetartiodactyla: Cetacea) from Brazil** (*Poster no. 30*)

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Cetaceans are highly susceptible to respiratory disease, which is one of the main causes of morbidity in this suborder. Thus characterising microscopic aspects of lung disease in cetaceans, its aetiology and the relationship with biological and epidemiological parameters (e.g. sex, age class, body condition, region and season), might help to understand disease pathogenesis and causes of death. In this study the lungs of 185 individuals, comprising 23 species of odontocetes from captivity, by-catch, live-stranded or found dead were examined. Carcasses were autopsied along the coast of Brazil from 1998 to 2015 and lung samples were submitted in 10 %

formalin to the Marine Mammal Tissue Bank (LAPCOM-VPT-FMVZ-USP). These were processed and stained with hematoxylin and eosin as routine and special histochemistry for microscopic examination. The major findings were: oedema (154/185; 83.2 %), congestion (143/185; 77.3 %), haemorrhage (106/185; 57.3 %), fibrosis (47/185; 25.4 %), subepithelial bronchial mineralisation (42/185; 22.7 %) and type II pneumocyte hyperplasia (38/185; 20.5 %). Findings with frequencies less than 20 % included atelectasis, bronchitis, vasculitis, pleuritis, granuloma/pyogranuloma, arterial tunica media hypertrophy, bronchial cartilage calcification, squamous metaplasia of bronchial epithelium, interstitial dystrophic mineralisation, bronchus associated lymphoid tissue hyperplasia, hemosiderosis, and extramedullary hematopoiesis. Evidences of neonatal respiratory distress (alveolar squames and/or meconium) was observed in 7/19 (36.8 %) calves and one fetus. Pneumonia was observed in 96/185 (51.9 %) cases, with two distinct pneumonic processes identified in 17/185 (9.2 %), totalising 113 different pulmonary inflammatory processes. Interstitial pneumonia was observed in 81/113 (71.7 %) cases followed by bronchopneumonia (29/113; 25.7 %) and bronchointerstitial pneumonia (3/113; 2.6 %). Pneumonia severity was moderate in 34/113 (30.1 %), moderate to severe in 9/113 (8 %) and severe in 6/113 (5.3 %) cases. Predominant inflammatory processes included mononuclear in 45/113 (39.8 %), mixed (mononuclear cells and granulocytes) in 40/113 (35.4 %), granulomatous/pyogranulomatous in 17/113 (15 %) and granulocytic in 11/113 (9.7 %). The aetiologic agent was identifiable in 25/113 (22.1 %) of the pneumonias: *i.e.* parasitic (19/113; 16.8 %), bacterial (4/113; 3.5 %) or mixed (both) in 2/113 (1.8 %). Significant differences of pneumonia were identified in males (66/112; 58.9 %) vs females (30/71; 42.2%),  $\chi^2 = 4.84$ ,  $p < 0.05$ ; adults (53/78; 67.9 %) vs juveniles (38/83; 45.8 %) and calves (4/18; 22.2 %),  $\chi^2 = 15.58$ ,  $p < 0.01$ ; and regions: south-eastern (51/80; 63.8 %), north-eastern (26/48; 54.2 %) and southern (19/56; 33.9 %),  $\chi^2 = 11.84$ ,  $p < 0.01$ . Our findings evidence an important prevalence of pneumonia in cetaceans from Brazil, providing valuable information that might help to establish baseline pulmonary pathology among these species and further aid in comparative studies.

## **Ranaviruses in wild amphibians in the Netherlands: the 2015 update**

(Poster no. 78)

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Ranavirus (genus *Ranavirus*, family *Iridoviridae*) infections in wild amphibians have been reported to date in nine European countries. In four countries (Spain, The Netherlands, France and Belgium), these ranaviruses were characterised as common midwife toad virus (CMTV) or CMTV-like ranaviruses. Ranavirus infection can be spread through trade and transport of amphibians, and the disease is one of the two notifiable amphibian diseases. Emerging ranaviruses may threaten amphibian communities in the wild, and therefore it is important to monitor the occurrence and spread of the disease in the wild.

Since the first detection in the Dutch wildlife in 2010, amphibian mortality events have been investigated annually and nationwide. To detect ranavirus in dead specimens, histopathological examination and diagnostics tests were performed (PCR and immunohistochemistry). The detected ranaviruses were subsequently partially or fully characterised molecularly and compared phylogenetically to ranavirus sequences from GenBank. Disease in the dead specimens was characterised by species and life stages affected and scoring of lesions.

In total, hundreds of dead amphibians from over 50 sites were investigated. Ranavirus-associated amphibian mortality events occurred at around a third of these sites. Fatal disease occurred in multiple amphibian species, but to different extent. Molecular characterisation showed differences in ranaviruses present in the Netherlands and, combined with spatiotemporal data, has provided evidence for an ongoing ranavirus epidemic, for which the most recent developments will be presented. The likelihood of continued spread from the index site was statistically quantified by calculating the discrete reproductive power of the identified outbreak using a nonhomogeneous birth process. These results show that continuous passive surveillance of amphibian mortality events can already provide a fair deal of information on ranavirus infections occurring in wild amphibian communities, while likely to be relatively feasible for many wildlife health centers in Europe. The data reiterate the need for paying more committed European attention to this multi-host disease.

