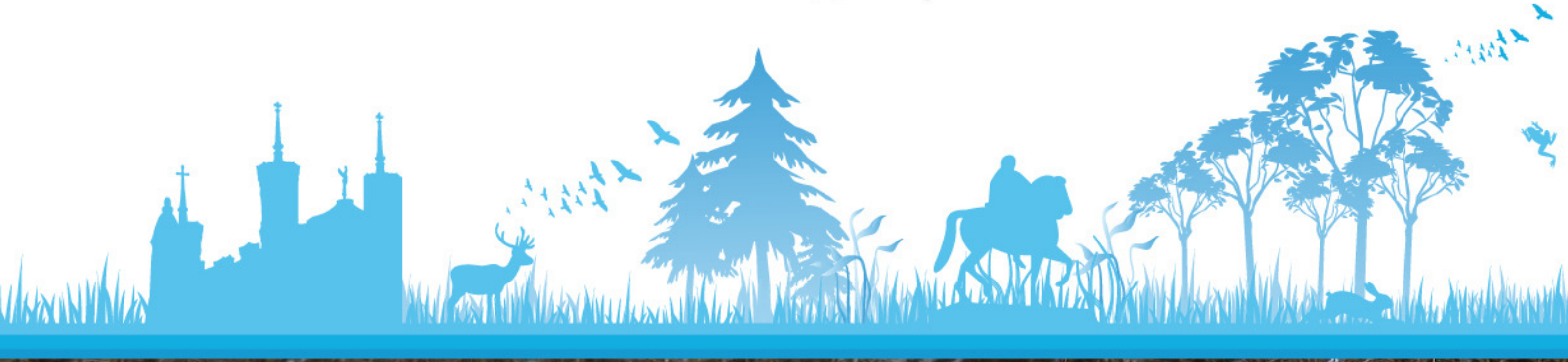

Joint 61st WDA/10th Biennial EWDA CONFERENCE

"convergence in wildlife health"

Lyon 23 - 27 July 2012

Proceedings

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Conference Overview

Welcome Address

Dr. Bernard VALLAT, Directeur général de l'Organisation Mondiale de la Santé Animale (OIE), 12 rue de Prony, 75017 Paris

The OIE welcomes participants to the “Convergence in wildlife health” Conference and is pleased to have been a partner for this successful event, alongside VetAgro Sup, the WDA and the EWDA.

Movements of animals and people enable pathogens to travel faster than the incubation period of the epizootic diseases they cause and the health risks for humans, domestic animals and wildlife are rapidly evolving. Growth of the human population, climate change and increased land use are all factors that must be urgently taken into account to safeguard biodiversity on all continents.

The Veterinary Services and veterinary teaching establishments must strengthen their capacities in the field of wildlife conservation and health management. New tools and new forms of collaboration and synergies need to be established between these services, wildlife specialists and users of the natural environment, which will in future provide valuable assistance in this field.

The OIE congratulates the organisers for having succeeded in bringing experts from all continents to Lyons to share their knowledge and help to provide a better response to the challenges posed by interactions between humans, wildlife and domestic animals. The OIE sincerely hopes that this conference will be a resounding success.

L'OIE souhaite la bienvenue aux participants de la conférence "convergence in wildlife health" et se félicite d'avoir été le partenaire de cet événement réussi, aux côtés de VetAgro Sup, de la WDA et de l'EWDA.

Les mouvements d’animaux et de personnes permettent aux pathogènes de se déplacer plus vite que la durée d’incubation des épizooties et les risques sanitaires pour l’’homme, les animaux domestiques et les animaux sauvages évoluent rapidement. L’augmentation de la population humaine, le changement climatique et l’utilisation accrue de terres sont de facteurs à prendre en compte en toute urgence afin de sauvegarder la biodiversité sur tous les continents.

Les Services vétérinaires et les établissements d’enseignement vétérinaire doivent renforcer leurs capacités dans la conservation et la gestion sanitaire de la faune sauvage. De nouveaux outils et de nouvelles formes de collaboration et de synergies sont à établir entre ces services, les spécialistes de la faune et les usagers de la nature qui constitueront à l’avenir une aide précieuse dans ce domaine.

L’OIE félicite les organisateurs d’avoir réussi à réunir à Lyon les experts de tous les continents, afin de partager leur savoir et de contribuer à mieux répondre aux défis posés par l’interaction des hommes, de la faune sauvage et des animaux domestiques. L’OIE forme ses vœux sincères pour que cette conférence soit un grand succès.

Dr Dolores Gavier-Widén, DVM, MSc, Associated Professor

President of the Wildlife Disease Association

The WDA is an international and multidisciplinary organization. All members of the WDA form a unique body of excellence in global wildlife disease and expertise in a vast range of ecosystems, hosts and aspects of health and disease of wildlife, involving field work to state-of-the-art technologies, and this is well represented in this conference. The WDA has held conferences annually since its foundation in 1952.

We are pleased to hold our 61st international conference in Lyon jointly with our European section, EWDA, the OIE and VetAgroSup.

On behalf of WDA, welcome to this conference, entitled “Convergence in wildlife health”.

Dr. Thijs Kuiken, DVM, PhD, DACVP, Professor of Comparative Pathology, Erasmus University Medical Centre, Rotterdam, The Netherlands

Chair, Scientific Committee of the 2012 Joint WDA EWDA Conference

Chair, European Wildlife Disease Association

Welcome to Lyon, where the confluence of the rivers Saone and Rhône mirrors the conference theme: convergence in wildlife health. The EWDA is proud to host this conference together with the WDA for more than 500 participants from all over the world. In this way, we jointly contribute to the much needed increase in knowledge on wildlife health, both in Europe and elsewhere.

Dr Stéphane MARTINOT, Directeur Général / Dean, VetAgro Sup, Marcy L'Etoile (France)

Dear colleagues, it is a great pleasure for our establishement, first veterinary school ever created in the world long time ago, to host the WDA international conference. During those days you will be delighted by a strong scientific program and a busy social program. Both of them will give you the opportunity to increase your knowledge and taste the French hospitality. Welcome in Lyon.

Conference Program

The Workshops will be held at VetAgro Sup, Veterinary Campus

The WDA meeting will be held at the Amphitheatre Merieux, ENS (downtown Lyon)

Sunday July 22nd

08:00-12:30	Business meetings (JWD editorial board) at the Novotel, Gerland
12:30-13:30	Lunch
13:30-18:00	Business meetings (WDA council, other) at the Novotel, Gerland

Monday July 23rd

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12:00-13:30	Lunch
13:30-16:00	Workshops
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11:30-11:45	[64] Developmental effects of field maternal transfer of lead on mallard ducklings	Rafael Mateo	80
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19:00	Banquet and Awards Ceremony Lyon City Hall / Salons de l'Hotel de Ville de Lyon		

Abstracts of key notes lectures and oral presentations

[1] ONE HEALTH

Aaron Bernstein, MD MPH
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For many years One Health, and other similar ideas, have been proffered as intuitive: that the health of all living things must be to some degree connected, and that by extension, strain upon one corner of the biosphere may strain another. The evidence bearing upon this postulate grows day by day and especially the fraction pertaining to human health. At every scale - from the sea of little known microbes that swarm over every nook of our bodies to the charismatic megafauna we know far better - science continues to delineate how our species fits into the tapestry of life. In many instances, as humanity pushes upon that tapestry, it rebounds and makes clear that having evolved within the web of life, we remain embedded in it. While the history of life is fraught with upheavals, the present is a time of unprecedented transformation, even in the brief context of human existence. This lecture will present several recent discoveries that demonstrate the interconnectedness of life and that given how little we know, we must be far more wise when it comes to doing business with the biosphere. To affect change, the consequences of biodiversity loss must be made personal, concrete, and, most importantly, reparable.

[2] HEALTH RISKS ASSOCIATED WITH THE TRADE OF PET MONKEYS IN PERU

A. Patricia Mendoza, DVM [1]*; Marieke H Rosenbaum, BSc [2]; Bruno Ghersi, DVM [3]; Nancy Cavero, BSc [1]; Yohani Ibañez, BSc [1]; Micaela De La Puente, BSc [1]; Miguel Sebastian [1]; Alberto Perez, DVM, MSC [1]; Jorge Nuñez, MSC [3]; Matthew Kasper, PHD [3]; William M. Switzer, PHD [4]; Joe Zunt, MD, MPH [5]; Joel Montgomery, PHD [4]; Marcela Uhart, DVM[1]

1 Wildlife Conservation Society, Lima, PERU
2 Cummings School of Veterinary Medicine at Tufts University, Grafton MA
3 Naval Medical Research Unit Six, Lima, PERU
4 Centers for Disease Control and Prevention, Atlanta, USA
5 University of Washington, Seattle, WA
**pmendoza@wcs.org*

New World primates (NWP) are the most common mammalian species involved in the wildlife trade in South America. In Peru, monkeys are commonly kept as pets, and repeated confiscations from the wildlife trade have led to over-crowding in zoos and rehabilitation facilities. As such, a rich human - NWP interface exists, that has not yet been systematically studied. Close contact among monkeys and humans may facilitate bidirectional pathogen transmission. Subsequently, releasing infected individuals back into the wild may negatively impact on wild populations. To determine the extent of pathogens harbored by NWP, we sampled 437 monkeys from 18 species at households, wetmarkets and zoos. Enteropathogenic bacteria were isolated from 45/249 (18%) fecal swabs (*Campylobacter* sp. = 10%; *Aeromonas* sp. = 7%). Gastrointestinal helminths or protozoa were found in 75/98 (76%) stool samples, with *Blastocystis* sp. (39%), *Entamoeba* sp. (35%), *Cryptosporidium* sp. (26%) and hookworms (33%) among the most frequent agents. Antibodies to Simian Foamy virus were detected in 60/157 (38.2%) serum samples, whereas Herpesvirus DNA was found in 5/78 (6.5%) blood samples. Oral and rectal swabs (n=52) were negative for Influenza virus. This study confirms the presence of zoonotic pathogens in traded monkeys in Peru. Enteric agents detected are also a potential disease source for other wild and domestic animals. Keeping monkeys as pets, or releasing them back into the wild, pose significant health risks to people and animals, and therefore should be strongly discouraged and controlled.

[3] ONE HEALTH SOLUTIONS TO MASS GLOBAL DECLINE AND EXTINCTION OF SPECIES: THE EXAMPLE OF THE INVESTIGATION OF CHYTRIDIOMYCOSIS

Lee F. Skerratt and Lee Berger
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A vital step towards mitigating the enigmatic decline of several hundred species of amphibians was determining its cause. The investigation progressed significantly when a “One Health” approach was adopted approximately 14 years after initial amphibian declines by herpetologist Keith McDonald. This then led to analysis of decline data by an epidemiologist and a tentative diagnosis of a novel pathogen spreading into naïve amphibian populations (Laurance et al 1996). A key factor in progress was incorporating prior knowledge of disease gained from studies of disease in animals including humans by epidemiologist Rick Speare. Recruiting a veterinary PhD student, Lee Berger, to conduct a pathological investigation of the outbreak at Big Tableland, Queensland, Australia, led to the discovery of chytridiomycosis and its impact on amphibians (Berger et al 1998). Additional epidemiological investigations revealed that the spread of chytridiomycosis is the worst disease affecting vertebrate biodiversity and has caused the severe decline of up to 200 species of amphibians globally with many of these now believed to be extinct (Skerratt et al 2007). Important determinants of the disease’s impact such as environmental preferences have been determined (Murray et al 2009, 2011). These findings are now being used to mitigate declines. This 30 year case study has shown the advantages of adopting a “One Health” framework for investigation of wildlife declines (Skerratt et al 2009).

[4] DYNAMICS OF THE TULAREMIA – WILDLIFE – HUMAN SYSTEM IN HUNGARY, CENTRAL EUROPE

Miklós Gyuranecz[1,7], Jenő Reiczigel[2], Katalin Krisztalovics[3], László Monse[4], Gabriella Kükedi Szabóné[5], Andrásné Szilágyi[3], Bálint Szépe[6], László Makrai[2], Tibor Magyar[1], and Károly Erdélyi[5]

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Francisella tularensis is a highly infectious zoonotic agent, but the epizootiology of tularemia is only partially understood. The aim of the current study was to analyze the dynamics of the tularemia – wildlife – human system. The study area included 3 counties in Hungary and the analyzed data (Spearman's rank correlation) represented 25 years. A 2-3 year cycling was characteristic for the analyzed data. The number of human tularemia cases showed significant correlation with the *F. tularensis* specific seroprevalence of European brown hares and with the population density of common voles. A significant negative correlation was observed between seroprevalence and population density of hares. Significant correlation was found between the cumulative precipitation between May to July and the number of human tularemia cases in 2 of the 3 counties. It is hypothesized that hares and ticks are the reservoirs during inter-epizootic periods, but during the cyclic peaks of high vole population densities; aggression, cannibalism and contamination of the environment through body discharges facilitate *F. tularensis* intra- and interspecific transmission including spillover to hares, eventually expanding local outbreaks to epizootic proportions. It is suspected that higher precipitation in summer effects increased tick activity and *F. tularensis* transmission. Finally it can be concluded that higher numbers of infection sources in the environment result in elevated numbers of human cases.

[5] WHITE STORKS (*CICONIA CICONIA*) AND RUBBISH DUMPS, THE GOOD THE BAD AND THE UGLY: FOOD, POLLUTANTS, AND PATHOGENS

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The white stork is a colonial species that has increased its population in Spain considerably due to the availability of rubbish dumps as continuous reliable food source. We studied four colonies of white storks, situated at (1) a functioning rubbish dump (2) a closured rubbish dump (3) a sheep farm (4) in a National Park. Faecal samples of 70 storks were collected at the nest, and body condition, blood samples and cloacal swabs were obtained from 103 nestlings at ringing. Nestlings from colonies at rubbish dumps had a significantly better body condition than nestlings from more natural colonies. In contrast methaemoglobulin concentration in the blood of nestlings from natural colonies was significantly lower in natural populations indicating exposure to substances causing oxidative stress at rubbish dumps. *Escherichia coli* isolated from white storks from rubbish dump colonies had a higher prevalence of virulence genes common to avian pathogenic *E. coli* (APEC). Resistance to the antimicrobials enrofloxacin, gentamicin and cefotaxim was significantly higher in the functioning rubbish dump (91.7, 81.3 and 87.1% respectively), than in the other colonies and 70% of the isolates showed resistance against all three antimicrobials, while multiresistance to antimicrobials was absent in *E. coli* isolates from storks from the National Park. The results highlight the risk of exposure of white storks to bacterial strains carrying resistance genes, when exposed to human residues.

[6] PREVALENCE OF *TRYPANOSOMA CRUZI* AND PATHOLOGICAL ALTERATIONS ON HUMANS AND ON RESERVOIR HOSTS FROM WESTERN MEXICO

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Deforestation in western Mexico has forced movement of triatomines and wild reservoir hosts of *Trypanosoma cruzi* to human dwellings. To estimate the impact on human and domestic animal health it was carried out search for *T. cruzi* in triatomines, humans and dogs and sylvatic animals an area of western Mexicio. Human, domestic dogs, opossums and rats were serologically tested for *T. cruzi* as well as for anatomic/pathological alterations. Infection rate for the only triatomine species (*Meccus longipennis*) was 72.95% (n = 122). Eight (3.92%, n = 204) humans, seven (5.3%, n = 131) dogs, 11 opossums (*Didelphis marsupialis*) (22%, n = 50) and 25 rats (*Rattus rattus*) (34.4%, n = 73) were positive to the presence of *T. cruzi*. One woman and two dogs showed some abnormalities on their hearts. Eight (66.66%) opossums and fifteen (64.00%) rats had some anatomic/pathological alterations (mostly splenomegaly). High infection rate on triatomines was incompatible with those detected on humans and dogs. Apparently, even when many triatomines are infected by *T. cruzi*, their peridomestic habits reduces vector-reservoir host contact, leading to have few infected humans and dogs. On the other hand, wild animals seem to be more affected by *T. cruzi* infections. Special attention has to be put on sylvatic animals, since they usually invade human dwellings, potentially carrying different *T. cruzi* strains, which could represent a higher risk of disease to human populations in the study area.

[7] SURVEILLANCE FOR AVIAN INFLUENZA IN WILD BIRDS THROUGHOUT THE UNITED STATES OF AMERICA, 2006-2011

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From 1 April 2006 through 31 March 2011, the United States Department of Agriculture, and state and tribal cooperators collected and tested 283,523 wild bird samples for avian influenza. Of those samples, 30.4%, 21.1%, 21.3%, and 27.2% were collected from the Atlantic, Pacific, Central, and Mississippi flyways, respectively. Samples were obtained from hunter-harvested birds (68.3%), live-bird sampling (30.0%), morbidity/mortality investigations (1.4%), and sentinel birds (0.3%). More than 265 wild bird species in all 50 states were sampled, but the majority (80.2%) was from dabbling ducks, geese, and swans. The apparent prevalence of low pathogenic avian influenza viruses (LPAIV) by matrix rRT-PCR was 9.7%, 10.9%, 12.4% and 15.5%, during biological years 2007-2010, respectively. Prevalence was lowest in the Atlantic Flyway during 2007 (22.7%), 2009 (18.7%), and 2010 (11.6%); during 2008, it was lowest in the Central Flyway (23.9%). Prevalence was highest in the Pacific Flyway during 2007 (27.8%), but during 2008-2010 it was highest in the Mississippi Flyway (27.0%, 35.2%, and 38.3%, respectively). Prevalence in waterfowl species was consistently highest in dabbling ducks followed by diving ducks, geese, and swans. The apparent prevalence of H5 and H7 subtypes across all species sampled were 0.7% and 0.1%, respectively. These data provide insights on the spatial and temporal distribution of LPAIV throughout the United States, and are being used to further knowledge on the ecology of LPAIV in wild birds.

[8] ACHIEVING COHERENCE IN REGULATORY RESPONSES TO AVIAN INFLUENZA

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Recognized links between infectious diseases in humans, animals and wildlife have led to increasing calls, both nationally and internationally, to reduce human vulnerability to infectious diseases by better integrating infectious disease control policies. The goals of this project were to investigate the extent to which the existing regulatory responses for infectious disease control in humans, animals, and wildlife are integrated and to examine ways to more closely align the legal and policy framework for infectious disease control with best practices in wildlife health. Using Avian Influenza as a case study and comparing multiple jurisdictions, the study found that government responses to avian influenza specifically, and infectious diseases generally, are heavily biased to the human impacts of infectious disease. Consequently, the key role of wildlife health in infectious disease emergence is neglected even though wildlife's role in emerging infectious diseases of concern to human populations is widely acknowledged. Similarly, a critical analysis of the significant role animal production in agriculture plays in infectious disease emergence is lacking. The study concludes that regulatory responses aimed at minimizing human impacts of Avian Influenza, including the economic loss associated with an animal health incident, often increase wildlife's vulnerability to infectious diseases and thus contribute to infectious disease emergence.