## **Serological evidence of influenza A viruses in Arctic carnivores**

(Poster no. 14)

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Influenza A viruses infect a variety of terrestrial and marine avian and mammalian species. Several influenza A viruses have been associated with different pinnipeds and cetaceans, however we have little information about the exposure of arctic carnivores which prey and/or scavenge on marine mammals. In order to detect serological evidence of influenza virus circulation in Arctic carnivores, we used a commercial competitive enzyme-linked immunosorbent assay screening serum samples from arctic foxes (*Vulpes lagopus*) (n = 43) and polar bears (*Ursus maritimus*) (n = 271) collected in Iceland and Svalbard, respectively. We detected antibodies against the nucleoprotein of influenza A virus in one arctic fox and six polar bears, the prevalence for each species being 2.3 % and 2.2 %, respectively. Interestingly, the positive Icelandic arctic fox belonged to the coastal ecotype that relies on the marine food web, while four polar bears were positive out of the 36 individuals captured on Nordaustlandet, the second largest island of the Svalbard archipelago. Further studies are required to characterise the influenza A virus subtypes present in these populations and to identify the reservoir host of influenza A virus in the high arctic. The low seroprevalence detected for both species in this study indicate that while influenza A virus circulate among the Arctic carnivore populations, they are not currently important pathogens for these species.

**Surveillance in wild boar - without hunters' acceptability a difficult task: a participatory study** (*Poster no. 89*)

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Classical Swine Fever (CSF) is a viral disease of domestic pigs and wild boar. CSF outbreaks may have massive economic consequences. In the past, CSF outbreaks in domestic pig farms could often be traced back to infected wild boar. Hence, disease surveillance is not only important in livestock but also essential in wild life.

Surveillance measures can only function if they are accepted by the key players in the system. Acceptability, which describes the willingness of persons to contribute, is often analysed using participatory methods. Participatory epidemiology enables the active involvement of key players in the processing of epidemiological issues. In the present study, we used participatory methods to evaluate the functionality and acceptability of CSF surveillance system in wild boar in Germany, which is highly dependent on the participation of hunters. Diagrams, proportional piling and scoring tools were introduced to investigate different aspects relating to the role of the hunters within the surveillance system for CSF in wild boar. The acceptability of alternative surveillance strategies was analysed with the help of visualisation tools. By conducting focus group discussions, potential vulnerabilities in the system were detected and feasible alternative surveillance strategies identified.

Trust in the current surveillance system is high, whereas the acceptability of the operation of the system is medium. Analysis of the acceptability of alternative surveillance strategies showed how risk-based surveillance approaches can be combined to develop strategies that have sufficient support and functionality. Furthermore, some surveillance strategies were clearly rejected by the hunters. Thus, the implementation of such strategies may be difficult.

Methods of participatory epidemiology can be used to evaluate the functionality and acceptability of existing surveillance plans for CSF among hunters and to optimise plans regarding their chances of successful implementation. Our study showed benefits and additional insights, which can result from the integration of participatory methods in the evaluation of surveillance. Participatory methods can be used to consider and include views and needs of important key figures during the design and the implementation of surveillance strategies.

**Potential transmissibility of chronic wasting disease to Korean water deer using *in vitro* amplification** (Poster no. 2)

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Chronic wasting disease (CWD) is one of groups of transmissible spongiform encephalopathies (TSEs) that cause fatal infectious neurodegenerative diseases in various species. CWD has been confirmed in free-ranging and farmed cervid in the North America. So far, there is no report of CWD transmission to wild deer population in Republic of Korea (ROK) after the outbreak of CWD in the farmed cervid. Korean water deer (*Hydropotes inermis argyropus*) is dominant wild cervid in ROK. Protein misfolding cyclic amplification with Teflon beads (PMCAb) is a highly efficient amplification method which reflects transmission barrier in animals. We studied that Korean water deer could support CWD transmission by using PMCAb. To address this, the normal brain homogenates from Korean water deer and elk were seeded with CWD prions from CWD infected elk, sika deer, and red deer for the PMCAb reactions. Interestingly, all three CWD prions we tested were amplified in both Korean water deer and elk. In contrast, the substrates from bovine transgenic mouse (TgBoPrP, Tg1896) failed to amplify CWD prions in the control experiment. Our results suggest that Korean water deer could be susceptible to CWD infection.

**Comparative analysis of various methods of anthelmintic medications injection (ivermectin and brovalzen) to bizons with dictyocauloses living in natural environment** (*Poster no. 104*)

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Dictyocauloses is one of the most spread parasite diseases among hoofed animals in Ukraine. Thus, the problems of development of diagnostic methods, research of course, pathologic anatomical changes and dictyocauloses treatment in hoofed animals, including the bizons living in natural environment, as well as their health improvement are very actual. Four bizons of 1 - 3 years died within one week at the territory of forest estate land. Autopsy showed that animals died due to asphyxia caused with respiratory organs blockage by sexually active helminthes Dictyocaulus. Lungs were enlarged, of pale-grey color, sometimes tumulus and "marbled", with areas of different hepatisation stages; atelectasis and vesicular emphysema were found. Bronchial and middle-wall lymph glands were enlarged significantly. After medical history and the reason of death were determined, it was stated that bizons had been constantly kept at the pinewood enclosure with the total area of 2,5 ha. The herd of bizons quantity of 13 heads was too big for such small territory. This area of forest was significantly fouled with animals' defecations. Huge amount of defecations has led to extinction of 20 % of trees at the area. Pilobolus, which leads to expansion of dictyocaulus larvae at the plants, were found in bulk on dried faeces. Ambient temperature reached 25 - 30 degrees that resulted in fast larvae development up to invasion stage. The research defined that all the animals were infected with dictyocauloses, e.g. extensiveness of helminthic invasion constituted 100 % and its intensiveness amounted at 9 - 12 Dictyocaulus viviparus larvae in vision of a microscope. It was suggested and tested several schemes for animals' treatment in combination with various antihelminthic medications: group feeding with brovalzen mixed with concentrated food items; individual distant injection of Ivermectine; individual feeding with brovalzen in form of feed attractions; group rearing with albendazole solution. However, the best results were achieved under application of 10 % water-and-albendazole solution, which was diluted in an appropriate quantity in compliance with the instructions in the drinking bowl, contained daily water portion for all the buffalos. Within 24 hours animals were receiving the necessary amount of medication that provided the treatment effect required. Within one year the medication was applied in four cycles. Each cycle consisted of two albendazole solution rearing with a week pause.

**Accuracy of blotting paper blood samples for serological tests to detect *Toxoplasma gondii* antibodies in semi-feral domestic cat** (Poster no. 97)

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Collection of blood on blotting paper (BP) is a promising sampling method for serological diagnosis in infectious wildlife diseases. BP blood samples are practical and easy to use under any field conditions. As only a small blood volume is required, they can also be minimally invasive for animals and allow repeated samples on a same individual during a short time period. If BP samplings begin to be largely used in wildlife health studies, validation of this method is always needed for many infectious diseases. The aim of the study was to evaluate the accuracy of BP blood samples to detect *Toxoplasma gondii* (*Tg*) antibodies in domestic cats in comparison to serum samples used as the putative gold standard. *Tg* is a felid parasite, also infecting all warm-blooded vertebrates around the world. Estimation of *Tg* exposure in cat populations based on *Tg* antibodies detection in serum samples is largely studied worldwide but little is known on the efficiency of BP samples. The present study was conducted on five populations of semi-feral cats located in eastern France. Whole blood (for serum) and BP blood were taken during four successive periods from 54, 38, 56 and 48 cats. Serological assays were performed using modified agglutination tests (MAT) and a titer of 1:25 were considered indicative of exposure to *Tg*. Prevalence in cats varied from 67 % to 71 % and from 63 % to 71 % with serum and BP blood samples respectively. Results obtained with the BP blood sampling method were highly concordant with those from the classical serum sampling method but the titers obtained from BP blood samples tended to be lower (45 % of all samples concerned), as previously reported in other studies. Elution of BP during processing may explain this result and may lead to a possible misclassification of individual infectious state and to an underestimation of prevalence in cat populations. Analysis showed that lowering the cut-off value from 25 to 10 for BP blood samples increased the concordance between the two sampling methods without decreasing the specificity. In conclusion, the use of BP blood samples provides a reliable alternative method to conventional serum sampling to detect *Tg* antibodies in domestic cats. However, lowering the cut-off value is recommended to prevent false negative samples and incorrect estimations of prevalence and incidence.

**Combined effects of season, age and demographic parameters on toxoplasmosis infection rates and oocysts excretions in semi-feral cat populations** (Poster no. 98)

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Toxoplasmosis is an infectious disease caused by the protozoan parasite *Toxoplasma gondii* (*Tg*) that can infect all warm-blooded animals worldwide. Felids, particularly domestic cats, are the definitive hosts. Cats become infected through the ingestion of a prey containing *Tg* tissue cysts. Once infected, they can excrete millions of oocysts which sporulate in the environment and become infectious for intermediate hosts. Despite numerous studies conducted on *Tg* transmission among cats, there is still a lack of knowledge about factors affecting infection dynamics in natural cat populations. In this study, we developed multi-event models to estimate cat infection rates in relation to age and season as well as survival rates. Models parameters were estimated from longitudinal captures-recaptures data of semi-feral domestic cats living around five dairy farms of eastern France. One hundred and twenty-seven cats, including 61 kittens born during the study, were captured every three months during two years. For each capture, a blood sample was collected and tested for the presence of *Tg* antibodies to determine cat infectious state. Models indicate a strong effect of the seasons on cat infections. Infection rates are significantly higher in fall than in spring and summer whereas no infection was detected in winter. Infection rates and the age of primary infection are highly variable between cat populations suggesting a differential exposure to *Tg* between farms. Age-specific infection rates were coupled with survival rates and fecundity parameters to estimate the annual rate of expected excretions by newly infected cats per farm. This rate differs between farms in accordance with the soil contamination by *Tg* previously measured in a jointed study. We discuss how season, environmental parasite load and demographic parameters influence simultaneously infection dynamic in cat populations but also how infection and population dynamics can both contribute to a runaway process of environmental contamination by *Tg* oocysts. More generally, our study highlights the importance to consider hosts infection and population dynamics jointly to understand spatial heterogeneity of environmental contamination by *Tg* and to prevent toxoplasmosis infection risks in rural areas.