[9] CLINICAL DENGUE VIRUS INFECTIONS IN ORANGUTANS

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Dengue viruses (DENV) are considered to have an asymptomatic sylvatic cycle in wild non-human primates and *Aedes* mosquitos. In humans, DENV infection can cause dengue fever, which may progress to fatal haemorrhagic shock syndrome (HSS).Wild orangutans have previously been documented with IgG antibodies against DENV, but not with clinical disease. Habitat destruction is the main threat for wild orangutans, and more than 600 are housed at the Nyaru Menteng reintroduction centre, in preparation for their release. The aims of this study were to document preliminary clinical and haematological data of 30 captive orangutans which tested positive in a commercial rapid test for DENV-specific IgM antibodies. The most common clinical signs of DENV infected orangutans were fever (> 38.5^{0}C) and lethargy. Mean haematological parameters showed lymphopenia, monocytosis, and anemia, but not thrombocytopenia. Twenty-one of these animals also harboured a *Plasmodium* infection, which exacerbated clinical signs, and included thrombocytopenia, although no clinical signs associated with HSS were seen. All 30 animals recovered following symptomatic treatment and, where indicated, with antimalarial drugs. Seven animals were treated for suspected secondary bacterial infections. Our results show that DENV infections do cause disease in naturally infected orangutans, exacerbated by co-infection with *Plasmodium spp*. Research to elucidate the DENV serotype(s), DENV ecology, and *Plasmodium spp* is ongoing.

[10] WEST NILE VIRUS AND WILD BIRDS DURING A MAJOR HUMAN DISEASE OUTBREAK, GREECE 2010-2011

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During 2010 and 2011 a major outbreak of human West Nile virus (WNV) infections occurred in Greece, with more than 350 laboratory-confirmed cases and 40 deaths. Our team conducted a serological and molecular surveillance on birds hunter-harvested prior during and after the epidemic period from the related areas. Serum and tissue samples from almost 300 birds, representing 2 resident avian species (*Corvus corvix, Pica pica*) were collected in mainland Greece during the hunting seasons of 2009-10, 2010-11 and 2011-2012. Almost 18% of the samples were positive for the presence of WNV IgG antibodies by IFA and could be confirmed by virus microneutralization test. Resident corvids hunter-harvested to the epicenter of the outbreak have been exposed to WNV at least eight months before the first human cases were reported. WNV lineage 2 was identified in a hunter-harvested Eurasian magpie, phylogenetically related to Austrian and Hungarian strains isolated in previous years from birds of prey, suggesting a southwards virus spread, possibly via birds' migration routes. Detected genetic determinants of virulence (E protein glycosylation, NS3 H249P) support the wild birds' potential role in the outbreak. A spatial analysis of the distribution of human cases and seropositive avian samples was also performed. A statistically significant correlation was observed between the place of stay of human patients and the dispersal range of seropositive hunter-harvested corvid samples.

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[11] CLIMATE CHANGE AND THE SPATIAL POPULATION DYNAMICS OF TICKS AT THEIR EXPANDING NORTHERN RANGE LIMIT

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Over the past two decades *Ixodes scapularis*, the main tick vector of Lyme disease in North America, has expanded its range northward from the USA to colonize new regions in southern Canada. This expansion has occurred during a period of climate warming; however, the degree to which climate change versus other processes such as host migration affect tick spread remains poorly understood. Our goal was to investigate the empirical relationship between climate, tick population growth, and resulting human exposure to Lyme disease risk in Canada. We fit Bayesian state-space models to passive surveillance data to model *I. scapularis* population growth in Quebec province from 1991-2008. We used competing models to evaluate the influence of temperature in the past three years and host dispersal of ticks on population growth rate. Both temperature and bird-mediated dispersal had positive effects on growth rate, with population increase occurring only above approx. 2250 cumulative annual degree days > 0℃. Prevalence of *Borrelia burgdorferi* in adult ticks was negatively related to tick density, supporting a lower risk of infection in nascent tick populations prior to *B. burgdorferi* establishment. Climate projections suggest that tick populations will increase exponentially in southern Canada in the coming decade within an increasing envelope of temperature suitability, resulting in new foci of disease risk where high densities of human and vector populations coincide.

[12] CORRELATION ON *YERSINIA PESTIS* CIRCULATION IN RODENT AND DOG: DOG SEROLOGY USED IN PLAGUE SURVEILLANCE IN MADAGASCAR

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Plague, a deadly infectious disease caused by *Yersinia pestis*, remains a public health problem in Madagascar with about thirty districts endemic for the disease. Seroprevalence among humans and rodents are not necessarily representative of *Y. pestis* transmission. In the years 80s, pet serology was used during plague investigation in many countries. This study aims at comparing the circulation of *Y. pestis* in rodents and dogs during plague transmission periods. Plague transmission parameters was determined for rodent and seroprevalence for both species. During epizootic period, dogs prevalence correlates with rodents' (r=0.887). There was relationship on dog exposure to *Y. pestis* during quiescent and epizootic periods ($\chi2 = 5.44$, p=0.020). Seroconversion negative-positive, was observed among surveyed dogs from endemic areas, indicating plague circulation in these foci. To our knowledge, it is the first comparison of *Y. pestis* circulation between rodents and dogs in natural settings. Dog serology survey gave evidence of human plague and was highlight plague risk in inactive focus.

[13] WILDLIFE-LIVESTOCK INTERACTIONS IN A WESTERN RANGELAND SETTING: QUANTIFYING DISEASE-RELEVANT CONTACT RATES

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Rangeland livestock in the western U.S. interact with abundant free-ranging wildlife populations (e.g. elk, deer and antelope), which may complicate the prevention and management of a foreign animal disease incursion in the intermountain West region of the U.S. The frequencies of livestock-wildlife interactions are poorly understood. This study estimates inter-specific contact rates using the largest-known dataset on cattle, elk, and deer locations, from the Starkey Project in eastern Oregon, USA. The Starkey Project is a 10,000-hectare fenced enclosure containing 500 cow-calf pairs, 200 elk, and 200 mule deer. Each year, roughly 41 cattle, 43 elk, and 21 deer are collared, and their locations are recorded every 30-60 minutes. Between 1993 and 2007, 849,443 spatial locations were collected for cattle; 1,427,707 for elk; and 489,118 for deer. We calculated spatial and temporal distances between all observed pairs of animal locations, as well as the proportion of pairwise observations occurring within a specified distance and time from each other. Of the 130-million pairs of animal locations separated in time by six hours or less, 2.2% of intraspecies pairs, and 0.04% of interspecies pairs involved animals located less than 100 meters apart. Contact rates between cattle, elk, and deer are relatively low, due perhaps to inter-specific avoidance. However, these rare events may be extremely important in the propagation of disease at the wildlife-livestock interface.

[14] ZOONOTICALLY IMPORTANT BACTERIAL DISEASES AMONG SPOTTED DEER (*AXIS AXIS*) IN WEST BENGAL, INDIA

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A survey study was conducted to study the incidence of zoonotically significant bacterial diseases in spotted deer in different national parks, sanctuaries and deer parks of West Bengal, India. The wild life survey was carried out in different National Parks, sanctuaries and deer parks in which information on the causes of mortality in spotted deer was obtained based on clinical and post-mortem examinations along with bacterial isolation studies from three National parks (Buxa, Gorumara and Sudarban), six wild life sanctuaries (Mahananda, Jaldapara, Bethuadahari, Bibhutibhushan, Ballavpur and Ramnabagan) and five deer parks (Kumari Kangshabati, Adina, Gorchumuk, Jaipur and Jhargram) respectively. In tuberculosis, mortality rates in spotted deer were 4.69% in Buxa National park, 6.33% in Gorumara National park, 0.33% in Sundarban National park, 8.64% in Mahananda Wild life sanctuary, 4.54% in Jaldapara Wild life sanctuary, 13.04% in Bethuadahari Wild life sanctuary, 8.60% in Bibhutibhusan Wild life sanctuary, 6.32% in Ballavpur Wild life sanctuary and 4.44% in Ramnabagan Wild life sanctuary, 6.38% in Kumari Kangshabati Deer park, 9.75% in Adina Deer park, 13.09% in Gorchumuk Deer park and 9.09% in Jhargram Deer park. The overall mortality rate in spotted deer due to tuberculosis was recorded to be higher in deer parks than sanctuaries and National parks. Deaths due to anthrax were recorded from Buxa National park (3.12%), Gorumara National park (3.79%), Sundarban National park (0.06%), Bethuadahari Wild life sanctuary (2.90%), Bibhutibhusan Wild life sanctuary (0.45%), Kumari Kangshabati Deer park (2.12%), Gorchumuk Deer park (4.34%) and Jhargram Deer park (3.63%) during the study period. The overall study revealed that the incidence and mortality caused due to diseases were more in Deer parks than in National parks and Wild life Sanctuaries.

[15] ECO-EPIDEMIOLOGY OF *CRYPTOSPORIDIUM* TRANSMISSION IN RELATION TO CHIMPANZEE HEALTH IN THE GREATER GOMBE ECOSYSTEM, TANZANIA

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Although responsible for numerous outbreaks of diarrheal disease in humans and domestic animals, how *Cryptosporidium* affects wildlife remains largely unknown. In light of this, we initiated a comprehensive assessment of the potential for transmission of *Cryptosporidium* from humans and domestic animals to chimpanzees and examined the effects of *Cryptosporidium* infection on chimpanzee health. We evaluated *Cryptosporidium* prevalence, determined sources of infection (via RFLP and sequence-based comparisons of *Cryptosporidium* recovered from chimpanzees, other wildlife, people, livestock, and watersheds), examined the frequency of illness associated with infection; and determined how specific social and ecological risk factors and/or concurrent infection with SIV affected illness. Results demonstrated an overall *Cryptosporidium* prevalence of eight percent with initial results suggesting no relationship between *Cryptosporidium* infection and SIV infection or illness. The two chimpanzee communities examined displayed distinct *Cryptosporidium* transmission pathways, with Mitumba (edge community) infected with *C. hominis*, a well-described human strain, and Kasakela (central community) infected with *C. suis*, a porcine variant presumably maintained in a sylvatic cycle. Interestingly, no chimpanzees were infected with *C. parvum* (a bovine variant) despite this species being detected frequently in goats and sheep sympatric to chimpanzee crop-raiding zones.

[16] LONGEVITY AND VIABILITY OF *BAYLISASCARIS PROCYONIS* EGGS IN SOIL

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Baylisascaris procyonis is a large zoonotic nematode that occurs in the small intestine of raccoons *Procyon lotor*), of which the larvae can cause visceral, ocular, and neural larva migrans within intermediate hosts. We investigated the likelihood of infected raccoons contaminating their environment with *B. procyonis*. Infected raccoon scats were placed in various climatic conditions and allowed to decay. Scats decayed in direct relation to rainfall. Complete decay required an equivalent of about 20 cm of rainfall for fresh scats and more for older, dryer scats. Upon decay, *B. procyonis* eggs contaminated the soil up to 49 cm from the scat. In addition, the quantity and viability of *B. procyonis* eggs were determined in 4 soil textures, 2 soil moistures, 2 soil canopies, and 4 soil depths up to 10 cm within 48 containerized plots during a 2 year period. *B. procyonis* eggs were collected from infected raccoons, and placed in 100 eggs/mL aliquots, and 1 aliquot was placed in the center of each 10 x 10 grid (100 grids) of each plot. Three randomly selected grids were sampled each month with a 2 x 10cm circumference soil tube; each soil sample was separated into 4 depths, and *B. procyonis* eggs were quantified under 100X magnification for each soil depth. Sandy soil texture and soil moisture increased permeability of *B. procyonis* eggs into the soil column. However, *B. procyonis* eggs did not exceed past 10 cm for any soil texture or moisture regime during the 2 year study. Eggs remained close to the soil surface in soil textures >60% clay. Egg viability was >93% for all soil textures, canopies, moistures, and depths. Infected raccoons can infect the environment with *B. procyonis* eggs, which remain near the soil surface and remain viable in the environment for at least 2 years.

[17] PANDEMIC H1N1 ISOLATED FROM NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*) OFF CENTRAL CALIFORNIA UPON RETURNING FROM THEIR ANNUAL MIGRATION TO THE BERING SEA, ALASKA

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Interspecies transmission of influenza A is an important factor in the evolution and ecology of influenza viruses. Marine mammals are in contact with a number of influenza reservoirs, including aquatic birds. Epizootics have been documented in harbour seals associated with H7N7 and H4N5 isolates, both of avian origin, and most recently with H3N8, also found in dogs, birds and horses; indicating that marine mammals may be important for transmission among avian and mammalian hosts. During surveillance off central California, two nasal swabs collected in April 2010 from free-ranging adult female Northern elephant seals (NES, n = 42) tested positive for influenza by RT-PCR. Virus was isolated and whole genome sequencing revealed that both corresponded to pandemic H1N1 (pH1N1) influenza that emerged in humans in 2009. The seals tested positive shortly after returning to the rookery following their migration, suggesting that exposure could have occurred at sea. Hemagglutination inhibition to detect influenza-specific antibodies showed samples collected early in 2010 were negative but by April animals began to test positive for antibodies to pH1N1, supporting molecular findings that pH1N1 transmission occurred among free-ranging NES. Viral replication and pathogenesis was indistinguishable from that induced by reference strains of pH1N1 in MDCK cells, mice and ferrets, however, was inefficient in human epithelial respiratory cells, thus indicating these may be NES adapted viruses.

[18] WIDESPREAD WILDLIFE HEALTH ASSESSMENT IN THE ARCTIC: THE CIRCUMARCTIC RANGIFER MONITORING AND ASSESSMENT MODEL

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Assessment and tracking of wildlife health in remote regions often includes a variety of logistical, financial, climatic and temporal obstacles. During International Polar Year, a team of wildlife users, managers, and researchers, did a collaborative, widespread assessment of the health of circumarctic wild migratory caribou and reindeer (*Rangifer tarandus* ssp.). We developed and implemented standardized protocols and synchronized sampling periods to facilitate cross-herd comparisons for a diverse array of health indicators. From 2007-09, we sampled more than 1000 animals from 17 herds resulting in a 'snapshot in time' for the health of *Rangifer* sp. in four circumpolar countries. Protocols for different levels of sampling intensity were refined to facilitate efficient, consistent, and scientifically robust sampling by subsistence hunters, wildlife technicians, and researchers. Concurrently, we compiled data on herd demographics, climate, and geography. Evaluation of these data, together with comparisons of diversity and abundance of pathogens within and across herds and seasons, has provided novel insights into evolution and ecology of pathogens, colonization events and spatial epidemiology. Further, the collaborative and inter-disciplinary approach has led to a more comprehensive assessment of population health. Implementation, efficacy, and outcomes of this broad-scale, multi-disciplinary, collaborative health assessment, will be discussed.

[19] IMPLICATIONS OF OUR INABILITY TO CONDUCT RISK ASSESSMENT FOR WILD SALMON HEALTH

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The Cohen Commission into the Disappearance of Fraser River Sockeye Salmon spent the last 2 years investigating reasons for declines in sockeye salmon (*Oncorhynchus nerka*) that lead to 3 consecutive years of fisheries closures. One hypothesized explanation for these declines was increased mortality and/or decreased fitness due to infectious and parasitic diseases from public or private aquaculture facilities. The author was retained by the Commission to assess the risks of public salmon enhancement facilities as sources of diseases that could have impacted wild fish. Risk assessment was not possible due to (i) the lack of knowledge of the role of disease in wild population regulation; (ii) the void of information on the probability of exposure of wild fish to pathogens derived from enhancement facilities and (ii) the lack of a shared conception of the meaning of the term health. A significant impediment to risk assessment was the historic reliance on pathogen focused definitions of health that fail to account for the social and ecological determinants that affect the fitness and abundance of wild species. The implications of these conclusions for fish and wildlife population health research and risk assessment will be discussed.

[20] WILDLIFE HEALTH SURVEILLANCE IN SWITZERLAND: CURRENT STRATEGIES AND EMERGING DISEASES

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The Centre for Fish and Wildlife Health is the Swiss national reference laboratory for wildlife health issues, with records dating back to the 1950s. Here we provide an overview of the current surveillance strategies and emerging pathogens detected in Swiss wildlife over the past decade. Our system combines: scanning surveillance (pathological examination of cases selected by field partners and of systematically collected carcasses of protected species), targeted surveillance (surveys for pathogens of wildlife, domestic animals or humans; questionnaire surveys) and interdisciplinary research. We routinely perform histopathology and ancillary tests, have developed a sample archive and regularly interact with field partners through teaching and presence in the fields. Some of the newly detected diseases and infectious agents in Swiss wildlife include babesiosis (*Babesia capreoli)*, coenurosis, paratuberculosis and various herpesviruses in ruminants; leptospirosis in beavers; *Mycoplasma hyopneumoniae* in wild boar; *Cytauxzoon* sp. in felids. *Brucella suis* in wild boar has increased in prevalence; sarcoptic mange and canine distemper have increased in geographic range, with a rising number of affected species. These records suggest that our methods efficiently detect previously unknown pathogens as well as changes in disease occurrence in wildlife. Regular and long-term contacts with field partners significantly contribute to their active participation in the surveillance.

[21] CAUSES OF MORTALITY AND DISEASES IN THE REINTRODUCED EUROPEAN BEAVER POPULATION IN SWITZERLAND FROM 1989 TO 2009

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The European beaver (*Castor fiber*) was successfully reintroduced to Switzerland between 1956-1977. We report the results of two decades of health surveillance in this reintroduced population which is now estimated to 1600 animals. Necropsy reports were available back to 1989. Carcass collection and investigations were intensified from 2007 to 2008, with systematic histological and coprological examinations. Overall, data on 246 dead beavers were available, including 193 necropsies. Causes of death were mostly non-infectious (80%) and due to traumas (traffic collisions, accidents in hydropower stations, poaching, dog attacks) or “death in the water”. Beavers found in the water typically presented lung lesions suggestive of the acute respiratory distress syndrome (ARDS) described in various animal species and humans. Non-infectious diseases further included a neoplasia and a ventricular septum defect. Fatal parasitic or bacterial diseases were diagnosed in 6% of the animals. Infections included echinococcosis (n=12) and yersiniosis (n=1). 40% of the investigated beavers harboured *Stichorchis subtriquetrus.* The ectoparasite *Platypsyllus castoris* was identified twice. Few beavers were weakly seropositive for *Francisella tularensis*. No diagnosis was achieved in 14% (autolysis, incomplete carcasses, unclear cases). This study suggests that Swiss beavers mainly die of anthropogenic causes. It also provides first data on the health of a free-ranging European beaver population.