**Molecular identification of *Paramphistomum leydeni* and *Paramphistomum cervi* in red deer (*Cervus elaphus*) in Croatia** (Poster no. 60)

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Paramphistomosis of domestic and wild ruminants is caused by ruminal flukes from *Paramphistomum* genus (Trematoda: Digenea) that parasitise in fore stomachs as adults and in intestines as juveniles, and may cause major economic losses. Given their worldwide distribution and indications that infections are becoming more common it seems that the importance of these parasites is underestimated. Even though it was considered that *Paramphistomum* flukes are present in Croatia, scientific data are rare. So far there is one report of *Paramphistomum cervi* and *P. microbothrium* in roe and red deer from 1966, and finding from 1990 that assumes *P. cervi* presence in red deer. In domestic animals the infection was registered only in 2006 and 2010 in sheep and cattle, but the identification of fluke species was not performed. The goal of our work was to identify the species of ruminal flukes which were found in one red deer (*Cervus elaphus*) in Croatia. Research was conducted on 23 samples of flukes found in the rumen of a hunted doe. Considering the fact that differentiation of *Paramphistomum* species based on morphological characteristics is insufficient, we performed the molecular analysis of ITS2 region of ribosomal DNA. A total of 320 base pairs of ITS2 region was amplified using primers GA1 (AGAACATCGACATCTTGAAC) (Anderson and Barker, 1998) and BD2 (TATGCTTAAATTCAGCGGGT) (Luton et al., 1992) and sequenced. We have determined that 22 samples were identical with *Paramphistomum leydeni* sequences from China, Uruguay and Ireland deposited in the GenBank, while one sample concurred with the *Paramphistomum cervi* sequence from China. This is the first report of *P. leydeni* in Croatia and first molecular confirmation of *P. cervi* presence. Our results do not exclude the presence of other *Paramphistomum* species in Croatia and considering the rising importance of these parasites it would be important to conduct a systematic research of distribution and prevalence of paramphistomosis both in wild and domestic ruminants.

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**Spotted fever group rickettsia species, *Anaplasma phagocytophilum* and *Borrelia* in immature *Ixodes* feeding on lizards in Algeria** (Poster no. 77)

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Our study was conducted in Séraïdi, Annaba (North East of Algeria) humid bioclimatic zone. Tick and lizard collection: this survey was conducted from 2012 to 2015. Lizards were hand captured in the site or using a special pitfall in the complex zone. Ticks were removed from each lizard using forceps and stored in 70 % ethanol for later identification to species and life stage using taxonomic keys (IPTM, version1.0 “AFPMB” and confirmed by Pasteur Institute of Algiers). DNA Extraction: DNA was extracted from ticks composed of males and unfed females, using the QIAmp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) and sequencing was done in Pasteur institute.

PCR results from 423 immature ticks showed that the presence of variety of pathogens: *Borrelia garinii*, *Coxiella burnetii*, *spotted Fever Group Rickettsia Species* and *Anaplasma phagocytophilum*.

The prediction of environmental risk related to the situation of our biotopes goes to an increased risk of transmission of pathogens in the study areas, because of the abundance of lizard's hosts and reservoirs, their high connectivity, and the presence of infected vectors that circulate between communities and landscape units.

**Assessment of hepatitis E virus circulation in wildlife and genetic characterisation of the virus strains in Greece** (*Poster no. 12*)

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Hepatitis E virus is an emerging non-enveloped positive strand RNA virus with worldwide distribution. Seven genotypes of the virus have been recognised. Genotypes 1 and 2 are found exclusively in humans, genotypes 3 and 4 are found in humans and animals, genotypes 5 and 6 have been detected in Japanese wild boar and genotype 7 has been isolated recently from dromedary camels. Wild and domestic animals have been identified as potential reservoirs of the virus. The study objective was to assess the virus circulation in the wild boar and cervid populations in Greece and to genetically characterise the isolated strains of the virus.

Whole blood and tissue samples were collected from hunter-harvested and/or found-dead animals, during the hunting seasons 2010 - 11 to 2014 - 15 in collaboration with local hunting associations. In total, 101 wild boars, 23 roe deer, 2 red deer and 1 fallow deer samples were collected and tested for antibodies against HEV and for the presence of HEV RNA. RT-PCR and sequencing was performed using primers which amplify a part from the ORF2 region of the HEV genome. Molecular evolutionary analyses were conducted on nucleotide sequences of HEV strains detected in wild boar in Greece and on HEV sequences that were retrieved from the EMBL database, using the programme MEGA 6. Phylogenetic distances were calculated by the Kimura-2-parameter method and unrooted trees generated based on the neighbor-joining method. A bootstrap analysis with 1,000 replicates was included.

Antibodies against HEV were detected in 14 samples (13,86 %). Nine samples from wild boars (8,91 %) were found positive and the presence of HEV nucleic acid was confirmed by sequencing. A different virus strain was genetically characterised from each of the nine wild boars and designated by the codes GRE-WB1 to GRE-WB9. The Greek strains from wild boars clustered with isolates from genotype 3. No antibodies or virus antigen was detected in samples from wild cervids. The results of the study confirm that HEV is circulating among wild boar populations in Greece. To our knowledge, this is the first report of detection of antibodies against HEV and the first characterisation of detected HEV RNA from wildlife in Greece. Presence of antibodies against HEV and HEV RNA in wild boar samples implies that wild boar can possibly act as a potential source of HEV infection in humans. Further studies are needed to fully elucidate the epidemiology of HEV in wildlife and the foodborne zoonotic transmission risks.

**Does koala retrovirus actually trigger disease?** (*Poster no. 88*)

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Koala retrovirus (KoRV) is an unusual virus in that it is currently undergoing the transition between being an infectious horizontally transmitted pathogen and an inherited vertically transmitted element in its host's genome. Despite much debate and circumstantial evidence we still don't have solid evidence that koala retrovirus actually triggers disease (neoplasia and immunosuppression) in individual animals or that it has impacts at a population level on koalas. This presentation will discuss data from a large epidemiology and next generation sequencing (transcriptome) study comparing animals from SE QLD in Northern Australia (who all have the inherited form of the virus) to those in South Australia (where there is a mixed prevalence of the virus). This data gives strong molecular and epidemiological evidence supporting KoRV as a driver of leukaemia, lymphoma and immunosuppression in koalas.