[22] DIFFERENTIAL AGE- AND SEX-SPECIFIC RATES OF CHRONIC WASTING DISEASE INFECTION IN WISCONSIN WHITE-TAILED DEER

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Chronic wasting disease (CWD) is a fatal prion-induced infectious disease affecting cervid populations, particularly in North America. The prolonged course and low detectable mortality in infected hosts make CWD a covert, yet significant, threat to ecologically and economically important species. We developed multi-state matrix models to evaluate infection rates for CWD in white-tailed deer in south-central Wisconsin (WI). We used age- and sex-specific prevalence from deer harvested during 2002-2011 to evaluate alternative models and estimate model parameters. Our findings indicate strong support for frequency-dependent, compared to density-dependent, transmission with rates of infection in adult males about 2.5 times higher than in adult females, and 3.5 times higher than in yearlings and fawns. This pattern suggests differential exposure among individuals, possibly related to deer social structure. Analyses of CWD spatial distribution showed that infected hosts are aggregated in the landscape, altering the rate of contact between infected and susceptible hosts. We investigated the effects of spatial disease aggregation and differential infection rates among age and sex stages on CWD dynamics and impacts on deer populations. We discuss the influence of these factors on alternative management actions to maintain a sustainable, healthy deer population in WI.

[23] PROGRESS TOWARDS THE DEVELOPMENT OF A CHLAMYDIAL VACCINE FOR KOALAS

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Wild koala populations throughout Australia continue to experience serious declines in numbers, leading to localised extinctions. Recent modelling shows that targeting chlamydial disease, among the many variables adversely affecting koala survival, would have the greatest potential impact. We are developing a *Chlamydia pecorum* recombinant MOMP based vaccine for immunising captive as well as wild koalas. In our recent trial we utilised a recombinant MOMP protein, cloned from a *C.pecorum* koala isolate, mixed with the adjuvant ISCOMATRIX. We vaccinated two groups of koalas, (i) wild caught animals that were clinically healthy and *Chlamydia* PCR negative, (ii) wild caught animals that had clinical signs of disease. Following vaccination, there was no increase in inflammatory pathological changes in animals previously infected with *Chlamydia*. Strong antibody (including neutralizing antibodies) and lymphocyte proliferation responses were recorded in all vaccinated koalas, both healthy and clinically diseased. Our data shows that an experimental chlamydial vaccine is safe to use in previously infected koalas, in that it does not worsen infection-associated lesions. Furthermore, the prototype vaccine is effective, as demonstrated by strong levels of neutralizing antibody and lymphocyte proliferation responses in both healthy and clinically diseased koalas.

[24] INSIGHTS INTO THE INTERPLAY BETWEEN ENVIRONMENT, STRESS, HEALTH, AND POPULATION PERFORMANCE IN ALBERTA GRIZZLY BEARS

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Although the future of grizzly bears in Alberta, Canada, has been of concern for several decades, recent research indicates the Alberta population is small, fragmented, and possibly declining. While human-caused mortality is considered the primary causative factor, little attention has been given to how the health of individual bears might also influence population performance. In 2005, we initiated a study in Alberta to determine relationships between environment, grizzly bear health, and population performance (demographic rates) to inform and enable effective land-use strategies. We proposed that negative effects of human-caused environmental change on grizzly bear populations may arise as a consequence of long-term physiological stress in individual bears. Through the directed efforts of a multi-disciplined team that included physical geographers, wildlife ecologists, stress physiologists, and veterinarians, and the data amassed from 503 handlings of 322 bears, we confirmed linkages between environment, chronic stress, health, and population performance. Our findings also indicate an application for “chronic stress biomarkers” as conservation tools to provide early warning of potential health problems in individual bears before population performance is reduced. However, because this application can be confounded by long-term health effects of capture and handling, the greatest promise lies in biomarkers that can be measured in samples collected with minimal invasiveness.

[25] TRANSMISSION AND FITNESS CONSEQUENCES OF CIRCOVIRUS INFECTION IN WILD AUSTRALIAN PARROTS

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Beak and Feather Disease Virus (BFDV) is a circovirus that commonly infects parrots and cockatoos, and is currently causing significant conservation concern around the world. Infection can result in feather and beak deformities, along with strong immunosuppression. Mortality is high among captive birds infected by BFDV, particularly in young birds. However, to date little is known about the ecology and epidemiology of this infection in wild populations where it is naturally present. We used quantitative PCR to test wild, breeding crimson rosellas (*Platycercus elegans*) sampled over an eight year period for BFDV. We tested nestlings and measured their growth and mortality to investigate the transmission of BFDV and it's effects on reproductive success. We also examined whether infections are distributed non-randomly across breeding pairs. BFDV infection was detected in all of our study populations, and was more common in juvenile birds. We detected BFDV in eggs, suggesting vertical transmission, and in nestlings. We found that infected birds tended to be paired together, indicating that transmission among breeding pairs may occur or that the virus may play a role in mate choice. However, we found little evidence that nestling growth or survival was affected by infection. Parrots are among the most threatened group of birds worldwide, and further studies are indicated to determine the fitness consequences of infection by BFDV in natural populations.

[26] RABBIT HAEMORRHAGIC DISEASE AND MYXOMATOSIS IN AN ONGOING 16-YEAR STUDY OF AN AUSTRALIAN RABBIT POPULATION

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Rabbit haemorrhagic disease (RHD) emerged as Australia’s new biological control agent for the European rabbit (*Oryctolagus cuniculus*) in 1995, and in the following year a mark-recapture study commenced at Turretfield, near Adelaide, South Australia. In the subsequent 16 years over 3,300 rabbits have been captured, with morphological measurements and a blood sample collected before release. Blood samples are analysed for antibodies to RHDV and myxoma. Nine myxomatosis and 11 RHD outbreaks have occurred on the site during this period. PCR of carcass tissue and DNA sequencing has provided RHDV capsid protein phylogenetic data and documentation on the evolution, persistence and influence of the various RHDV strains. Since 2006 there has been a general recovery in rabbit numbers and RHD outbreaks have changed from largely biannual to annual with increased seroconversions and reduced mortality. RHD outbreaks are now earlier in the year and more prolonged than previously recorded on the site. These results offer valuable insights into virus evolution and the influence these diseases have on rabbit populations at other Australian and international locations.

[27] FOOD RESOURCES, PARASITE INFECTION AND POPULATION GROWTH IN FIELD VOLES (*MICROTUS AGRESTIS*)

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It has been proposed that the effects of parasites on wildlife are conditioned on food restriction. However, this has rarely been tested experimentally. Field voles (*Microtus agrestis*) harbour numerous helminth species, which may have adverse effects on their life history. Little is known about the association between these infections and individual body condition or population dynamics and to what extent food resources influences them. This study aims to test the hypothesis that low food resource availability reduces the health state of field voles, predisposing them to parasite infection, which in turn reduces their physiological condition even further. To test this, we carried out a field experiment in thirty-two, 20 × 25 m adjoining enclosures. Seven pairs of voles were introduced to each enclosure, with eight enclosures being assigned to each of four treatments: 1) food supplementation, 2) antiparasite treatment 3) food supplementation and antiparasite treatment and 4) control. Vole numbers were monitored by monthly live trapping, during which all individuals were sampled for blood and faeces to assess physiological condition, immune function and parasite load. Faecal floatations allowed comparison of burdens of *Heligmosomoide*, *Eimeria*, and Anoplocephalidae with the physiological condition and immune function of individuals and population densities in the enclosures. Results of the experiment will be discussed.

[28] GENETIC DIVERSITY OF TRYPANOSOMES IN WESTERN AUSTRALIAN MARSUPIALS AND THE POTENTIAL OF MIXED INFECTIONS AS A CONTRIBUTORY FACTOR IN THE POPULATION DECLINE OF BRUSH-TAILED BETTONG (*BETTONGIA PENICILLATA*)

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Trypanosomes are blood parasites commonly found infecting Australian marsupials. Despite studies having helped to better understand the ecology of Australian trypanosomes, their full diversity and health impact on Australian wildlife is poorly understood. We investigated the genetic variability, tissue distribution and pathogenicity of trypanosomes circulating in 9 species of Western Australian marsupials. 18SrDNA and gGAPDH loci were used to detect *Trypanosoma* spp. infection in 192 blood samples by PCR followed by sequencing. We also assessed the presence of trypanosomes in 183 tissues from 42 carcasses of sick-euthanized and road-kill marsupials. PCR results showed that all marsupials were positive for *Trypanosoma* spp. with a rate of infection of 52% in blood and 58% in tissue. Inferred phylogenetic trees revealed the presence of 7 genotypes that clustered in 3 clades: *T. copemani*, *T. gilleti* and *T. cruzi*. All trypanosomes exhibited a preferential tissue tropism for skeletal muscle, oesophagus and lung. Mixed infections were only found in tissues of the endangered marsupial, Brush-tailed Bettong; histology of these tissues showed marked multifocal neutrophilic exudation, myositis, and prominent skeletal muscle degeneration in the oesophageal skeletal musculature and heart. Our results demonstrate a lack of host specificity of Australian trypanosomes and suggest the interaction of different genotypes/species in mixed infections may play an important role in pathogenicity.

[29] ASSESSMENT OF OXALATE NEPHROSIS IN A POPULATION OF SOUTH AUSTRALIAN KOALAS (*PHASCOLARCTOS CINEREUS*)

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The major cause of morbidity in the Mt Lofty Ranges koala population near Adelaide, South Australia is kidney disease, and renal crystals consistent with calcium oxalate have been observed in some individuals. Oxalate nephrosis is not well reported in koalas and is uncommon in other koala populations in Australia. The present study aimed to assess the prevalence of oxalate nephrosis in this population, and determine whether age, sex or wild/captive origin were predisposing factors. In addition, renal function of affected koalas was compared with that of other populations (Kangaroo Island and Queensland). 51 koalas from the Mt Lofty Ranges population were examined at necropsy between March 2008 and October 2010. Of these, 55% showed gross and/or histological evidence of oxalate nephrosis. Koalas < 2 years old represented 62% of those affected, although no significant association between disease and age was found to occur. In addition, there were no differences found between sexes or wild/captive origin. Nearly all koalas with oxalate nephrosis showed azotaemia (elevated plasma creatinine and urea) and all individuals with concurrent urine samples showed a USG < 1.035, consistent with renal insufficiency. These findings show that oxalate nephrosis is a major disease of this koala population and should be considered in assessments of koala population health to facilitate management and conservation.

[30] RESPIRATORY DISEASE SURVEILLANCE IN GEOGRAPHICALLY REMOTE POPULATIONS OF RED SQUIRRELS (*SCIURUS VULGARIS*)

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Red squirrel populations in Britain have declined greatly in recent decades and are now fragmented. Published data on the health status of these populations is minimal. Necropsies, with histopathological and in selected cases, microbiological examinations were performed on 163 red squirrels from Isle of Wight, Jersey, NW England and Scotland; 40% were killed by road traffic or predators e.g. cats and dogs, but most were found dead in or near gardens. Significant pathological lesions, excluding any due to trauma, were seen in the lungs of 48 squirrels. Bacterial infections were predominant (17) and included coccal pneumonia (7), purulent bronchopneumonia (5, with 2 due to *Bordetella bronchiseptica*), and inhalation pneumonia (3). Fungal lesions included pleurisy due to *Mucor hiemalis*, necrotising pneumonia caused by an unidentified fungus and granulomata in a case of adiaspiromycosis. Five squirrels had pulmonary foci of metaplastic bone or cartridge. Toxoplasmosis cases (12) showed varying degrees of pneumonitis. Other notable conditions included chylothorax (1) and neoplasia, with single cases of pulmonary carcinoma, lymphoma and adenoma. *Hepatozoon* sp. schizonts were present in the lungs of 26% of squirrels overall but there was often no associated pathology. Conclusion: some red squirrel populations suffer significant mortality due to human activities (road traffic, pet ownership) but respiratory disease, mostly infectious in origin, is also a common cause of mortality.

[31] ASSESSING THE HEALTH STATUS OF RECIPIENT AND TRANSLOCATED POPULATIONS FOLLOWING TRANSLOCATION

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Translocation programmes give rise to risks of disease in the recipient (conspecific and sympatric) and translocated populations due to novel encounters between hosts and parasites and the effects of non-infectious disease agents such as toxins. However, diseased and dead free-living animals, particularly small and cryptic animals, are difficult to detect and may be quickly scavenged. Therefore pre- and post-release monitoring of population size in conjunction with assessment of health status provides information of value in determining the impact of disease on the affected populations. We assessed the health of translocated and recipient populations following eight translocation programmes in England, involving species of invertebrate, amphibian, bird and mammal, using clinical examinations, pathological examinations and measures of population size. For example in the pool frog (*Pelophylax lessonae*) translocation programme, clinical examinations were carried out on pool frog, common frog (*Rana temporaria*) and common toad (*Bufo bufo*) populations and population size was monitored through visual surveys and capture-mark-recapture. None of the diseases so far detected are predicted to affect the population numbers of anurans and population monitoring has revealed relatively stable populations of pool frogs, common frogs and common toads at the translocation site. Monitoring population dynamics may assist in the interpretation of post-translocation health assessment.

[32] SPREAD OF HPAIV H5N1 FROM SOUTH CHINA AGRO-ECOSYSTEMS THROUGH WILD BIRDS MIGRATION: A MULTIDISCIPLINARY APPROACH

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Agricultural intensification in South China has increased densities of domestic ducks raised on intensively irrigated paddy fields, an important factor of the persistence of H5N1 highly pathogenic avian influenza virus (AIV) persistence. Some major wild bird congregation wetlands in South China have been partly turned into paddy fields where millions of free-grazing domestic ducks are raised. This may facilitate the contact and the circulation of AIV between poultry and wild waterbirds, reservoir of AIV. In this study we combined epidemiological, ecological, agricultural and virological data to investigate the potential role of wild birds in the long-distance spread of H5N1 HPAIV virus from South China. We used new technologies such as satellite-tracking of Wild birds, GPS tracking of domestic ducks, remote sensing of irrigated paddy fields, phylogenetic analysis of H5N1 strains isolated in wild and domestic birds. Our results show that: (i) wild birds share paddy fields, wetlands and AIV strains with free-grazing domestic ducks when wintering in South China, (ii) spring migration of wild birds from South China match spatially and temporally with the long-distance spread of HPAIV H5N1, (iii) epidemiological and virological data support the role of wild birds in this long-distance spread. This multidisciplinary study brings together different elements in favour of the long distance spread of H5N1 AIV from South China agro-ecosystems through wild ducks migration.

[33] ANIMAL MIGRATION AND INFECTIOUS DISEASE RISK

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Long-distance migrations are spectacular and have long fascinated biologists, yet a little-studied consequence of migration is its effects on infectious disease processes. This talk will review a growing number of examples of how annual migrations can impact infectious disease spread, with a special focus on interactions between monarch butterflies and a protozoan parasites as a global case study. Monarchs occur worldwide and are best known for their bird-like migrations in eastern North America. The protozoan *Ophryocystis elektroscirrha* is transmitted both vertically and horizontally and causes debilitating infections in monarchs. Past work showed that parasite prevalence and virulence are highest in non-migratory monarch populations and that parasites have negative effects on monarch survival. More recent work used a combination of field studies and captive experiments to address two interrelated questions: (1) Do infected wild monarchs have lower migratory success than healthy butterflies? (2) Do migratory monarchs experience fitness tradeoffs between immune defense and energy reserves? Findings show that infected butterflies travel shorter distances and have lower migration success than uninfected adults. Although innate immune defenses were not costly to captive monarchs, we found tradeoffs between defense and lipid reserves among fall migrating adults. These results are consistent with several recent studies on the relationship between migration and infection in wild birds. Given that many animal migrations are imperiled by human activities, studies of pathogen dynamics in migratory species are urgently needed to predict future disease risks for wildlife and humans alike.

[34] INFLUENZA VIRUS AND MIGRATORY BIRDS: CONVERGENCE OF WITHIN- AND BETWEEN-HOST DYNAMICS

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Need influenza virus be avirulent to be maintained in wild bird populations? Although a positive answer may result from the “conventional wisdom” of a long co-evolutionary history between influenza viruses and wild birds, there is currently little understanding of this central question in pathogen ecology. We used different approaches to address it. First, epidemiological analyses of the spatio-temporal distribution of outbreaks of highly pathogenic avian influenza virus (HPAIV) H5N1 were performed to detect correlations between wild bird movements and virus spread. Second, experimental infections of a shorebird species in migratory and non-migratory state with HPAIV H5N1 were carried out to evaluate the impact of this virus on birds’ health and shedding. Third, we determined the impact of influenza virus pathology in different bird organs on its population-level fitness by use of a recently developed cross-scale modelling framework. Overall, the main results of these studies indicate that being of the HPAIV phenotype may not preclude influenza virus from spreading over relatively large distances. Furthermore, viruses causing clinical signs predisposing for viral transmission, e.g. diarrhea, may be evolutionary favoured for long-term maintenance in the population. These results highlight the subtlety of the definition of pathogenicity and the unique evolutionary and epidemiological dynamics that arise from the convergence of two scales: within and beyond the individual host.

[35] TRANSMISSION ECOLOGY OF SIN NOMBRE HANTAVIRUS IN NORTH AMERICAN DEERMOUSE POPULATIONS IN OUTDOOR ENCLOSURES

WDA Student Research Recognition Award

Karoun H. Bagamian
Population Biology, Ecology & Evolution Program
Emory University & Medical Ecology Unit, Viral Special Pathogens Branch
U. S. Centers for Disease Control and Prevention

Since the inception of the multidisciplinary field of disease ecology in 1979, ecologists, veterinarians, and public health researchers have explored natural disease systems and attempted to build predictive models of disease. Models of directly transmitted pathogens often predict that increased host population densities result in increased disease, but mark-recapture data from well-studied rodent-virus host-pathogen systems have reported conflicting results. Concurrently, these field studies have identified the importance of seasonality, host physiology and population processes on infection dynamics. Traditionally, transmission information is deduced from disease prevalence data, or determined in artificial laboratory settings, both of which do not adequately illustrate the natural progression of disease through a host population, and often separate ecological factors from within-host pathological and immunological factors. We address these discrepancies and explore questions about the role of host population density, seasonality, and host aggression on disease transmission by conducting manipulative field transmission experiments using North American deermice (*Peromyscus maniculatus*) naturally infected with Sin Nombre virus (SNV) in outdoor enclosures. This interdisciplinary project uses ecological, molecular, and immunological approaches to understand SNV infection and transmission in a natural host-pathogen system. We show that seasonality and host heterogeneities in behavior and viral infection load may have a stronger influence on disease transmission dynamics than host population density. We describe the first successful SNV transmission experiment in a closed deer-mouse population. Also, we introduce a new sub-specialty of disease ecology - transmission ecology.

[36] ECO-EPIDEMIOLOGY OF *FASCIOLOIDES MAGNA* IN ELK OF THE CANADIAN ROCKIES IN A WILDLIFE-LIVESTOCK SYSTEM

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Fascioloides magna, a liver fluke of elk (*Cervus elaphus canadensis*) and deer, is transmissible to livestock. Cattle are dead-end hosts in which the infection does not become patent. We explored the effect of cattle grazing on the distribution of *F. magna* in free-ranging elk in Alberta. Fresh fecal samples were collected from 640 elk from 10 herds that were exposed (n=5, H1-H5) or not (n=5, H6-H10) to cattle. We recovered *F. magna* eggs with Flukefinder®. Elk herd home ranges were estimated from telemetry data; environmental variables (e.g. vegetation type, stream density, elevation) were determined for each home range, as well as the intensity of interactions with cattle, and used as herd-level variables in a multilevel logistic regression analysis. The 5 elk herds exposed to cattle had lower proportions of positive samples (H1: 0%, H2: 0%, H3: 0%, H4: 1.4%, H5: 0%) than the unexposed herds (H6: 75%, H7: 5.1%, H8: 29%, H9: 27%, H10: 71%). Univariate analysis suggested a protective effect of cattle presence ($p<0.001$). This effect was still significant after adjusting for environmental variables in the multilevel logistic regression. Decisions regarding wildlife and livestock management often result in modifying the level of interaction between species. Understanding the dynamics of multi-host parasite infections is essential for wildlife managers and the livestock industry to anticipate the outcomes of these management decisions.

[37] ROLE OF THE INVASIVE AFRICAN CLAWED FROG (*XENOPUS LAEVIS*) AS VECTOR OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN CHILE

EWDA Student Travel Grant

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Amphibian chytridiomycosis, due to *Batrachochytrium dendrobatidis* (*Bd*), causes global amphibian population declines and species extinctions. Current evidence indicates recent global spread from an enzootic focus, possibly initially via trade in the African clawed frog (*Xenopus laevis*). *Xenopus laevis* has been feral in Chile since the 1970s. Recently *Bd* has been identified in wild populations. The epidemiology and potential impact of *Bd* on sympatric amphibians, however, have not been studied. Here, we investigate the role of *X. laevis* as a vector of *Bd* to two native sympatric species, the Chilean frog (*Calyptocephalella gayi*) and the four–eyed toad (*Pleurodema thaul*). Within a biosecure mesocosm, *Bd*–free *C. gayi* and *P. thaul* were separately co–housed with naturally *Bd*–infected *X. laevis*. On a weekly basis over 5 weeks, skin swabs were taken for *Bd* detection using qPCR. Infection occurred in 10/15 (66.7%) *P. thaul* and 12/15 (80%) *C. gayi*. All infected native individuals had low infections (0.2–685 GE), except one *C. gayi* (4.009 GE). One *P. thaul* and eight *C. gayi* died during the study; but none was associated with chytridiomycosis. In contrast, all *X. laevis* died; 27/30 (90%) had severe infection (10.270–131.300 GE) and all exhibited signs consistent with chytridiomycosis. Our results demonstrate the transmission of *Bd* from an invasive species to native anurans. Further research is required to understand the impacts of the infection on *X. laevis* and native anurans in Chile.

[38] DEER KEDS (*LIPOPTENA CERVI*) – INFESTATION INTENSITY, HABITAT AND EFFECT ON MOOSE (*ALCES ALCES*)

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A hematophagous ectoparasite of cervids, the deer ked (*Lipoptena cervi*), is currently spreading in Scandinavia. In Norway, keds are now invading the south-eastern part of the country. The widely distributed moose (*Alces alces*) is the main host and abundant deer ked populations are considered a major obstacle for human outdoor activity during the swarming period in fall. Knowledge of the relationship between moose, habitat and deer ked can potentially enable wildlife management authorities to regulate ked burden to wildlife and humans in a local area. The objectives of our study were to (i) determine deer ked infestation intensity on moose, (ii) evaluate if landscape characters and local moose population density are determinants of deer ked abundance on moose and (iii) evaluate to what extent ked abundance has an effect on the moose. The study area was a moose management area of 2,097 km^2, about 23 km east of Oslo, Norway. Hunters collected 350 skin pieces from the neck of moose during the regular hunting season in 2010. At the laboratory, the coat was removed and all deer keds counted. Infestation intensity was compared with intrinsic moose variables (age group, gender, carcass weights, hair cortisol levels) and extrinsic landscape variables (forest tree composition, forest age, latitude, longitude, altitude) surrounding each kill site. We found that forest tree composition, forest age, latitude, moose population density and moose age were determinants of deer ked abundance on moose.

[39] EXPLORING PERSISTENCE MECHANISMS OF HENIPAVIRUSES AND LYSSAVIRUSES IN ISOLATED ISLAND POPULATIONS OF AFRICAN FRUIT BATS

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Isolated islands provide valuable opportunities to study the persistence of viruses in wildlife populations, including population size thresholds such as the critical community size. The straw-coloured fruit bat, *Eidolon helvum*, has been identified as a reservoir for henipaviruses and Lagos bat virus (LBV) in continental Africa. Here, we sampled from an island populations of *E. helvum* fruit bats in the Gulf of Guinea to investigate the presence of these viruses in isolated island populations. Henipavirus and LBV serological analyses were undertaken samples collected from three islands in 2010, and the most isolated island was revisited in 2011. Given the isolation of these island fruit bats and the lack of connectivity with other populations, it was expected that populations would be too small to allow persistence of any viruses that cause acute, immunising infections. Contrary to expectations, island individuals displayed clear evidence for exposure to both viruses. Combined with data from continental Africa, age-specific modeling of seroprevalences has provided insight into viral transmission and maintenance. *E. helvum* is known to roost close in proximity to human populations across continental Africa and are a source of bushmeat, therefore these results could have important public health implications.

[40] GRIZZLY BEARS SHOW HORMONAL RESPONSES TO CHANGES IN SALMON CONSUMPTION AND ABUNDANCE

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Stress and reproductive hormones are valuable indicators of wildlife health and provide insight into life history trade-offs. In coastal British Columbia, Canada, grizzly bears evolved with spawning salmon as an abundant but spatially and temporally constrained food source. Recent and dramatic declines in salmon might have negative consequences on bear health and ultimately fitness. We predicted that hormone levels in hair, a tissue that reflects long-term steroid levels, would vary in response to salmon consumption and abundance, indicators of nutritional and socio-ecological contexts, respectively. Our samples were collected from hair snags (n=37) and government sampling of hunted bears (n=38) between 2004 and 2010. Employing genetics and stable isotope analysis, we quantified the dietary salmon of individuals. We measured cortisol and testosterone in hair using enzyme immunoassays. Cortisol was elevated following years when bears consumed below average amounts of salmon (p=0.001), suggesting that bears might be nutritionally stressed. In males, increasing testosterone was correlated with low salmon abundance (r=-0.88, p=0.01) but not salmon consumption (r=0.01, p=0.98). Elevated testosterone might be an adaptive response to increased competition in years of low salmon abundance. If salmon declines continue, chronically elevated testosterone and cortisol could negatively affect bear health; this knowledge could inform bear and fisheries management practices.

[41] INVESTIGATIONS ON THE OCCURRENCE OF BOVINE TUBERCULOSIS IN WILDLIFE AND ON THE RISK OF A RESERVOIR EMERGENCE IN WILD UNGULATES IN SWITZERLAND AND THE PRINCIPALITY LIECHTENSTEIN

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Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* or *M. caprae* has recently (re-)emerged in livestock and wildlife in all countries bordering Switzerland (CH) and the Principality of Liechtenstein (FL). Our aims were (1) to assess the occurrence of bTB in red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) in risk areas in CH/FL and to reinforce general surveillance in all wild mammals; (2) to evaluate the risk of a bTB reservoir formation in wild ungulates in CH/FL. Tissue samples collected from 2009 to 2011 from 434 hunted red deer and wild boars and from 8 ungulates with TB-like lesions were tested by a direct real-time PCR and culture to detect mycobacteria of the *M. tuberculosis* complex (MTBC). Suspect colonies were identified by real-time PCR and genotyping followed by spoligotyping. Data on risk factors for bTB maintenance within wild populations were retrieved from the literature and by interviews in CH/FL. MTBC were detected in 6 wild boars (3.6%, 95% CI 1.4-7.8) and none of the red deer (0%, 0-1.4). *M. microti* was confirmed in 2/6 positive wild boars. Main risk factors for bTB maintenance worldwide (high population densities, aggregation and intensive wildlife management) are largely absent in CH/FL. *M. bovis* and *M. caprae* were not detected but we report for the first time MTBC mycobacteria in Swiss wild boar. Conditions for a reservoir establishment seem unfavorable but increasing wild boar densities and unofficial feeding of wild ruminants represents a risk.

[42] EPIDEMIOLOGICAL AND PATHOLOGICAL INVESTIGATIONS ON *MYCOPLASMA HYOPNEUMONIAE* INFECTION IN WILD BOAR (*SUS SCROFA*) IN SWITZERLAND

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Enzootic pneumonia [EP] caused by *Mycoplasma hyopneumoniae* [M.hyop] was successfully eradicated from the domestic pig population in Switzerland. However, subsequent recurrent outbreaks suggested the wild boar as a source of reinfection. The goals of this study are (1) to assess whether wild boar represent a reservoir for M.hyop and to identify risk factors for infection, and (2) to investigate whether M.hyop infection is associated with lung lesions in wild boar. From April 2010 to February 2011 we evaluated different materials (nasal swabs [NS], bronchial swabs [BS] of fresh and thawed lungs), gathered preliminary prevalence data in 6 regions and developed a scoring scheme for macroscopic lung lesions. Samples from 181 wild boars were tested by real time PCR. Lungs were macroscopically evaluated and classified using criteria reported in domestic pigs. BS from fresh lung showed the highest sensitivity but difference with NS was not significant. Overall prevalence was 42% (CI 32-52%). Significant differences were observed between age classes and geographical regions. No association was found between the presence of macroscopic lung lesions and positive PCR-results of BS but severity of lesions seemed to increase with M.hyop load. Due to logistical benefits and expected better participation quote of field partners, we selected NS as sampling material for subsequent sampling rounds. Nevertheless, we continue to collect a limited number of lungs for histopathological examination.

[43] THE MULTIFACTORIAL AETIOLOGY OF UROGENITAL CARCINOMA IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*): A CASE CONTROL STUDY

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Urogenital carcinoma has an unusually high prevalence in California sea lions (*Zalophus californianus*). It is likely that the aetiology is multifactorial, as contaminants, inbreeding and herpesviruses have been linked with this cancer to date. This research focuses on two areas; genetic predisposition and association with a herpesvirus. By genotyping DNA extracted from skin samples from 40 urogenital carcinoma cases and 73 control animals, we identified that certain genotypes at microsatellite SGPv11 are at a significantly increased risk of developing carcinoma (crude odds ratio 4.12; 95% CI 1.3,13.14; p=0.015). SGPv11 is found within an intron of the gene HPSE2 which codes for the protein heparanase 2. HPSE2 and HPSE1 share about 40% sequence identity in humans. As HPSE1 gene expression is associated with cancer metastasis there is considerable clinical interest in HPSE2. The significant relationship between SGPv11 genotype and cancer offers a starting point for investigating the role of heparanase 2 in urogenital carcinoma. We are currently characterising splice variants and measuring the transcription of heparanase 2 from cancer cases compared to controls. Using PCR screening of DNA extracted from urogenital tissue from a subset of the same study animals (19 carcinoma and 38 controls), we have determined that the presence of the gammaherpesvirus, Otarine herpesvirus-1, was not significantly related to cancer occurance (Fishers exact test p=0.167) as previously suggested.

[44] PATHOLOGY OF MACROPODS LIVING IN A HIGH FLUORIDE ENVIRONMENT

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Particulate and gaseous fluoride emissions contaminate vegetation surrounding fluoride-emitting industry, with potential for skeletal and dental disease following long-term consumption by herbivorous wildlife. Eastern grey kangaroos (*Macropus giganteus*) resident near an aluminium smelter in southeastern Australia have been affected by chronic fluoride toxicosis. In the current study we aim to extend the investigation to other species of macropods. We discuss dental and skeletal pathology in relation to species, age, bone fluoride concentration and location relative to the emission source. Necropsy examinations of red-necked wallabies (*Macropus rufogriseus*) and swamp wallabies (*Wallabia bicolor*) from the smelter site have revealed skeletal and dental lesions consistent with fluorosis in *M. rufogriseus*, but not in *W. bicolor*. Through better understanding of how fluoride exposure leads to fluorosis in macropods, including further description of the associated epidemiology and pathology, we aim to produce management options that may prevent disease in macropods resulting from industrial fluoride emissions.

[45] INTRANASAL OXYGEN OR PARTIAL REVERSAL WITH ATIPAMEZOLE FOR TREATMENT OF HYPOXEMIA IN FREE-RANGING MOOSE (*ALCES ALCES*) IMMOBILIZED WITH ETORPHINE-ACEPROMAZINE-XYLAZINE

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Hypoxemia is a risk in wildlife immobilization, especially with potent opioids and α_2 agonists. Treatment of hypoxemia was evaluated in 33 adult free-ranging moose (*Alces alces*) darted from a helicopter with 3.37 mg etorphine, 15 mg acepromazine and 75 mg xylazine. Intranasal oxygen at a flow rate of 4L/min and an early reversal of xylazine with 7.5 mg atipamezole were evaluated, with four treatment regimes; intranasal oxygen (n=10), atipamezole intramuscularly (n=6), atipamezole intravenously (n=10), or a combination of atipamezole intravenously and intranasal oxygen (n=7). Arterial blood was analyzed immediately with an i-STAT®1 Portable Clinical Analyzer 10-30 minutes after darting, and again 15 minutes after institution of treatment. Twenty-six animals were hypoxemic before treatment (PaO_2 <80 mmHg), with ten animals markedly hypoxemic (PaO_2 40-60 mmHg) and three animals severely hypoxemic (PaO_2 <40 mmHg). Intranasal oxygen treated hypoxemia (increased PaO_2 >80 mmHg) in nine of ten animals (range PaO_2 91-185 mmHg). Atipamezole intramuscularly showed few improvements. Atipamezole intravenously treated hypoxemia in two animals (range PaO_2 82-86 mmHg), and increased PaO_2 (mean increase 15 mmHg) in additional six animals although they remained hypoxemic. The combination of atipamezole intravenously and intranasal oxygen treated hypoxemia in six of seven animals (range PaO_2 81-124 mmHg). Our study shows that intranasal oxygen effectively treated hypoxemia in immobilized moose.

[46] DEVELOPMENT OF MULTIPLEX SEROLOGY PLATFORMS (BIO-PLEX) FOR SURVEILLANCE OF DISEASES IN WILD BOARS IN EU

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The potential of a multiplex serology test based on Luminex technology, where all antibodies are simultaneously detected in a single assay was investigated as a serodiagnostic tool for surveillance of diseases in wild boar population in Europe. The performance of «in-house" separate ELISAs which use relatively crude bacterial and cultured virus lysates as capture antigens was compared to the multiplex assay where the same antigens were covalently bound to the magnetic fluorescent beads used in the Luminex platform. Thirty six different bacterial, viral and parasitic antigens were tested with known hyper immune sera and with negative sera in serial dilutions. A panel of 483 wild boar sera of known positive and negative status for *M. Bovis, Brucella spp,* Hepatitis E virus and Aujeszky were used for validation purposes. The MPB83, BP 26, HEV 272 and Aujeszky gE components of the multiplex test respectively, showed similar sensitivities and specificities to the separate "in-house" ELISAs. In general, bacterial components performed well and the performance of the viral components was less successful which was attributed to relatively low signal strength for these antigens, leading to higher assay variability and a reduced ability to distinguish positive and negative samples compared to the "in-house" ELISAs. The use of recombinant proteins was investigated and was shown to lead to an appreciable high signal strength compared to the use of crude antigen in the Luminex system. The performance of the antigens in the Luminex assay was comparable to the WildTech serology array where the same antigens were spotted onto microarray strips.

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[47] ACTIVE DISEASE SURVEILLANCE SYSTEM FOR MOUNTAIN GORILLAS AND CONSERVATION STAFF AT THE VOLCANOES NATIONAL PARK, RWANDA

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Mountain Gorillas are found only in two places in the world and one of the locations is the Virunga Massif comprising the Volcanoes National Park of Rwanda, Virunga National Park of the Democratic Republic of Congo, and the Mgahinga National Park of Uganda. The population of critically endangered mountain gorillas living in Virunga Massif has grown by 26.3% to approximately 480 individuals in the past seven years (Mountain Gorilla Census, 2010). Studies have shown that people, cattle, and mountain gorillas can be infected by common pathogens. Viral screening performed on samples collected from wild Gorillas also reveals a range of antibody titers to select human pathogens, such as hepatitis and influenza. The implication is that there is increased risk of disease transmission from humans to gorilla and vice versa. These underscore the importance of a “one-health” initiative. We used a standardized data collection system known as IMPACT. IMPACT is a web-based syndromic health monitoring system designed to help identify a health problem. The system uses a “Clinical Decision Tree” model to assess the level of risk based on the number of animals affected. If the risk is low with few animals affected, as in the case of scabies mite mange; veterinary personnel respond appropriately. If the risk is high with many animals sick, as in the case of measles or TB, the model recommends a coordinated action plan that calls for outside technical and logistical support; this action plan is known as the Contingency Plan. Data generated through IMPACT in 2009 revealed an outbreak of a respiratory infection in the *Hirwa* group of Gorillas. Eleven of the twelve gorillas presented with moderate to severe respiratory infection and two died (CFR 17%). Multiplex polymerase chain reaction (PCR) analysis for the presence of respiratory pathogens revealed *Human Metapneumovirus* (HMPV) in serum, lung tissue, and throat, nose, anus and vagina swabs. A total of 440 park personnel and their families participated in the Conservation Personnel Health. Fifty five percent (55%) of the park workers reported defecating in the park. Laboratory examination revealed varying levels of different parasite among those enrolled in the program; the common organisms detected included *Ascaris lumbricoides*, *Entamoeba coli* (21%), *Entamoeba histolitica* (7%), *Giardia lamblia (*15 %), T*richomonasintestinalis* (12%), and Trichuris *trichuria* (3%). The Joint surveillance of gorilla and human diseases is important in reducing the risk of zoonotic disease transmission between employees and the park’s mountain gorillas. It also helps Gorilla doctors understand the incidence of possible transmissible diseases that could threaten the health of the endangered mountain gorillas and therefore promotes conservation efforts and the “one- health” initiative.