## Enteric pathogens of zoonotic concern in non-human primates in Sri Lanka

(Poster no. 36)

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Zoonotic disease is a two-way street where humans and other animals are interchanging pathogens. We investigated the occurrence of the potentially zoonotic *Campylobacter* spp., *Salmonella* spp. and group A rotaviruses in faecal samples from free-ranging toque macaques and tufted gray langurs in Sri Lanka. Samples were opportunistically collected from primate troops with close human contact at five sites. Standardised culturing was used to detect the bacteria and an ELISA-based dipstick test was used for detection of group A rotaviruses antigens. Genotyping was performed using pulse field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) and the isolates' sensitivity to selected antibiotics was tested with VetMIC<sup>TM</sup> (National Veterinary Institute, Uppsala, Sweden) panels Camp EU, CLIN GN and GN-mo (version 4).

All 98 samples tested negative for rotavirus. The 40 samples from gray langurs were also all negative for *Campylobacter* spp. and *Salmonella* spp. Of the 58 samples collected from toque macaques, *C. jejuni* was isolated from ten, *C. coli* from four and *Salmonella enterica enterica* subsp. Virchow from two of the samples. The fact that neither of the bacteria were isolated from tufted gray langur samples could reflect a true difference between the primate species. However, this should be interpreted in the light of a relatively small sample size. Resistance to ampicillin, ciprofloxacin, nalidixic acid and tetracycline was identified in four *C. jejuni* isolates, of which three were multidrug resistant. In addition, all *C. jejuni* showed undetectable MIC-values to colistin, while all *C. coli* were sensitive to the substance. All *C. coli* were resistant to ampicillin. The *S. Virchow* isolates were sensitive to all antibiotics tested for. Six strains of *C. jejuni* were identified using PFGE and MLST clonal complexes were assigned to all isolates. Sequence types were assigned to seven out of ten *C. jejuni*. The detection of antibiotic resistant zoonotic bacteria in free-ranging toque macaques with close human contact may have implications for both non-human primate conservation and public health in Sri Lanka and beyond.

**Novel viruses of rodents and shrews** (*Poster no. 68*)

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Recent work with a large sample collection of European rodents and shrews using a degenerate PCR screening approach has identified a number of novel viruses. These include a number of viruses closely related to the alphacoronavirus Lucheng virus, recently described in China, indicating that these viruses are widespread across Eurasian rodents. In addition arenavirus outbreaks in zoological facilities in cosympatric populations of rodents and primates have been traced historically. Ongoing work will include the expansion of the screening programme to bornaviruses as well as the development of pseudotype virus assays for serological screening of the identified viruses at a population level.

**Geographical distribution and analysis of *Leishmania* infection in European Brown Hares, dogs and humans and inference for the phylogenetic position of the *Leishmania* sequences in Thessaly, central Greece** (Poster no. 49)

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Leishmaniasis is a mandatory notifiable disease in Greece, endemic in most islands and coastal regions of Greece. Human leishmaniasis cases presented higher incidence during the last years in Thessaly, central Greece. This fact prompted us to investigate the geographical distribution of *Leishmania* infected European brown hares, dogs and humans in relation to environmental parameters and to infer the phylogenetic position of animal and human *Leishmania* parasites detected in Greece.

Whole blood and bone marrow samples collected from children which attended the main hospital of the University of Thessaly with clinical signs compatible with Leishmaniasis, as well as canine lymph node aspirates and hare spleen samples were examined by ITS1-nPCR. Four *Leishmania* PCR positive samples from children, three from dogs and seven from hares were sequenced and used for molecular evolutionary analyses in conjunction with *Leishmania* sequences that were retrieved from the EMBL database, using the programme MEGA 6. Phylogenetic distances were calculated by the Kimura-2-parameter method and

unrooted trees generated based on the neighbor-joining method. A bootstrap analysis with 1,000 replicates was included. The regions where the *Leishmania* infected hares and dogs and the human Leishmaniasis cases were recorded, were used as background information and analysed using Geographical Information System (GIS).

The phylogenetic analysis performed on 60 *Leishmania* ITS1 sequences, including the fourteen Greek sequences, revealed that the homology of the nucleotide sequences between the Greek *Leishmania* parasites was 99.1 %. The homology of the nucleotide sequences between the Greek *Leishmania* parasites and strains belonging to *L. donovani* complex retrieved from GenBank, was 98.9 %.

The *Leishmania* positive hares were found in shrubland with pasture land and in agroforestry formations with a mean altitude of 350 m above sea level and a mean distance of 500m from villages and towns. Most *Leishmania* infected dogs lived in urban and rural areas (irrigated and non-irrigated land) at low altitude. The majority of the human cases was found in discontinuous urban fabric and permanently irrigated land with a mean altitude of 171 m.

The similarities detected between *Leishmania* parasites detected in hare, canine and human samples could possibly indicate an overlap of the transmission cycles of *Leishmania* spp. The investigation of the role of wild mammals in the transmission cycle of *Leishmania* spp needs further elucidation in order to reach conclusions regarding the epidemiology of the disease and the implementation of preventive measures for the protection of public health.