(ABSTRACT TRUNCATED)

[48] HETEROGENEITY IN THE RISK OF *MYCOBACTERIUM BOVIS* TRANSMISSION IN EURASIAN BADGER (*MELES MELES*) CUBS

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Within a socially structured population, the behaviour of certain infected individuals can have a disproportionately large effect on transmission dynamics and the spatio-temporal distribution of infection. Endemic infection with *Mycobacterium bovis* in Eurasian badger (*Meles meles*) populations in the UK and Ireland is an important source of bovine tuberculosis in cattle. The organisation of badger populations into social groups and differential rates of contact amongst group members can give rise to heterogeneity in transmission risks. Here we examine the risk of infection acquisition and progression in badger cubs in a high density population of endemically infected wild badgers in south-west England, in relation to the infection status of adult group members. We observed variation in both the risk of infection and over-winter mortality in cubs, with the highest risk being associated with the presence of an infectious breeding female. However, despite considerable annual variation during our study period from 1982 to 2010, the proportion of high-risk social groups with infectious breeding females remained below 30%. We discuss how our findings underpin the persistence of infection at both a social group and population level, and the potential implications for disease management strategies.

[49] STATE-DEPENDENT MODELLING REVEALS KEY EPIDEMIOLOGICAL PARAMETERS FOR BOVINE TUBERCULOSIS IN BADGERS

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Epidemiological models help to predict optimal control strategies in wildlife-host disease systems. Key parameters include disease-induced mortality and rates of disease transmission among hosts. We applied multi-state mark-recapture analysis to 22 years of data from a population of Eurasian badgers (*Meles meles*) naturally infected with bovine tuberculosis (TB; *Mycobacterium bovis*). This study estimated apparent transmission, disease progression and variation in survival among disease states. These are important parameters known to influence disease prevalence and rates of cattle herd breakdowns in current modelling simulations. We proposed a new categorisation of badgers based on the results of diagnostic tests: uninfected, ELISA positive, one-site excretor, multi-site excretor. This alternative categorisation improved model fit when compared to classifications used in previous studies. We found a higher risk of infection and rapid disease progression in male badgers, coupled with state-dependent increases in mortality. This could promote sex-biases in the risk of transmission to cattle, and may influence optimal control strategies for TB in badger populations.

[50] COMPARISON BETWEEN PESTIVIRUS INFECTIONS IN WILD AND DOMESTIC RUMINANTS IN THE FRENCH SOUTHERN ALPS SUGGEST THAT INTERSPECIES TRANSMISSION MAY OCCUR

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In alpine pasture, interspecies transmission has recently been incriminated in the epidemiology of pestivirus infection. The aim of this study was to investigate pestivirus infections and transmission between wild and domestic ruminants sharing pastures in the French Southern Alps. Individual sero-prevalence reached 76.5% (95% confidence interval [95% CI]: [74.2 – 78.8%]) of the 1383 sheep tested, 38.7% (95% CI: [33.8 – 43.9%]) of the 369 chamois (*Rupicapra rupicapra*), 28.7% (95% CI: [17.4 – 38.1%]) of the 72 roe deer (*Capreolus capreolus*) and 22.2% (95% CI: [6.5 – 37.9%]) of the 27 mouflons (*Ovis musimon*) tested. Three pestivirus strains were isolated from sheep and were classified in the BDV-3, BDV-Tunisian and BDV-6 genotypes. For the first time, one strain (named RUPI-05) was isolated from an alpine chamois and clustered in the BDV-6 genotype, showing 92% of similarities (in the 5'UTR region) with the ovine strain isolated in the same area. Comparative virus neutralization tests showed that sheep had higher titers than chamois for all domestic strains tested but lower for the RUPI-05 strain. These results indicate a very high and unexpected seroprevalence in domestic ruminants and a high seroprevalence in wild ones. Besides, a genetic continuity among the circulating viral strains was observed between the ruminant species, confirming the occurrence of interspecies transmission. Further studies are needed to attribute a specific epidemiological role to each animal species.

[51] IMMUNIZATION AGAINST BLUETONGUE VIRUS SEROTYPES 1 AND 8 PROTECTS EXPERIMENTALLY INOCULATED SPANISH IBEX (*CAPRA PYRENAICA*)

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Inclusion of wildlife in vaccination control programs may be considered in emerging diseases such as Bluetongue (BT). BT is an infectious viral disease of domestic and wild ruminants caused by bluetongue virus (BTV; *Orbivirus, Reoviridae*), which is transmitted by blood-feeding midges of the genus *Culicoides* (*Diptera, Ceratopogonidae*). Up to now, twenty-six BTV serotypes have been described. Outbreaks involving either BTV serotypes 1 (BTV-1) and 8 (BTV-8) have challenged Europe in the last years. In the present study, a susceptible species such as Spanish ibex (*Capra pyrenaica*) was vaccinated and challenged against BTV. Four out of fifteen ibexes were immunized with a single dose of inactivated vaccine against BTV-1, four against BTV-8 and seven ibexes were non vaccinated controls. Seven ibexes (four vaccinated and three controls) were inoculated with each BTV serotype. Viraemia, antibody and IFN-gamma responses were evaluated until 28 days after inoculation (dpi). The vaccinated ibexes showed significantly ($P<0.05$) higher neutralizing antibody levels after vaccination compared to the non vaccinated ones. The non vaccinated ibexes remained seronegative until challenge and showed neutralizing antibodies from 7 dpi. BTV RNA was detected in the blood of non vaccinated ibexes from 2 dpi to the end of the study (28 dpi) and in target tissue samples obtained at necropsy (8 and 28 dpi). Clinical signs were unapparent and no gross lesions were found at necropsy. The present results further confirm the susceptibility of Spanish ibex to asymptomatic BTV-1 and BTV-8 infection. Moreover, one single dose of monovalent vaccine prevents viraemia of both BTV-1 and BTV-8. It also suggests that pathogenesis and host immune response may vary among the different BTV strains, and that Spanish ibex can contribute to the maintaining of BTV confirmed by viraemia detected until 28 dpi and probably for longer periods.

[52] THE EFFECT OF ENVIRONMENT AND DOMESTICATION ON *ICHTHYOPHTHIRIUS MULTIFILIIS* INFESTATION IN BALTIC SALMON REARED FOR RESTOCKING

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Restocking is a widely used practice for maintaining threatened feral fish populations. However, reared fish have high mortality in the wild, and parasitic infections cause losses during rearing. Enriching the standard rearing conditions and avoiding domestication selection have been suggested to improve fish survival and health. The goal of this study was to explore, how the rearing environment and the genetic origin of the fish affect the parasite load in the settings used for fish farming. Baltic salmon parr originating from farmed and wild broodfish were reared in 16 outdoor 50 m^2 concrete tanks, fish density being 40 fish/m^2. Eight tanks were enriched with shelters and by varying the waterflow. While a natural *I. multifiliis* infection was occurring and being treated, skin mucus scrapings from 271 fish were examined and the parasites counted under a light microscope. In fish reared in the enriched tanks the prevalence of *I. multifiliis* after the formalin treatment was 47 % in “farmed” and 16 % in “wild”, while respective proportions were 68 % and 52 % in the standard tanks. The lower prevalence among “wild” (ANOVA, $P<0.01$) and among the enriched tanks ($P<0.05$) is hardly a chance; the origin and method of rearing both contributed to the prevalence of infection. The results show that adding diversity in fishes’ farming environment might have potential in the prevention of certain diseases. These effects on health and the connections to immunity need to be studied further.

[53] AMPHIBIAN HEALTH IN RICE FIELDS IN COSTA RICA: WHAT ROLE DO PESTICIDES PLAY?

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Humans modify their environment in many ways, but one of the most important for amphibians is destruction of wetlands and conversion to human land uses. While some propose rice fields as an adequate alternative habitat for displaced amphibians, this idea has never been rigorously tested in the field. I am conducting an in-depth study of *Bufo marinus* population health within contrasting land use types characterized by two pesticide regimes: conventional rice farms and organic rice farms. The health and immune function status of *Bufo marinus* is assessed through body condition, corticosterone levels, response to PHA, as well as lungworm, tick and gastrointestinal parasite diversity and abundance. Based on body condition scores, fat body measurements and paratoid gland size, *Bufo marinus* have significantly lower condition scores in conventional rice fields. Interestingly, females are generally more heavily affected than males. However, lungworm (*Rhabdias* spp.) and adult trematode loads are higher in organic rice farms than in conventional rice, likely due to the effects of pesticides on intermediate hosts or free-living lifestages of these parasites. Yet gastrointestinal nematode abundance was higher in fields treated with pesticide, which may indicate immunosuppression. This data suggests that pesticide use is having a negative effect on the condition of amphibians living in rice fields that outweighs a release from parasite pressure, which may translate into a loss of fitness.

[54] ASSESSING CLASSICAL SWINE FEVER CONTROL MEASURES UNDER UNCERTAIN VIRAL CONSTRAINTS

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Classical Swine Fever (CSF) is a viral disease in wild boars and domestic pigs causing huge economic impact on livestock industries. Due to of CSF endemicity in wild boar populations in several European countries, huge effort is paid on CSF control in wild boar by oral mass vaccination. However, few is known about the efficacy of the applied measures to control or eradicate the disease. We implemented a spatially-explicit, individual-based wild boar population model, coupled with a CSF virus model on the level of individual traits. Over a range of case mortality and duration of the infectious period (the virulence), we compared alternative spatial baiting strategies regarding their performance, measured by final size of the infected area and long-term persistence. Our analysis showed that high success in virus eradication as well as prevention of disease spread was only possible with preventive vaccination in terms of baiting in front of the epidemic wave. Buffered vaccination was completely sufficient to exploit the effect of vaccination of the entire area, which translates strategic needs into a practical management plan. A buffer radius corresponding disease spread of one year revealed suitable to fully exploit the potential of oral mass vaccination. Although preventive baiting strategies are not yet implemented in the field due to EU legislation but with marker vaccines in sight, we recommend buffered baiting of the area with infected animals.

[55] EFFECTIVENESS OF EXCLUSION MEASURES FOR PREVENTING MULTI-HOST INTERACTION AT WATER SOURCES UNDER DRY CONDITIONS

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Tuberculosis (TB) is endemic in Eurasian wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in south-central Spain and evidence suggests transmission to domestic cattle. Known risk factors for TB at the interface between livestock and wild ungulates include temporal and spatial overlap, particularly around waterholes during summer. We evaluated the effectiveness of selective exclusion measures for reducing direct and indirect contact between cattle and wild ungulates at waterholes. We first monitored 6 waterholes with infrared digital cameras at a TB positive farm to quantify interactions. We then assigned 3 waterholes to be "cattle only" and 3 to be "wildlife only". Cattle only waterholes were surrounded with a wildlife-proof fence (2 m high) and a cattle-specific gate. Wildlife only waterholes were surrounded by a fence that wild ungulates could breach but cattle could not (1.2 m high). Deer and wild boar, respectively, easily jumped or undercrossed this fence. Cattle only fences were 100% effective at preventing wildlife access to waterholes. Wildlife only fences were 100% effective in preventing cattle access to waterholes and did not impede wildlife use. Many cows learned to operate the cattle-specific gate quickly and others followed and learned from them. Within 2 weeks 75.2% actively entered and exited through the cattle only gate. We demonstrate how simple, low-cost fencing strategies can serve as biosecurity measures to substantially reduce direct and indirect contact between cattle and wild ungulates, serving to reduce the potential for TB transmission.

[56] COSTS AND BENEFITS OF WILDLIFE DISEASE CONTROL

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Disease in wildlife populations can have adverse impacts on the health of domestic animals and humans, with consequent costs to our agricultural and public health systems. Wildlife diseases may also have profound impacts on species of conservation concern and hence reduce the benefits that global biodiversity brings to humankind. However, interventions to control wildlife diseases, such as culling, vaccination and changing contact behaviour, can have both positive and negative effects. Behavioural and demographic responses of wildlife populations to intervention can give rise to complex epidemiological outcomes. In addition, the manipulation of a single host population for the purposes of disease control can have far-reaching effects on other components of the ecosystem, with potential implications for epidemiology, economics and conservation. There is a strong case for the use of ecological impact assessment approaches to determine the likely outcomes of proposed interventions to manage disease in wildlife populations.

[57] GLOBAL BIOLOGICAL OPPORTUNITIES AND CHALLENGES IN THE ORAL RABIES VACCINATION OF CARNIVORES

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Rabies serves as a significant historical model for disease management in wildlife. By example, over the past three decades, significant progress has occurred in the prevention and control of rabies by oral vaccination of free-ranging wild meso-carnivores, predominantly in Europe and North America. Large regions are now free of rabies virus perpetuation among major reservoirs. Obvious biological benefits have accrued for humans, domestic animals, and wildlife, including endangered species. Opportunities for greater extension into developing countries would augment plans for global canine rabies elimination. However, additional challenges remain. Rare cases of vaccine-associated disease have been documented. Moreover, all licensed vaccines are based either on rabies virus or recombinant vaccinia virus, and not all species are immunized effectively by current products. Costs may preclude distribution of appropriate density of vaccine-laden baits to reach continental-wide targets. Few models can predict accurately the potential ecological consequences of removing mortality factors in particular populations. Application of next generation adenoviruses, rabies viruses, and other agents, combined with targeted immuno-contraceptive strategies, may provide substantial opportunities for additional novel progress in this arena.

[58] BCG VACCINATION REDUCES RISK OF TUBERCULOSIS INFECTION IN VACCINATED BADGERS AND UNVACCINATED BADGER CUBS

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The control of tuberculosis (TB) in cattle in Britain and Ireland is hindered by persistent infection in wild badgers (*Meles meles*). Vaccination with Bacillus Calmette-Guérin (BCG) has been shown to reduce the severity and progression of experimentally induced TB in captive badgers. Analysis of data from a four-year clinical field study, conducted at the social group level, suggested a similar, direct protective effect of BCG in a wild badger population. Here we present new evidence from the same study identifying both a direct beneficial effect of vaccination in individual badgers and an indirect protective effect in unvaccinated cubs. We show that intramuscular injection of BCG significantly reduced the risk of free-living vaccinated individuals testing positive to both a diagnostic test combination for more progressed infection and a more sensitive panel of tests for the detection of infection *per se*. In addition, we show the risk of unvaccinated badger cubs, but not adults, testing positive to the same panel of diagnostic tests decreased significantly as the vaccinated proportion of their social group increased. Together, these results provide additional insights into the nature of the protective effect of BCG vaccination of wild badgers in their natural social setting. Our findings should be considered in light of the relatively short time scale over which the beneficial effects of vaccination were observed.

[59] BOVINE TUBERCULOSIS AND FELINE IMMUNODEFICIENCY VIRUS CO-INFECTION IN LIONS: A POSSIBLE THREAT FOR CONSERVATION

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Bovine tuberculosis (BTB), caused by *Mycobacterium bovis* (*M. bovis*), is a disease that was introduced relatively recently into the Kruger National Park (KNP) lion population, where feline immunodeficiency virus (FIV) has long been endemic. In humans, co-infection with *M. tuberculosis* and human immunodeficiency virus increases disease burden. If BTB were to reach high levels of prevalence in lions and if similar worsening effects would exist between FIV and BTB as for their human equivalents, this could pose a serious lion conservation problem. Data were collected of 671 lions in KNP during the period 1993-2008 for spatio-temporal analysis of both FIV and BTB, and to assess whether a similar relation between the two diseases exists in lions. We found that BTB prevalence in the south was higher than in the north (72% vs 19%) and increased over time in the north (0% to 41%). No significant spatio-temporal differences were seen for FIV. Both infections affected blood protein values, FIV in a more pronounced way than BTB. The effect of co-infection on these values, however, was always less than additive. Though 30% of the lions were co-infected, there was no evidence for a synergistic relation as in their human counterparts. Whether this results from different immunopathogeneses remains to be determined. Though no changes in the lion population age distribution were found, the spatio-temporal increase of BTB itself may pose a risk for lion conservation.

[60] VACCINE APPROACHES AGAINST DEVIL FACIAL TUMOUR DISEASE

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The Tasmanian devil (*Sarcophilus harrisii*) is facing extinction due to a transmissible cancer, devil facial tumour disease (DFTD). This cancer is a cell allograft that is not rejected by the devil's immune system. The mechanism of tumour immune escape is not well understood. We have investigated different strategies to produce an effective vaccine against DFTD. Four healthy Tasmanian devils were immunised against inactivated DFTD tumour cells (immunised twice four weeks apart). The four devils produce weak antibody and cytotoxic responses *in vitro*. Two of these devils were challenged with live tumour cells and developed DFTD tumours five weeks after challenge, indicating that the responses against the tumour were not protective. To increase potential antigens on the cell surface of DFTD cells, we produced devil cytokine-rich supernatants, by incubating devil lymphocytes with the mitogen Concanavalin A (Con A sup). We next treated DFTD tumour cells with this Con A sup for 48 hours. Treated DFTD cells were used as the immunogen in a DFTD-affected devil (this devil was immunised using the same protocol described above). Abundant T-lymphocytes were identified in the tumour mass seven days after the first immunisation, indicating adequate cytotoxic responses were activated. The tumour mass reduced more than 80% in size in five weeks. These results suggest that devil cytokines can up-regulate antigens on DFTD cells that are suitable targets for the devils' immune system.

[61] ASSESSING THE MULTI-SECTORAL IMPACTS OF FOOT AND MOUTH DISEASE AND CURRENT APPROACHES TO ITS MANAGEMENT AND CONTROL IN SOUTHERN AFRICA

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Foot and mouth disease (FMD) in southern Africa presents three fundamental challenges to policy makers: (1) the SAT viruses predominantly involved are maintained by free-living wildlife populations, especially African buffalo and are not eradicable without wildlife depopulation and enhancement of prohibitively expensive and environmentally destructive fencing systems; (2) current approaches to FMD management constrain rural economic development in the region; and (3) for both demographic and technical reasons, current approaches are failing to adequately control the disease. We suggest an alternative approach to FMD management and associated trade standards which meets existing international norms and has the potential to ameliorate the conflicts between disease control and rural development. Successfully addressing these challenges has become particularly important with the establishment of transfrontier conservation areas (TFCAs) and as the OIE/FAO Progressive Control Pathway for FMD (PCP-FMD) is being developed in support of a comprehensive Global Strategy. Failure to develop a more holistic, effective and contextually appropriate approach that takes into account southern Africa’s unique circumstances will perpetuate the current situation and limit the potential for sustainable rural development that includes both the livestock and wildlife conservation sectors.

[62] EXPERIMENTAL ANTI-FUNGAL DRUG TRIALS TO INCREASE HIBERNATION SURVIVAL OF WHITE NOSE SYNDROME INFECTED BATS

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White nose syndrome (WNS) is a devastating fungal disease causing unprecedented mortality in several species of North American hibernating bats. With mortality rates up to 100% in some hibernacula, and continuing pathogen spread across the continent, several bat species may be at risk of extinction if no intervention is taken. In an effort to reduce WNS mortality in hibernating bats we are investigating anti-fungal drug treatments in naturally infected little brown bats (*Myotis lucifugus*) during captive hibernation. An aqueous sonicated suspension of terbinafine HCl was given subcutaneously to groups of WNS infected bats at four dosages from 6 mg/kg to 200 mg/kg daily for 10 days, then bats were placed in environmental chambers kept at hibernation conditions. There was no significant difference in survival time between terbinafine treated bats and control bats. In a second trial, voriconazole (V-Fend®, Pfizer) was administered subcutaneously at 3 dosages for 5 days to WNS infected bats. Voriconazole treated bats exhibited adverse drug effects after the first dose, and death within the treatment period for the lowest dose administered. WNS infected controls for this experiment survived hibernation, suggesting timing of treatment application and varying hibernation conditions may increase survival. Further study of captive hibernation conditions and antifungal drug therapies and protocol timing are needed to provide intervention strategies for this devastating disease.

[63] ENVIRONMENTAL AND DEMOGRAPHIC DETERMINANTS OF AVIAN INFLUENZA VIRUSES IN WATERFOWL ACROSS THE CONTIGUOUS UNITED STATES

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A first step towards understanding where and when avian influenza virus (AIV) might emerge from North American waterfowl is to identify environmental and demographic determinants of infection in their populations. Using AIV surveillance data collected from 19,965 wild waterfowl across the contiguous United States between October 2006 and September 2009 we fit logistic regression models relating the infection status of individuals sampled on their breeding grounds to demographic characteristics, temperature, and interval apparent prevalence during the preceding overwintering season at the local watershed scale. We found strong support for sex, age, and species differences in the probability an individual tested positive for AIV. In addition, for every seven days that the local minimum temperature fell below zero, the chance an individual would test positive increased by 5.9%. We also found a 12% increase in the chance an individual would test positive during the breeding season for every 10% increase in the interval apparent prevalence during the prior overwintering season. These results suggest that viral deposition in water and sub-freezing temperatures during the overwintering season may act as drivers of individual level infection risk during the subsequent breeding season. Further study is needed to identify how these drivers might interact with other, host specific infection determinants, such as species phylogeny, immunological status, and behavioral characteristics.

[64] DEVELOPMENTAL EFFECTS OF FIELD MATERNAL TRANSFER OF LEAD ON MALLARD DUCKLINGS

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The prevalence of Pb shot ingestion in mallards (*Anas platyrhynchos*) from the Ebro delta (NE Spain) was 30% before the Pb shot ban in 2001, and it is still 14% in 2011-12. Pb can produce sublethal effects on several biological functions in birds, including the reproduction and the nestling development. Here we have studied the effects of the maternal transfer of Pb through the egg to the duckling under field conditions. Eggs of mallard ducks (n=44) were collected from independent nests and incubated artificially. Pb levels were measured in eggshells and blood of ducklings, and these were correlated with δ-ALAD activity, plasma biochemistry, oxidative stress biomarkers, cellular (PHA reaction) and humoral (SRBC agglutination) immune functions, and results of three neurobehavioral tests performed at different days of age. Ducklings with blood Pb levels above 180 ng/ml died during the first 7 days post hatching. Eggshell Pb levels were significantly correlated with blood Pb levels in ducklings at day 3, but not at day 28. δ-ALAD activity and cellular immune function were negatively correlated with Pb levels in eggshell and blood of mallard ducklings. The rest of the studied parameters were not correlated with blood or eggshell Pb levels. The use field eggs may give more realistic results than experimental studies, because it permits to evaluate field Pb exposures in which other toxicants may be also present.

[65] INTRANASAL CHRONIC WASTING DISEASE (CWD) INOCULATION OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

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Chronic wasting disease (CWD) is a persistent problem in populations of both wild and captive cervids in North America. Prevalence rates continue to increase and the disease continues to spread, positive animals are documented in new areas each year. Indirect transmission can occur via environmental contamination and is thought to occur by the oral and/or intranasal route. Oral transmission has been experimentally demonstrated and although intranasal transmission has been postulated, it has not been tested. Prions have been shown to strongly adsorb to clay particles and upon oral inoculation the prion/clay combination exhibits increased infectivity in rodent models. Deer and elk naturally inhale dust while foraging and performing other behaviors in their environment. We therefore hypothesized that contaminated dust particles may be a viable mode of intranasal exposure to CWD. To test this hypothesis, CWD-positive brain homogenate was mixed with montmorillonite clay, dried, re-powdered and intranasally inoculated into white-tailed deer once a week for 6 weeks. Deer were euthanized at 3, 3.5, 4 and 6 mo. post initial inoculation and tissues were examined for CWD. Our results demonstrate that CWD can be efficiently transmitted intranasally utilizing montmorillonite dust particles as carriers and that the intranasal route is a viable route of exposure.

[66] THE ROLE OF POLYMORPHISMS IN MHC (CLASS II) IN THE PATHOGENESIS OF EUROPEAN BROWN HARE SYNDROME (EBHS)

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To determine the presence of EBHSV, its phylogenetic position, and possible correlation with hares' MHC genotype, a total of 170 liver samples were obtained from hares in Denmark between 2004 and 2009. Macroscopical and histopathological findings consistent with EBHS were found in 24 (14.1%) of hares, 35 (20.6%) had liver lesions not typical of EBHS, 50 (29.4%) had other lesions and 61 (35.9%) had no lesions. Samples were tested for viral RNA by RT-PCR and the products were sequenced. The genetic variation in exon 2 DQA gene of MHC was assessed in order to detect potential susceptible or resistant genotypes. EBHSV was detected in 65 (38.2%) of the 170 samples tested. Analysis of sequence variation of DQA exon 2 revealed the occurrence of 7 different alleles. Three alleles were identical with those previously detected in Europe (*Leeu-DQA*14, 30* and *42*) and 4 were unique for Denmark (*Leeu-DQA*51-54*). Susceptibility to the EBHSV was defined as the occurrence of deaths in association with the intensity of histopathological lesions typical to the EBHS in liver. There was a significant difference in alleles' distribution within EBHSV positive hares between those found dead with severe histopathological lesions and those found sick or apparently healthy. Although very difficult to prove any direct association between a specific DQA allele and EBHSV susceptibility or resistance, our results indicate a clear implication of the allele *Leeu-DQA*30* to EBHSV susceptibility.

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[67] SEROLOGICAL RESPONSE TO *TREPONEMA PALLIDUM* INFECTION IN OLIVE BABOONS

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Although humoral antibodies and CD8+ cytotoxic T-cells are relatively ineffective at clearing *T. pallidum* infection, hosts mount a strong antibody response to the bacterium. Virtually nothing is known about antibody response in naturally occurring simian infections, however. Sixty-three olive baboons (*Papio anubis*) were sampled to investigate a genital ulcerative disease caused by *T. pallidum* at Lake Manyara National Park in Tanzania. The spirochete was confirmed in 38 individuals by IHC and PCR methods. Different serological tests were used to measure anti-*T. pallidum* IgM and IgG. Results of a quantitative and qualitative gelatin particle agglutination assay, fluorescence-*Treponema*-antibody absorption test (FTA), IgM/G immunoblots, as well as two rapid tests were compared and correlated to PCR results. Antibody titres in infected animals were generally high, with mean anti-*T. pallidum* IgM+IgG titres of 1:2.94E+04±SE 9.87E+03 in the initial clinical stage, 1:2.17E+05±SE 1.83E+05 in the moderate stage, and 1.78E+06±SE 1.38E+06 in the severe stage. Sensitivity (Sen) and positive predictive values (PP) for the gelatin particle agglutination assay (Sen 100%, PP 95%), the FTA IgG (Sen 100%, PP 98%) and the immunoblot IgG (Sen 100%, PP 93%) are reliable, whereas the specificity and negative prediction values need more testing. Data on changes in IgG subclass composition over the course of infection, obtained using an IgG subclass ELISA, will also be presented.

[68] CAUSES OF MORTALITY IN WHITE-NOSE SYNDROME IN LITTLE BROWN BATS (*MYOTIS LUCIFIGUS*)

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White-nose syndrome (WNS) has been reported to have caused the death of millions of bats in eastern North America since first being detected in NewYork State in 2006. The fungus *Geomyces destructans* (Gd) has been shown to be the cause of WNS but interestingly Gd has also been detected in Europe without evidence of significant mortality. The mechanism by which Gd causes mortality in bats has not yet been determined. We exposed *Myotis lucifugus* to North American and European isolates of Gd during torpor under laboratory conditions which resulted in characteristic lesions and mortality. European Gd caused more rapid mortality than North American Gd indicating a lack of virulence factors in EUGd does not explain the absence of mortality in European bats. Both Gd isolates caused an increase in frequency of arousal in bats compared to non-exposed control bats. This resulted in depletion of energy reserves which likely contributed to mortality. In addition, alterations in hematocrit, and serum chemical and electrolyte parameters, suggest altered fluid fluxes across the damaged epidermis of the wing may also contribute to mortality. In a few cases, local invasion of Gd infected skin by the bacteria *Serratia* sp. and *Hafnia alvei* followed by bacteremia and septicemia may also have contributed to death. These are chitinase producing bacteria commonly found in the gut of insectivorous bats.

[69] DRIVERS OF HONEY BEE COLONY DECLINES AND LOSSES

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Over the last three decades scientists have published a series of articles reporting two phenomena affecting global amphibian populations: enigmatic population declines and gross deformities. During the 1980s and 1990s three key hypotheses were proposed for the cause of these issues: climate change, pesticides (specifically atrazine), and UV-B radiation. It is now clear that the majority of 'enigmatic' declines were caused by the emerging fungal disease chytridiomycosis, and that trematode infections are strongly implicated in amphibian deformities. In this talk we present evidence that a similar situation may be occurring with the recent increase in honey bee colony losses. We will present a review of current hypotheses, including pathogens, pesticides and multifactorial causes. Our results highlight the role of *Varroa destructor* and it's transmission through the trade in the losses occurring in Europe and Canada and indicate there are urgent management issues that need to be addressed for a now commercially produced species that is facing several emerging diseases. Finally we present underlying problems with the term "Colony Collapse Disorder". We propose that significantly more effort needs to be focused on the role of *V. destructor* in global bee health issues and development of appropriate management and treatment strategies.

[70] USE OF NEXT-GENERATION SEQUENCING TECHNOLOGY TO DETECT UNKNOWN VIRUSES IN WILDLIFE

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Viral infections are an important cause of disease in humans, domestic animals, and wildlife. However, our knowledge of viruses that infect humans and animals is still incomplete and many diseases with unknown etiology may be caused by as yet unidentified viruses. To identify unknown viruses, we perform random amplification of viral nucleic acids in combination with next-generation sequencing. Using this technique, we have identified multiple unknown viruses in humans and animals which could not be detected using conventional methods. For example, we have identified novel Astroviruses in diarrhoea samples from European roe deer (*Capreolus capreolus*; Smits *et al* JGenVirol, 2010). In addition, in the bursa of a herring gull (*Larus argentatus*), we have identified sequences from a novel adenovirus and an adeno-associated virus, from which the presence of the first virus was expected based on histopathological examination. These examples indicate that next-generation sequencing can be used to expand our knowledge of the virus diversity present in animals. Cooperation between researchers performing field studies, disease monitoring and diagnosis, and laboratory studies are required to obtain the optimal benefit from these novel techniques.

[71] FIRST REPORT OF RIFT VALLEY FEVER IN TWO WILDLIFE SPECIES IN THE ETOSHA NATIONAL PARK IN NAMIBIA

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Rift Valley Fever is a zoonotic mosquito-borne viral disease causing severe epidemics in Sub-Saharan Africa. RVF is found throughout most of the African continent and outbreaks are probably often underreported because of surveillance systems deficiencies. Wildlife has however rarely been considered as a potential reservoir host for the virus. An opportunity to investigate the role wildlife play in Rift Valley Fever epidemiology arose when, in May 2011, an animal diseases surveillance program was organized in the Etosha National Park, Namibia, during the RVF outbreak in Oshikoto region, as reported by OIE (WAHID). In less than two months 200 springbok (*Antidorcas marsupialis*) and 50 blue wildebeest (*Connochaetes taurinus*) were sampled. This study reports prevalence of anti-RVFV IgG and IgM antibodies, detected with two commercial competitive ELISA kit, and identification of viral RNA by Real-time RT-PCR. IgG antibody prevalence was high both in springboks, 35% (70/200) and in wildebeests, 24% (12/50). IgM and virus RNA were instead observed only in springboks, with prevalence of 15% (30/200) and 9% (17/200) respectively. The viraemic period in livestock can be as short as 6-18 hours or can last up to 6-8 days, but they never become carriers. These data provide evidence that wild ruminants can be infected by RVFV and further studies are need to determine their role in the epidemiology of the disease and whether they can be used as sentinels prior to a noticeable outbreak.

[72] FIRST DETECTION OF A BETA HERPESVIRUS IN A RUMINANT: *CAPREOLUS HERPESVIRUS* 1 (CAPRHV1)

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Herpesviridae is a family of DNA viruses comprising the subfamilies of alpha-, beta- and gammaherpesvirinae. These viruses have surfaced as significant pathogens of multiple hosts, ranging from invertebrates to primates. Several alpha- and gammaherpesvirinae have been detected in wild and domestic ruminants in association with disease, while to our knowledge no member of the beta- subfamily has been detected in these hosts to date. An adult roe deer (*Capreolus capreolus*) from the Canton Bern, Switzerland was found apathic, “star gazing” and displaying erratic behavior. The animal was shot by a game warden and submitted for post-mortem investigation. The animal was in good nutritional condition and no significant gross findings were observed at necropsy. Histologically a monolateral corneal degeneration and necrosis with intranuclear inclusions was observed, along with a mild non-suppurative encephalitis. The tissue lesions were suggestive of herpesvirus infection, which was later confirmed by nested PCR and sequencing. Sequence analysis revealed the presence of an undescribed member of the betaherpesvirinae subfamily, with the proposed name of *Capreolus herpesvirus* 1 (CaprHV1). This is the first report of a beta herpesvirus infection in a ruminant. The presence of lesions consistent with a herpesvirus infection (eye and brain disease) is suggestive of a pathogenic nature. The significance of this agent in wild and domestic ruminants remains to be assessed.

[73] IMMOBILIZATION WITH BUTORPHANOL-AZAPERONE-MEDETOMIDINE IN DESERT AND ROCKY MOUNTAIN BIGHORN SHEEP: RESPONSE TO OXYGEN THERAPY

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Reversible immobilization is desirable for capture of free-ranging wildlife. The drug combination butorphanol-azaperone-medetomidine (BAM) has been physiologically evaluated only in a few species. We studied BAM for immobilization of free-ranging Rocky Mountain (35-71 kg, *n*=7) and desert bighorn sheep (52-57 kg, *n*=5) by ground darting in Canada and USA. A standard dose of 1 ml BAM (27.3 mg B, 9.1 mg A, 10.9 mg M) was used independent of age. Arterial blood samples were collected before and during intranasal oxygen supplementation (6 L/min) and immediately analyzed in the field. For reversal, atipamezole, tolazoline and naltrexone were injected intramuscularly. Recumbency occurred within 3-15 min of initial darting in 10 sheep, whereas two animals required additional drugs. Ear and tail movements, salivation and bloating were observed in some. All sheep developed marked hypoxemia (PaO_2 20-54 mmHg) and mild to marked hypercapnia ($PaCO_2$ 46-65 mmHg). Oxygen therapy was efficient in desert bighorn sheep (PaO_2 124-167 mmHg) and Rocky Mountain lambs and yearlings (PaO_2 112-179 mmHg), whereas Rocky Mountain subadults (2 years old) remained hypoxemic (PaO_2 35-77 mmHg). Recoveries were smooth and calm with sheep walking 2-16 min after administration of reversal drugs. Sedation relapse was observed in one desert bighorn sheep. BAM was successfully used for reversible immobilization of bighorn sheep but further study is needed for treatment of hypoxemia.

[74] IMPORTANCE OF HEALTH PROTOCOLS: CASE OF RELEASING PROGRAMS OF PSITTACINES IN COSTA RICA

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In Latin America, few releasing programs of psittacines have a well-established health protocol since they are based almost exclusively on ethological aspects, but only in a few cases infectious diseases are monitored. In addition, the institutions which are responsible for monitoring projects normally do not handle information properly and some do not have reliable data on the presence or absence of infectious agents or the feasibility of releases, leaving the process to the discretion of the institutions. The goal of this study was to determine if viral diseases, specifically circovirus and polyomavirus, are present in rescue centres working with the release of psittacine birds in Costa Rica. During 2006 and 2007, samples from 59 psittacine birds were collected in three rescue centres in Costa Rica. Of these, 52.5% were positive by PCR to polyomavirus, but none to circovirus. Our results demonstrate the importance of including the health status of psittacines in a release protocol, especially considering the risk of spreading infectious agents to the wild and in the future. Many species could depend on the gene pool of birds that are in captivity. The results obtained will be used to develop a protocol that is mandatory in each process of liberation in Costa Rica. Further studies are needed to determine the prevalence of these viruses in free-living populations.