## Detection of Bluetongue virus in wild cervids in Greece (Poster no. 15)

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Bluetongue is a vector-borne viral disease of domesticated or wild ruminants, which affects clinically mainly sheep and is a significant cause of financial losses in affected farms. The disease is caused by *Bluetongue virus* (BTV), a member of the genus *Orbivirus* (family *Reoviridae*). BTV includes 26 serotypes (1–26). The virus is principally transmitted by biting midges of the genus *Culicoides*. In Greece the first outbreaks occurred in the east Aegean Greek islands in the late 1970s while a large outbreak took place in Greece and the other countries of south-east Europe in 2014. This study aimed to investigate the presence of *Bluetongue virus* in wild cervids in Greece during 2010 to 2015.

Spleen and whole blood samples were collected from hunter-harvested and/or found-dead cervids during the hunting season 2010 - 11 to 2014 - 15 in collaboration with local hunting associations. Samples from 68 roe deer, two red deer and one fallow deer were examined for the presence of antibodies against BTV and BTV RNA. A one-step RT-PCR was performed, according to an OIE prescribed protocol for international trade using primers to amplifying a 274 bp region of VP5 (NS1) gene of the virus. Data regarding location of each sampling site were analysed with Geographical Information System (GIS) using the software ArcGIS Desktop 10.1.

Antibodies against BTV were detected in seven roe deer. In addition, four samples were found positive by PCR and the presence of HEV RNA was confirmed by sequencing. Three samples were collected in Koziakas hunting area (total surface 483 km<sup>2</sup>) and one was collected in Drama in Northern Greece (dead animal). All four samples were collected from roe deer. According to GIS analysis, there are 545 permanent livestock farms in the Koziakas hunting area, out of which 478 are sheep and goat farms numbering 44,215 animals (range 8 - 415). Cattle population

reaches 5,849 animals. Taking into account the presence of sheep flocks/goat herds in semi-nomadic state moving through the area, these numbers are even higher. Within a buffer zone of three kilometers from the sites where the positive samples were collected, there were 25, 24 and 73 livestock farms respectively. Mean distance of PCR positive roe deer samples from livestock farms was  $840 \pm 108$  m. These findings support the potential of wild cervids as BTV maintenance and/or spill-over hosts, considering co-existence of domestic and wild ruminants. However, the possibility that wild cervids are BTV reservoirs in Greece warrants further investigation.

**First phocine distemper virus case in the Netherlands since 2002 presents as ‘old seal encephalitis’** (Poster no. 17)

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In 1988 and 2002, two phocine distemper virus (PDV) epizootics among harbour seals (*Phoca vitulina*) in north-western Europe caused the death of tens of thousands seals. Phocine distemper virus belongs to the genus *Morbillivirus* of the family *Paramyxoviridae*. Infected harbour seals develop acute necrotising broncho-interstitial pneumonia. In related canine distemper virus and human measles virus, rare persistent infections in the central nervous system have been reported previously, known as old dog encephalitis and subacute sclerosing panencephalitis, respectively.

We report the first case of PDV infection in a harbour seal since the last epizootic in 2002. A 15-year-old severely emaciated and apathic male harbour seal stranded on the Dutch island Texel on October 14th, 2014, and was euthanised for humane reasons the same day. Based on the number of annual growth layers in the teeth, the seal was estimated as 15 years of age, implying it was 3-year-old during the 2002 PDV epizootic. Histopathological examination revealed a severe non-suppurative encephalitis. Immunohistochemical staining using an antibody against closely related canine distemper virus demonstrated the presence of morbillivirus antigen in the brain, but not in other organs. This was confirmed by PCR using primers targeting a conserved part of the PDV phosphoprotein gene. Phylogenetic analyses of the partial phosphoprotein and full-length haemagglutinin gene sequences demonstrated clustering with PDV strains from the 2002 outbreak. Attempts to isolate the virus using Vero.DogSLAMtag cells were unsuccessful.

Taken together, seal age, histopathological findings and nucleotide sequence analyses suggest viral persistence of a PDV/2002 strain in the central nervous system of this seal. Hence, this case may be regarded as the first report substantiating the evidence for ‘old seal encephalitis’, a rare representation of persistent PDV infection.

**Antimicrobial resistances in wildlife: insights from a Mediterranean biodiversity hotspot, the Camargue (Southern France)** (*Poster no. 32*)

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Antimicrobial resistance spread is of major concern for human health and associated with growing economical issues. While it is increasingly hypothesised that wildlife could play an important role in antimicrobial resistant bacteria dynamics, empirical data remain scarce at the moment. As an introduction we will give a systematic review we led to present the elements of response that literature can bring concerning four questions: i) Which resistant bacteria are most frequently observed in wildlife? ii) How are resistant bacteria exchanged between wildlife and the other compartments involved? iii) In which habitats are those resistant bacteria found? iv) Are resistances associated with some ecological traits of the host?

In a second part we will present the first results of our ongoing project aiming at characterising the antimicrobial resistant bacteria carried by gulls in the Camargue (Southern France) and understanding their dynamics. We collected cloacal swabs from two gull species: yellow-legged gulls (*Larus michahellis*) that live in close contact with humans and slender-billed gulls (*Chroicocephalus genei*) that feed at sea. Following culture on selective media we notably isolated carbapenem resistant *Escherichia coli* and *Vibrio cholerae* strains as well as cephalosporin resistant *Proteus mirabilis* strains. We will discuss these results in the light of gull ecology as well as of the genetic comparison between the strains we identified and those that were isolated from humans in the study region.