[75] REASSESSING THE RISKS OF THE REINTRODUCTION OF SAND LIZARDS (*LACERTA AGILIS*) TO THE HEALTH OF FREE-LIVING REPTILES

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A reintroduction programme for captive bred sand lizards (*Lacerta agilis*) to sites in England and Wales has been in progress since 1969. In 2005, the opportunistic pathogen *Serratia marcescens*, a bacterium not yet recorded in free-living reptiles in the United Kingdom, was isolated in pure culture from the intestinal tract of three out of four juvenile captive bred sand lizards examined post-mortem as part of an investigation of a mortality outbreak in captivity. To determine if the reintroduction of sand lizards infected with *Serratia marcescens* posed a significant disease threat to free-living populations of sand lizards and other reptile species, we investigated whether this bacterium was present in free-living sand lizards through the collection of cloacal swabs for bacteriology from 199 free-living sand lizards from Dorset, UK. *Serratia marcescens* was detected in 2% of free-living sand lizards. It was concluded that reintroduction of captive sand lizards harbouring *Serratia marcescens* was unlikely to represent a significant risk to the health of free-living populations of reptiles, on the basis that the bacterium is already present in the wild. This study represents an example of how the risk of reintroduction programmes to the health of free-living populations can be reassessed after the programme has commenced.

[76] EFFECTS OF MULTIPLE POLLUTANTS ON A SINGLE HOST

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For years, toxicological evaluation of hazardous substances has relied mostly upon individual evaluation of compounds. This approach is still the most widely used and products arriving on the market undergo a full evaluation of their potential toxic effects (acute, chronic, reproductive, mutagenic, carcinogenic etc.). It is generally accepted, though, that these compounds will eventually be mixed in the environment and reach plant, animals or humans as combined mixtures. Conceptually, toxicology has moved from the older idea of additive, synergistic or antagonistic effects of mixtures to a more generalized view of additive, more/less than additive (interaction), independent action of chemicals. Both the US and the EU are currently developing strategies to evaluate combined effects of pollutants under these assumptions for humans and environmental purposes. Whenever possible, assessment is based on mechanism of action (MoA), QSAR (quantitative Structure Activity Relatioship) and Threshold of Toxicological Concern (TTC) for individual compounds. This information should be available as much as possible in order to classify compounds in groups of compounds sharing similarities, thus limiting the evaluation to the "simple" additive effect or even reducing the various compounds to a single mixture with its own TTC. The last interesting concept in this field is the "species sensitivity distribution" (SSD), which is very useful to predict a level of concern for any given species based on available data. Several examples of current investigations will be developed, including specific data regarding wildlife (PCBs, anticoagulant rodenticides).

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[77] IMPACTS OF FARMING INTENSIFICATION ON WILDLIFE AND ECOSYSTEM HEALTH

Lindsey Mccrickard[1,2,7], Scott H. Newman[1,2], Borja Heredia[1,3], Edgar Kaeslin[1,2], Kathryn Campbell[1,4], Ruth Cromie[1,5,6]

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In 2009 the world surpassed 1 billion people living in a state of chronic hunger. Current agricultural productivity allows us to feed only a fraction of the world's population. Farming intensification of both crops and livestock is required in order to meet the needs of the world's growing population. By pursuing intensification, producers are able to produce higher quantities of food within a smaller area, although this can come at the cost of degrading the local environment and impacting wildlife populations. These intensive systems, when not appropriately managed, can lead to the emergence of new diseases, pathogens jumping hosts, or severe degradation of the environment causing biodiversity loss. This presentation will outline three specific case examples that highlight the impacts farming intensification can have on disease emergence and wildlife within wetland, grassland and forest ecosystems. Safeguarding food security and concurrently protecting natural resources and ecological health highlights the One Health approach required to achieve success with low impact farming intensification practices as one potential solution.

[78] THE SCIENTIFIC TASK FORCE ON WILDLIFE AND ECOSYSTEM HEALTH: PROMOTING ONE HEALTH ACROSS SECTORS

Lindsey Mccrickard[1, 2], Scott Newman[1], Borja Heredia[1], Ruth Cromie[1], David Stroud[1], Martin Wikelski[1], Kathryn Campbell[1], Dae-Sung Yu[1], Thomas Manyibe Nyariki[1]
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One of the greatest challenges of the 21st century is ensuring global food security and sustainable natural resource management. Various methods of coping with food insecurity have been utilized including intensification and expansion of livestock farming and aquaculture, increasing pressure on forest-derived species as a food source, and farming of broader diversity of wildlife species and at higher densities. To date, a balanced approach has not been found, resulting in unhealthy ecological and agricultural systems that provide opportunities for pathogen jumping species, expanding ranges, or emerging and increased non-infectious diseases including nutritional and toxic conditions. The One Health framework, a concept that states the areas of human, livestock, wildlife, and environmental health are all highly interconnected, serves as an excellent approach to resolving complex multidimensional issues like this. The Scientific Task Force on Wildlife and Ecosystem Health, co-convened by UNEP CMS and FAO, works to ensure that disease dynamics are considered in the broader context of sustainable resource management, agricultural production, food security, socio-economic development and environmental protection and wildlife conservation. The Task Force facilitates coordination, information-sharing, and communication between relevant organizations at a global level.

[79] CIBZ: ONE HEALTH IN SOUTH AMERICA FROM THEORY TO PRACTICE

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South America has huge wildlife diversity; however there is scarce data available about diseases and limited specific resources (human and founding) for health management. Buin Zoo Conservation and Research Department (CIBZ) were created in 2010 with "One Health" as philosophy, and the mission to address the wildlife health management based on scientific criteria. Our goal is to serve as a tool to answer questions such as What, Who, Where and When in wildlife diseases research and develop management proposals, covered by interdisciplinary and interinstitutional working networks. We have created three programs on wildlife: education and training, disease surveillance, and health issues for management in the region. After two years of work, through the first program we have accessed to more than 400 students and professionals in the region in different issues regarding wildlife and zoo animals. Through the second program we established a serum and samples bank for develop health screening on more than 500 captive wild mammals, for different infectious pathogens (*Brucella abortus*, Canine Distemper Virus, MAP, *Leptospira interrogans*, MRSA, Bovine viral diarrhea virus, *Salmonella sp*., *Toxoplasma gondii*). We are currently working to provide information on the health status of four endangered mammals' species in the region. Through these efforts we pursue increasing standards levels of wildlife health management in natural and artificial environments in South America.

[80] A ONE HEALTH APPROACH TO WILDLIFE DISEASE SURVEILLANCE IN AUSTRALIA

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The Australian Wildlife Health Network (AWHN) links and informs people who deal with wildlife disease. We work with federal and state conservation, agriculture and human health departments, universities, zoos, private practitioners and wildlife carers, hunting industries and diagnostic pathology services, and others with an interest in wildlife health. AWHN provides Australia with a unique framework for managing wildlife health and disease in the One Health arena. Wildlife surveillance and incident response is particularly challenging. Activities not only require coordination across jurisdictions, but across multiple agencies, organizations and industries, often with loosely defined stakeholder groups. Wildlife disease surveillance is part of Australia's national animal health information system and used by animal, human health and environment agencies to inform policy and for better management of any disease that has wildlife as part of its ecology. AWHN improves communication and coordination; through technical advice, facilitation and professional support; ensuring better investigation and management of wildlife health in support of human and animal health, biodiversity and trade. Rapid and timely access to information and a network of wildlife health professionals in Australia is crucial to our decision making and to enable coordinated response to disease incidents. This paper will discuss wildlife networking in a One Health Space using three examples.

[81] DATA STANDARDIZATION AND WILDLIFE HEALTH DATA QUALITY

Tammie O’Rourke[1,8], Damien O. Joly[1], Jonathan Palmer[2], Sarah Olson[1], Melissa Manhas[1], Daniel O'Rourke[1], Peter Rabinowitz[3], Matthew Scotch[4], Tracey Goldstein[5], Christine Kreuder Johnson[5], David Wolking[5], Matt Lebreton[6], William Karesh[7], Jonna Mazet[5]

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Data standardization is critical to digital data collection systems to ensure data consistency and efficiency. Other benefits of maintaining data standards include increased data compatibility, reduced data redundancy, improved data access for reporting and data analysis and allows for the data to be easily aggregated and compared using various dimensions. However, data standards can also impose constraints on a project and must be approached collaboratively and with an evolutionary approach. The USAID Emerging Pandemic Threats - PREDICT project is a multi-country endeavour led by the University of California - Davis Wildlife Health Center to develop the global capacity to detect emerging zoonotic diseases of wildlife origin. Data standards are imperative to the projects' information collection process due to the complexity of collecting surveillance data, test results and genomic data from 5 organizations and 23 countries. The Global Animal Information System (GAINS) development team, in collaboration with the wildlife field staff and senior management, has developed a set of standard terminology, database collection templates and an extensive database structure to facilitate in the data requirements for the project. These standards have been adopted by the participating 5 organizations and has become invaluable to the success of the data collection initiative of the PREDICT project. The resulting datawarehouse is the source of extensive, quality data for monitoring the progress of the project, as well as ancillary data mining and analysis for assessing numerous wildlife health objectives.

[82] COLLABORATIVE APPROACHES TO IMPLEMENTING ONE HEALTH PROGRAMS

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The problems of a global society are increasingly complex and interdependent and, consequently, are not isolated to particular groups or disciplines. Many of the environmental problems we face today are characterized by such complexity. Issues and phenomena such as climate change, maintenance of biodiversity, pollution, and one health are not only biologically complex, but are technically and socially complex on a global scale. Partnerships and collaborations at local, national, regional and global levels are a critical component of the One Health approach to controlling infectious diseases. Such partnerships require participation by local and national government agencies, non-governmental organizations, industry and universities, and international organizations such as the OIE, FAO, and WHO. We believe that effective, enduring collaborations on detecting and managing infectious diseases, similar to other sciences, are formed primarily through a transdisciplinary approach. Transdisciplinarity grounds complex issues in their ecological and social context, and enable decision makers to reach across agencies and disciplines to strengthen the basis for sustainable ecosystems, health, and development policies. We review the primary barriers and keys to successful collaboration, the main types of collaboration used in science, and discuss why transdisciplinary approaches are essential in addressing complex issues such as emerging infectious diseases.

[83] MONITORING WILDLIFE DISEASES TO ADDRESS WILDLIFE CONSERVATION AND PUBLIC HEALTH CONCERNS IN BOLIVIA

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Despite their importance for conservation and zoonotic potential, few studies on wildlife diseases have been undertaken in Bolivia. Since 2010, health evaluations were carried out on three ungulate species, which are part of the Tsimane´ indigenous communities diet. Exposure to brucellosis was detected in 23% of *Tayassu pecari* and 21% of *Pecari tajacu. Fasciola hepatica* was identified in 83% of *Mazama americana* while *Ascaris suum* (6%) and cysticercosis antibodies (14%) were present in *Tayassu pecari*. Even though further information is required to determine if and how these pathogens might affect wildlife populations and humans, efforts are underway to improve hygiene practices among indigenous peoples, and to create awareness on the need to monitor wildlife diseases at high-risk interfaces. Additionally, wildlife disease diagnostic capacities are being enhanced and collaboration with public and animal health government agencies is being facilitated. As a result, in 2012 a flavivirus epizootic was promptly detected in a wild *Alouatta sara* population from Santa Cruz, allowing for the implementation of preventive measures to protect surrounding human populations from a possible yellow fever outbreak. These actions have prompted recognition of wildlife disease surveillance needs as the National Plan for Integrated Zoonotic Disease Management in Bolivia, a penta-ministerial effort, is being developed.

[84] WILD MEAT, LIVELIHOODS, AND SUSTAINABILITY: A ONE HEALTH APPROACH TOWARDS AN INTEGRATED APPROACH FOR THE MANAGEMENT OF EMERGING RISKS ASSOCIATED WITH THE USE OF WILD ANIMALS AND THEIR PRODUCTS

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The sustainable use of wildlife has traditionally contributed to food security in developing countries. It provides a protein source and generates income supporting the livelihoods of local communities. Extractive use of wild animals, however, has increased rapidly over recent decades, moving from local use towards large - scale commercial enterprises, fostered by human population growth, urbanization and globalization. This has led to unsustainable exploitation of the resource, and to the development of informal markets, which lack health safeguards and undermine local populations' interests. It also involves associated risks: the depletion of a resource contributing to food security and ecosystem health; an increased exposure of humans, domestic and wild animals to pathogens; and a threat to biodiversity and species conservation in general. Sustainable use of wild animals and their products requires considering food, health, ecological, economic and cultural dimensions. Prevention and control should be based on a multidisciplinary and collaborative approach taking into account multiple science - based assessments. This programme relies on the development of a holistic methodology to support the development of national policies for the sustainable management of wildlife resources by local communities, including food security, public health, food safety, environment and development aspects. It is clearly an operational application of the One Health approach.

[85] ONE HEALTH INITIATIVE: UNITING HUMAN, VETERINARY AND ENVIRONMENTAL MEDICINE

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The WDA's One Health orientation brings an important dimension with a focus on the role of wildlife in zoonotic diseases and in conservation.

Mission

Human and animal healths are linked. The One Health Initiative (OHI) promotes, improves, and defends health and well-being of all species by enhancing cooperation and collaboration between physicians, veterinarians, and other scientific health professionals and by promoting strengths in leadership and management.

Vision: OHI is dedicated to improving the lives of all species—human, animal and plant.

One Health shall be achieved by joint efforts in:

- education
- communication among professionals
- clinical care
- cross-species disease surveillance and transmission prevention and control
- comparative medicine research
- development of new diagnostic methods, medicines and vaccines
- informing and educating political leaders and the public

The OHI website (http://www.onehealthinitiative.com) is a global repository for all OHI news and information. The OHI website has a daily average of over 300 visitors from over 130 countries, with open, free access to all, worldwide.

Any health scientist may be added to one and/or both lists (contact: kkm@onehealthinitiative.com).

[86] INFORMATION CENTER OF WILDLIFE DISEASES IN BRAZIL: PARTICIPATORY MONITORING FOR BUILDING SENTINEL MODELS FOR HUMAN HEALTH AND BIODIVERSITY CONSERVATION

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Monitoring and building predictive models for zoonotic diseases are a difficult task especially when they originate from biodiversity. In Brazil, the monitoring of disease rates, as mortality and morbidity are conducted by the Ministry of Health for human diseases and the Ministry of Agriculture and Livestock in the case of disease of farmed animals. The prevention mechanisms are mostly focused on immunization, specific education programs and a few other prophylactic measures without any approach with complex ecosystems modeling. Seeking to fill this gap and monitor the emergence and reemergence of diseases arising from the Brazilian biodiversity, the Oswaldo Cruz Foundation is building an "Information Center of Wildlife Diseases" to monitor etiological agents with potential disruption of biological barrier to humans. The occurrence of vectors, hosts, etiological agents and environmental changes will be accompanied through a participatory information system built by the concepts of citizen science and crowding computing powered by information posted by cellphones or internet. The geo-referenced reporting of occurrences made by population and municipal health, environment and agriculture services, connected with a specialist's board and network diagnostic laboratories will provide reliable information for health and conservation politics and database for complex predictive and sentinel models.

[87] THE SMITHSONIAN-MASON SCHOOL OF CONSERVATION: TRANSFORMING CONSERVATION BIOLOGY LEARNING IN THE 21ST CENTURY

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The complexity of today's world and the ever-evolving threats to biodiversity demand forward-looking approaches to conservation biology. In response, George Mason University and the Smithsonian Institution have forged an exciting partnership to establish the Smithsonian-Mason School of Conservation (SMSC). Its approach to learning establishes a benchmark for innovative education for current and future generations of global conservation professionals, leaders, and practitioners. The SMSC offers a range of residential, hands-on and trans-disciplinary programs in conservation for undergraduates, graduate students and professionals from around the world. Highly qualified instructors, including Smithsonian scientists, Mason faculty, and colleagues from national and international organizations expose students to compelling research and immerse them in the practice of conservation. Participants thrive in an atmosphere of creative, analytical thinking on how to solve some of the most intractable conservation problems facing society today. The SMSC offers its graduates the opportunity to become part of a lifelong, global community that supports visionary thinking and leadership in conservation.

[88] CAUSE-SPECIFIC MORTALITY STUDIES: ARE DISEASES BEING UNDERESTIMATED?

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Describing the relative frequency of different causes of mortality within a population is an important component of conservation research. This review aims to assess to what extent diseases are considered in this type of studies. Of 76 articles, 23 had veterinary participation (VP; 6 of them had a coauthor affiliated to a veterinary institution and in other 17, carcasses were sent for necropsy to a pathologist). Diseases were more frequently mentioned in the Introduction or considered among the potential mortality factors in articles with (49% and 87%, respectively) than without (9% and 17%) VP. Predation was identified as the main mortality factor in both groups, but it was more frequent among works without VP (63% vs 39%). Disease was the most frequent cause in 4 studies, all of them with VP. Mean frequency of disease was 8% in studies with and 17% in studies without VP, and 32% if a coauthor was affiliated to a veterinary institution. No differences were found in the frequency of unknown causes between studies with (20%) or without (14%) VP. Twenty-two studies, all of them without VP (41.5% of this group), included mortality factors under terms such as “natural causes”, “starvation”, or “malnutrition”, and 8 of them identified one of these causes as the most frequent mortality factor. None of the above-mentioned factors improved over time. I conclude that the relative importance of diseases is underestimated in many ecology studies, what can affect conservation strategies.

[89] EVALUATION OF SANITARY STATUS OF DOMESTIC AND WILD FAUNA THAT SHARE PATHOLOGIES AND POTENTIAL HABITAT WITH THE IBERIAN LYNX – PRELIMINARY RESULTS - PROJECT LIFE+ ENHANCING HABITAT FOR THE IBERIAN LYNX AND BLACK VULTURE IN THE SOUTHEAST OF PORTUGAL

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Wildlife diseases can represent a serious conservation threat for free-living populations of endangered species. Small, geographically isolated and genetically depleted populations, suffer from a progressive loss of diversity that potentially increases their susceptibility and decreases their response to infectious agents. Coordinated programs to evaluate the sanitary status of animal populations are very important to the success in conservation of endangered species small populations, such as the Iberian Lynx. Since healthy reproductive individuals are essential in viable populations, it is crucial to better understand the threat and potential impact that agent diseases might pose to the species. This evaluation is especially important on animals that share the same habitat in areas of potential occurrence of the Iberian Lynx. For this purpose, an epidemiological survey was drawn, consisting on a sample collection and laboratory testing of biological material from domestic and wild animals to detect and quantify the presence of pathogenic agents that might affect the Iberian Lynx. A better understanding of diseases' transmission and distribution in these populations will allow a more precise targeting of control measures, assuring adequate habitats for the species presence and coexistence with other wild and domestic animals. The information gathered will allow the elaboration of a monitoring/control plan for the major pathologies found.