Our results are alarming enough to encourage determining the contamination source of the bacteria we identified. They also demonstrate that wildlife cannot be neglected if we are to meet the challenge of antimicrobial resistance control.

## **Sarcoptic mange infection in red foxes (*Vulpes vulpes*) in Belgium**

(Poster no. 61)

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Eight cases of sarcoptic mange in foxes were reported during summer 2014 (n = 3) and winter 2015 (n = 5) in Belgium. All animals came from the same restricted forest zones near urbanised areas and some of them were discovered in private gardens (Forest District of Thuin, Hainaut, Belgium). Three of them were found dead, the others were shot for sanitary reasons. At necropsy, all of them presented large areas of alopecia and extensive skin lesions such as hyperkeratosis and suppurative lacerations. Animals presented poor body condition and some of them were severely emaciated (absence of visceral fat). Mange has profound influences on population since, if untreated, death follows in four to six months. In the present cases, cutaneous scrapings and histopathological examinations were systematically performed. Numerous parasites were observed in skin scrapings and marked dermo-epidermatitis with several parasites was observed in histopathological sections. These results suggest an outbreak of sarcoptic mange in red foxes in a limited area of the country. This is a first record for the country. Furthermore, investigations on lungs and digestive tracts performed on three of these foxes revealed they were also infected by parasites transmissible to pets and/or humans: *Angiostrongylus vasorum* was observed in the respiratory tract of one fox whereas *Toxocara canis*, *Uncinaria stenocephala*, *Taenia* spp. and *Echinococcus multilocularis* worms were detected in the digestive tract.

In conclusion, urban foxes represent a source of parasites of public health and veterinary importance and foxes surveillance should be strengthened in these areas.

**Anthropogenic caused injuries and illnesses in free-living white-tailed sea eagles (*Haliaeetus albicilla*) submitted to a wildlife centre in Northern Germany (Poster no. 99)**

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White-tailed sea eagles (*Haliaeetus albicilla*) were almost extinct in the German federal state of Schleswig-Holstein in the 1980s, with only 4 breeding pairs being left. But due to intensive protection measurements the population has steadily grown up to 84 breeding pairs in 2015 ([www.projektgruppeseeadlerschutz.de](http://www.projektgruppeseeadlerschutz.de)). Since the year 2000 nearly all of the free-living white-tailed sea eagles that are found sick or injured in or near Schleswig-Holstein are submitted to the rehabilitation centre of the Wildlife Park Eekholt. Altogether 47 eagles were examined and treated there between June 2000 and March 2016 in close cooperation with the small animal clinic of the Freie Universität Berlin.

In our presentation we give an overview of the causes of injury or disease in these birds. Most of the submitted eagles suffered from trauma (n = 22, 47 %), in many cases caused by wind turbines (n = 10). More than a quarter of the eagles (n = 13, 28 %) were intoxicated e.g. by lead (n = 6), organophosphates/ carbamates (n = 2) or zink (n = 1) or showed signs of intoxication (n = 4). Four eagles (8 %) showed permanent feather disorders like pinching off syndrome. In seven cases (15 %) the origin of disease remained unclear. One young eagle (2 %) was submitted malnourished due to disturbances at the breeding place. Eagles that had to be euthanised because of a poor prognosis or that died during rehabilitation usually underwent a post mortem examination at the IZW.

At least half of the white-tailed sea eagles that were submitted to the rehabilitation centre of the Wildlife Park Eekholt in the last 16 years were injured or damaged as a result of anthropogenic environmental changes.

**Canine adenovirus type 1 in red foxes (*Vulpes vulpes*) in the UK: epidemiology, pathology and evidence of inapparent infection** (Poster no. 27)

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Canine adenovirus type 1 (CAV-1) causes infectious canine hepatitis (ICH) in canids, including free-ranging red foxes (*Vulpes vulpes*). We have investigated outbreaks of ICH among red foxes in wildlife hospitals in Scotland and have defined the clinical features and pathology of these cases. Furthermore, we have examined the comparative pathology of ICH in dogs and foxes by immunohistochemistry using markers for viral proteins, apoptosis and proliferation. A molecular and serological survey was conducted to investigate whether red foxes are a significant wildlife reservoir of CAV-1 in the UK. The findings indicate that the seroprevalence of CAV-1 among foxes is higher than has previously been reported in Europe, and that a number of foxes without evidence of clinical disease due to ICH are also infected with CAV-1 in a range of tissues. Some infected animals also shed CAV-1 in urine. These animals are a potential source of infection for conspecifics and other susceptible species, including domestic dogs. Post mortem examination revealed that none of the infected red foxes had gross or histological evidence of ICH. It is hypothesised that a mechanism of host evasion/viral persistence exists in CAV-1 infections, allowing the virus to persist in host tissues for an extended period of time.

**Molecular detection of hemoprotozoa agents in wild Orinoco goose (*Neochen jubata*) in Brazil** (Poster no. 54)

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Arthropod-borne hemoprotozoa parasites are incriminated as causative agents of negative effects on host fitness, including reproductive success and immune responses in avian species. Recently, the role of migratory birds in the dispersion of vector-borne pathogens has been investigated, as reservoirs or carriers. In this context, the present research aimed to verify the occurrence of hemoprotozoa agents (*Haemoproteus* sp., *Plasmodium* sp. and *Babesia* sp.) in wild Orinoco goose (*Neochen jubata*) captured in the Araguaia River Valley Region, Goiás, Brazil. Sixty-two blood samples were collected by puncture of ulnar vein, in the years 2010 (21 birds), 2013 (20 birds) and 2014 (21 birds). Blood samples were submitted to DNA extraction using a commercial kit and nested PCR assays targeting cytochrome B gene of haemosporidia (*Haemoproteus* sp., *Plasmodium* sp., and *Leucocytozoon* sp) and 18S rRNA of *Babesia* sp. following previously described protocols. Purified amplicons were submitted to sequencing and phylogenetic analysis. Twenty-five (25.8 %) out of 62 geese were positive for *Haemoproteus* sp., and six (6.45 %) for *Plasmodium* sp. Not one of the samples was positive for *Leucocytozoon* spp. Six (9.6 %) geese were positive for *Babesia* spp. Phylogenetic analysis based on cytochrome b gene positioned the sequences obtained closely to *H. macrovacuolatus* from South America and others *Haemoproteus* sp. detected in birds, and *Plasmodium* sp. detected in Brazilian birds. Based on the 18S rRNA gene, the *Babesia* sp. sequences obtained were positioned closely to *Babesia vogeli* detected in dogs from Brazil and South America. The present study showed that Orinoco geese are exposed to infection by hemoprotozoa agents. This is the first molecular detection of *Babesia vogeli* in birds.

**Serological evidence of exposure to *Toxoplasma gondii* and *Neospora caninum* in wild Orinoco goose (*Neochen jubata*) in Brazil** (Poster no. 52)

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*Toxoplasma gondii* and *Neospora caninum* are apicomplexan intracellular protozoa that affects several animal species, including humans. While felines act as definitive hosts for *T. gondii*, canids are responsible for oocysts shedding of *N. caninum* in the environment. The present work aimed at investigating the occurrence of IgG antibodies to *T. gondii* and *N. caninum* in wild Orinoco goose (*Neochen jubata*), by Indirect Fluorescent Antibody Test (IFAT). Between 2010 and 2014, 62 geese were captured and sampled in Araguaia River, state of Goiás, central-western Brazil. NC-1 *N. caninum* and RH *T. gondii* strains tachyzoites were used as antigens in IFAT assays. Twenty (32.3 %) out of 62 sampled geese were seropositive to *T. gondii*, showing titres ranging from 40 to 640. On the other hand, only 6 (9.7 %) were seropositive to *N. caninum*, showing titres of 20 in IFAT. Four (6.5 %) animals were seropositive to both *T. gondii* and *N. caninum*. In conclusion, wild Orinoco goose seems to be more exposed to *T. gondii* than to *N. caninum*.

## **Idiopathic facial lesions in wolverines (*Gulo gulo*) from Norway and Finland**

(Poster no. 74)

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Three wolverines shot in Norway since 2008 and one filmed in Eastern Finland in 2014 had similar lesions: All four individuals seem to have lost the soft, hairless part of their nose and much of their lips, exposing their nasal conchae and cavity, as well as and their teeth, and giving them a grotesque appearance. The lesions seem to be bilateral symmetrical. Necropsy has been performed on only one of the individuals. In this, the lesions were old and mostly healed. Genetic analysis showed that two of the Norwegian wolverines were closely related, while the third individual was not. The three Norwegian wolverines were shot geographically close to each other, suggesting that they may have been exposed to similar environmental factors, but the Finnish observation disturbs this line of thought.

Wolverines often fight with each other and other animals, and they do live a hard life where they are exposed to physical trauma and harsh and cold weather. However, the bilateral symmetrical nature of these lesions and the similarity between animals, suggest that these lesions are caused by an intrinsic factor. We speculate if it can be (a) some kind of autoimmune disease causing a photosensitive nasal dermatitis or a photosensitisation dermatitis caused by (b) a kind of porphyria or (c) intake of large amounts of photodynamic phylloerythrin from sheep that have died of bog asphodel (*Narthecium ossifragum*) poisoning.

***Leishmania infantum* in wild lagomorphs: an epidemiological survey in Italy**  
(Poster no. 50)

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*Leishmania infantum* is the etiological agent of zoonotic visceral leishmaniasis (ZVL). In Northern Italy the epidemiology of ZVL has experienced profound changes in the last decades as far as territorial expansion and involvement of different animal species.

We tested by PCR, the spleen of 222 lagomorphs (n = 10 wild rabbits *Oryctolagus cuniculus*, n = 108 hare *Lepus europaeus* and n = 104 Eastern cottontails *Sylvilagus floridanus*). The animals were culled during regular hunting activities between 2008 and 2014 and come from 10 different regions of Italy. A specific PCR protocol was used to amplify a 145bp fragment of the kinetoplastid DNA of *L. infantum*. All positive amplicons were sequenced to confirm species identification.

A total of 51 animals tested positive by PCR with an overall prevalence (P) of infection of 22.97 % (CI 95 % 17.93 - 28.94). The highest prevalence was recorded in rabbits: P = 30 % (CI95 % 10.78 % - 60.32 %) followed by *S. floridanus* with 28 of 104 animals infected and a prevalence of 26.92 % (CI95 % 19.33 % - 36.16 %). The lowest prevalence was recorded in brown hare (P = 18.52 %; CI95 % 12.32 % - 26.88 %) with 20 infected animals out of 108.

Despite not being statistically significant, the higher prevalence recorded in rabbits and cottontails can be attributed to the more limited home-range of these two species compared to *L. europaeus* which can favor the spread of infection as it better copes with the behavioural attitude of phlebotomine sandflies vectors.

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