[90] THE HARMONIZED MONITORING PROGRAM OF SELECTED ZOONOTIC DISEASES OF WILDLIFE IN LOMBARDY, NORTHERN ITALY

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In Italy, as the rest of Europe, changes in agricultural land use and in wildlife management practices have influenced the population dynamics of wildlife, often leading to an overabundance of species in several areas. In Lombardy, a Northern region laid between the Alps and the Po river, the interest in the detection of wildlife infectious diseases has considerably grown in the last years, and monitoring control programs in wildlife have been carried out for more than 10 years. Based on this experience and on the knowledge of the health status of wild populations, we planned an harmonized monitoring program toward zoonotic diseases providing a common approach in all the areas involved in the project, from sampling schemes to diagnostic laboratory methods. Specific diagnostic protocols were developed for each species, in particular for those that are under specific hunting planning, such as wild ungulates, hare and red fox. Different contexts can influence the diseases transmission between wildlife, livestock and human and can increase the spread of pathogens. The adoption of specific monitoring programs is needful to identify changes in their occurrence and also to detect unusual wildlife mortality. Moreover only a structured plan allows to optimize the necessary resources, and also to promptly react to new and emerging wildlife diseases.

[91] SPATIAL APPROACH FOR THE ASSESSMENT OF BORRELIOSIS BURDEN IN BELGIUM: A MULTI-HOST PATHOGEN AND A GENERALIST VECTOR

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Lyme borreliosis is a major concern in parts of the world but precise distribution of the pathogen is mostly unknown. In Belgium, the disease burden in human is difficult to assess as cases are not automatically notified and disease burden on mammals, birds and domestic animals is largely overlooked. In this context, this study identifies, to the best of current knowledge, the distribution of Lyme disease pathogen in Belgium and discusses ecological interactions and environmental state which could have led to increase in the disease burden. An extensive geographical review of the distribution recorded more than 300 sites where pathogens/positive serologies occur, by geo-locating historical and recent records from the literature, unpublished dataset and other surveys. If the distribution seems more concentrated regionally according to human cases records, pathogen in cattle are more spread out in the country. The variety of host and disease burden seems wider than previously thought. Occurrence of the pathogen in ticks specific for birds in Belgium, bats in United Kingdom and hedgehogs in Switzerland suggest successful pathogen life cycle in specialised niche with transmission by specialised vector. However, co-occurrence of the generalist *Ixodes ricinus* species on those same hosts serves as a bridge to transfer pathogens between host groups in a same area. The increase abundance of ticks might be linked to disequilibrium of ecosystem and reduction of ticks natural predators.

[92] TICK-BORNE AGENTS IN WILD ANIMALS FROM AMAZON, BRAZIL: PARTIAL RESULTS

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Wild animals have been considered as potential reservoirs of pathogens and/or zoonotic agents and could represent a valuable source of information about the diversity of parasites. The objective of this study was to verify the presence of tick-borne diseases in free living wildlife animals in the states of Mato Grosso and Pará, Amazon region of Brazil. For this purpose, samples of lungs and spleen were collected from hunted wild animals from indigenous communities (Mato Grosso) or road-killed animals (Pará) from September 2010 to November 2011. Animal tissues were stored at -20°C and sent for DNA extraction and PCR targeting a 18S rRNA-gene fragment for detection of Piroplasmida hemoparasites. From 165 samples, 4.2% (n=7) were positive and the products were subjected to DNA sequencing and analysis of similarity with sequences available in GenBank. Five sequences amplified from different vertebrate hosts (*Dasyprocta agouti, Dasypus novemcinctus, Mazama americana*, *Tayassu pecary* and *Didelphis marsupialis*) showed 96% similarity with the genus *Theileria*. One sample from *Leopardus pardalis* was most similar (98%) to *Cytauxzoon felis;* and a sample from *Cuniculus paca* was most similar (93%) to *Babesia* sp. Preliminary results suggest a high diversity of hemoparasites from the Order Piroplasmida in wild mammals from the Amazon region.

[93] SMALL MAMMAL DIVERSITY AND *LEISHMANIA* INFECTION ACROSS A FOREST COVER GRADIENT IN SOUTHERN COSTA RICA

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Anthropogenic activities have transformed the landscape leading to gradients of forest cover worldwide. These fragmented and heterogeneous landscape patterns have been associated with the emergence and transmission of Cutaneous Leishmaniasis in Southern Costa Rica. One possible mechanism behind the emergence of this neglected tropical disease is the change in the biodiversity and abundance of mammal species, that can serve as reservoirs of Leishmania parasites, and that is mediated by concurrent changes in the landscape. Here, we present results of one year of small mammal species sampling using the lineal transect sampling effort to estimate demographic parameters from wildlife populations employed along several plots across a forest cover gradient in southern Costa Rica. We think the use of this sampling methodology can improve our understanding of the transmission dynamics of this disease by providing demographic information necessary to understand the eco-epidemiology of this pathogen in the reservoirs. Anthropogenic activities have transformed the landscape leading to gradients of forest cover worldwide. These fragmented and heterogeneous landscape patterns have been associated with the emergence and transmission of Cutaneous Leishmaniasis (CL) in southern Costa Rica and Panama. Understanding the fragmentation effect over reservoir mammals' population dynamics will help to explain a possible mechanism behind the emergence of this neglected tropical disease. Potential changes in mammals' biodiversity and abundance can influence levels of leishmania parasites in this reservoir population. However, in order to establish epidemiological baseline data, it is necessary to find out more about the species identified as CL reservoirs in areas where human incidence of the disease has been well reported. Such variables are mammal population size, abundance or prevalence of CL parasites within the mammals' reservoir population under study. We choose as a sampling method using the lineal transects sampling effort, to estimate demographic parameters from wildlife populations employed along several plots across a gradient forest cover at Bajo de Savegre, southern Costa Rica.

[94] WILDCOMS (WILDLIFE DISEASE & CONTAMINANT MONITORING AND SURVEILLANCE): A NEW KNOWLEDGE EXCHANGE NETWORK

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Disease and contaminants can pose major risks to wildlife and Man. Disease can be a natural driver that affects wildlife populations but some diseases warrant particular attention because: they have been precipitated by anthropogenic change, of their severity, their threat to rare species or because they are transmitted to people. The risk that environmental contaminants can pose to wildlife has been repeatedly demonstrated and a classic example is the impact of organochlorines on otters in Britain. The Wildlife Disease & Contaminant Monitoring and Surveillance (WILDCOMS) network (http://www.wildcoms.org.uk/) brings together nine major UK surveillance schemes that monitor one or more sentinel species for multiple diseases and/or contaminants. The overall aim is to foster and facilitate knowledge exchange, harmonisation towards best practice, greater sharing of resources, and productive collaboration between the different surveillance schemes and their many end-users. In particular, WILDCOMS will provide an integrated, national overview of environmental disease and contaminant risk and maximise dissemination of such information.

[95] POPULATION STRUCTURE AND BODY CONDITIONS RELATED TO FEEDING BEHAVIOR OF YELLOW BABOONS AT MIKUMI NATIONAL PARK, TANZANIA

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Comparison on population structure and body condition score (BCS) as a health parameter in relation to feeding behaviour was carried out for three known troops of yellow baboons (*Papio cyanocephalus*) at Mikumi National Park. The troop included (i) Mwanambogo - which obtain its feeds entirely in nature, (ii) Lyambangali - mostly using household garbage around the park headquarters and (iii) Kikoboga - regularly found scavenging along the Dar es salaam-Iringa highway. In all these, troop size, sex, age and BCS of each individual encountered was noted, determined and recorded. During the study, a total of 176 baboons were observed and counted. Large troop size (n= 61, 34.7%) was observed for Lyambangali followed by Mwanambogo (n= 59, 33.5%) and Kikoboga (n= 56, 31.8%) respectively. In terms of BCS, 85% (n =52) of Lyambangali troops scored very good as compared to about 75% (n =44) for Kikoboga and Mwanambogo respectively. Household garbage appears to supply sufficient feeds to Lyambangali troop as embodied by good body condition as well as small female to offspring ratio. The dependence on household and roadside garbage for Lyambangali and Mwanambogo troops is an emerging problem, which is already a nuisance to the park residents as well as tourists. It also increases the risk of disease transmission. The study recommends protective measures to be developed and applied to prevent the aberrant behaviour in yellow baboons at Mikumi National Park.

[96] HEALTH STATUS OF WILD GREY-CROWNED CENTRAL AMERICAN SQUIRREL MONKEYS (*SAIMIRI OERSTEDII CITRINELLUS*) BY THE MANUEL ANTONIO NATIONAL PARK, COSTA RICA

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In Costa Rica, the Grey-crowned Central American squirrel monkey (*Saimiri oerstedii citrinellus*, SOC) is considered an endangered subspecies, because of high population isolation, land use practices, diseases, pesticides and electrocutions. The SOC populations live mainly in the Manuel Antonio National Park (MANP)–and surrounding fragmented and altered areas, where there is high tourist visitation year around. This study was carried out to determine the health status of SOC in and near MANP. During 2010 and 2011, eight individuals (5 females and 3 males; 5 adults, 2 juveniles and 1 infant) were captured by temporary chemical immobilization. A physical examination was performed and samples for clinical, parasitological, mycological and virological analysis were taken. All animals scored 3 at a 1 to 5 body condition scale (where 5 were the best); hematocrit and hemoglobin values were between 30-41% and 10.4-11.8g/dl, respectively. Endoparasites were found: four fecal samples were positive to *Strongyloides* sp., 2 to Strongylida, 1 to Spiruridea eggs, but no ectoparasites were detected in any of the animals. In one ear swab, *Malassezia pachydermatis* and *Candida spp.* were detected. Blood samples were analyzed by PCR to determine presence of Plasmodium (*P. falciparum*, *P. vivax*, *P. malariae*), Dengue Virus (types 1, 2, 3 and 4), West Nile Virus and Equine Encephalitis Virus (Venezuelan, East, West). Only one female was positive to *P. falciparum*.

[97] SEROLOGICAL AND MOLECULAR DETECTION OF WEST NILE VIRUS IN NON-HUMAN PRIMATES FROM COSTA RICA

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West Nile Virus (WNV) was introduced in the Northeastern United States in 1999. In Costa Rica, according to the National Service for Animal Health (SENASA), WNV was first documented in 2009, and is currently considered endemic across the country. In order to assess the distribution of WNV in wild non human primates, the four occurring wild species were captured and released throughout the country for collection of samples between 2000 and 2011. The wild species are the howler monkey (*Alouatta palliata,* HM), the white-faced capuchin (*Cebus capucinus,* WFC), the spider monkey (*Ateles geoffroyi,* SM), and the titi monkey (*Saimiri oerstedii,* TM). Samples included 146 sera and plasma, that were analyzed using a competitive ELISA (ID-Vet Innovative Diagnostics, France); and 169 blood samples that were analyzed using RT-PCR. Forty five samples (30%) showed antibodies against WNV. The distribution by species was: 22 of 97 HM, 20 of 36 WFC, 3 of 10 SM, and 0 of 1 TM. The positive individuals where captured on the years 2001 (10), 2002 (7), 2008 (1), 2010 (4), and 2011 (23). A total of 4 blood samples yielded positive results in the RT-PCR test, they were taken in 2011 from wild HM troops located in Guanacaste, and sites were the forest cover is highly fragmented. These results are the first report of serological and molecular detection of WNV in non-human primates from Costa Rica, and show the importance of early detection and surveillance for national wildlife health programs.

[98] EXPOSURE TO SELECTED PATHOGENS IN FREE-RANGING OWL MONKEYS (*AOTUS AZARAI AZARAI*) IN FORMOSA, ARGENTINA

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The owl monkeys *(Aotus azarai azarai)* of the Argentinean Chaco are an excellent model to evaluate the potential effects of fragmentation on population dynamics and health since they inhabit both continuous gallery forests and forest islands surrounded by human-modified cattle-grazing grasslands. To compare the health status of owl monkey groups living in both habitat types, between February and April 2011, eight adult owl monkeys were immobilized in a gallery forest within our core study site at Estancia Guaycolec in Formosa. Blood samples were collected and screened for selected pathogens. All animals were negative for hemoparasites on blood smears, and microfilaria by Knott test. Evidence of exposure to yellow fever (serology) and *Manzonella ozzardi* (PCR) was also negative. One animal was positive to *Trypanosoma cruzi* by kDNA-PCR (kinetoplast DNA) but negative by Sat-DNA-PCR (nuclear DNA). Because the latter method is more specific for *T.cruzi*, it is possible that a different *Trypasonoma* spp. was detected. *Leishmania Viannia braziliensis* DNA was found in four owl monkeys by PCR-RFLP (Restriction Fragment Length Polymorphism) and sequencing. To the best of our knowledge, this is the first report of *L.V. braziliensis* in this species. Comparisons between these findings and those of owl monkeys in forest islands will shed light on the influence of fragmentation on population health, which will in turn contribute to guide owl monkey conservation strategies.

[99] SURVEY OF INFECTIONS TRANSMISSIBLE BETWEEN BABOONS AND HUMANS IN CAPE TOWN, SOUTH AFRICA

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Baboons on the Cape Peninsula in South Africa exhibit substantial spatial overlap with urban, agricultural and tourist areas and consequently have frequent contact with people. Sharing space and food poses a risk of the baboons passing infection to, or gaining infection from, humans. The aim of this study was to ascertain whether human diseases are present in this baboon population. A sample of 27 baboons from five troops was screened for 10 zoonotic infections of public health importance. The majority of baboons (56%) tested positive for antibodies reactive or cross-reactive to human cytomegalovirus, hepatitis A virus or Epstein-Barr virus. There was considerable variation in virus immunity among baboon troops, suggesting that zoonotic infection prevalence in baboons may be positively correlated with the proportion of urban land in their habitat. No evidence of infection with measles, polio, tuberculosis, or pathogenic faecal bacteria was found. We conclude that baboons on the Cape Peninsula pose a low but potential risk for transmitting zoonoses and that they might be at risk from anthroponoses. The information gained from this study is assisting the development of baboon management plans to minimise infectious disease risks to humans and baboons.

[100] IMPLEMENTING ONE HEALTH IN GOMBE NATIONAL PARK, TANZANIA

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Many chimpanzee populations are threatened by loss of habitat due to human encroachment. This results in 1) genetic and spatial isolation of endangered chimpanzee populations, and 2) increased human-wildlife contact. Genetic isolation leads to a potential decrease in long-term viability of the population, while increased human-wildlife contact leads to an increased risk of disease transmission between chimpanzees and humans. Wildlife managers in parks containing great apes perceive that disease outbreaks have been, and continue to be, a significant threat to conservation goals for wild apes. Many documented or perceived disease outbreaks in chimpanzees are suspected to be the result of close contact with humans, but definitive evidence is scarce. On the other hand, there is at least one well documented emerging infectious disease of humans transmitted from chimpanzees – HIV-1. Gombe National Park, Tanzania currently hosts the longest continuous study of wild chimpanzees (Jane Goodall Institute), spanning 52 years. It is Tanzania's smallest National Park (35Km^2), and is surrounded by a high-density of humans. These characteristics make Gombe both the perfect storm for human – non-human primate disease transmission, as well as the perfect wild laboratory for the creation and implementation of the One Health paradigm. A 10-year review of the design, implementation and results (including the discovery of pathogenic SIV) of a One Health program in/around Gombe follows.

[101] INTERACTION BETWEEN HOST DISPERSAL AND PARASITE SPATIAL STRUCTURING AND SPREAD IN LONG-TAILED MACAQUES

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The relationship between host dispersal and parasite spatial structuring is one of ultimate importance in understanding infectious disease transmission. On the Indonesian island of Bali, long-tailed macaques (*Macaca fascicularis*) thrive in populations associated with large Hindu temple complexes across the island. The interactions of macaques within and between temple sites create a dynamic system to study the role of host population structure on parasite burdens. Using GIS tools combined with genetic analyses of 15 macaque populations, we apply three analytical approaches to understand these relationships. In the first approach, we make a direct comparison between genetic and geographical distance using Mantel tests. In the second approach, we incorporate GIS data for Bali in two spatial genetic analysis tools – kriging and wombling – to understand how macaque utilization of the anthropogenic landscape limits or enhances dispersal and gene flow. Finally, we incorporate parasite burden into our analysis to evaluate how well spatial and genetic structuring of host populations corresponds to parasite distributions to identify potential barriers and corridors for parasite transmission and spread.

[102] SURVEY OF *LEISHMANIA CHAGASI* IN NEOTROPICAL PRIMATES FROM THE STATE OF SÃO PAULO, BRAZIL

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American visceral leishmaniasis (AVL) caused by *Leishmania (L.) chagasi* is a worldwide zoonosis with impact on public health due to morbidity, mortality and high incidence rates. Several studies on the role of wild mammals as AVL reservoirs have been conducted. However, little is known about the importance of neotropical primates (NTP) in the epidemiological chain. The goal of this study was to investigate the occurrence of *L. chagasi* infection in free ranging NTP from the Atlantic rain forest (n = 95) and from Rescue Centers (n= 56) in the state of São Paulo, Brazil, using serological tests (ELISA and IFI), immunohistochemistry (IHC) of intact skin and PCR-RFLP of blood samples. The results showed that 81% (17/21) of PCR-RFLP positive animals were *Callithrix* sp. Serological test indicated that 3.6% (2/56) of RC-NTP and 10.5% (10/95) of FR NTP were positive for IgG anti-*Leishmania* spp. Moreover, 13.9% (21/151) were PCR positive for *L. chagasi,* being 5.4% (3/56) from RC and 19% (18/95) from FR NTP. All animals were clinically healthy and no parasites were found through skin histopathology and IHC. The present results suggest transmission of *L. (L.) chagasi* among NTP from Atlantic Forest and Rescue Centers in the state of São Paulo, which could be an important public-health problem. We believe that further studies, like those on xenodiagnoses, should be conducted in order to clarify the role that NTP play in this important zoonotic process.

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[103] THE HUSBANDRY, WELFARE AND HEALTH OF CAPTIVE AFRICAN CIVETS (*VIVERA CIVETICA*) IN WESTERN ETHIOPA

The OIE kindly provided a full support to the conference attendance of this participant

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A study was carried out in Jimma zone, western Ethiopia to evaluate the husbandry and health of captive African civets (*Vivera civetica*). Wild civets were found in the wild in all 13 of the districts in the zone, although traditional civet-keeping was practiced in only five. Civet management practices were determined via the use of a questionnaire survey of 15 farms; containing a total of 107 civets. Health was assessed by routine clinical examination, and examination of faecal and blood smears of 55 civets selected at random. All civets were male and over one year of age, with a mean weight of 12.5 ± 0.79 kg. Fifteen percent were in poor body condition, and only 13% had a good body condition score. An average of 7.13 civets was kept on each farm. Farmers obtained wild civets by either trapping them themselves, purchasing them from dealers or a combination of both. The civets were housed separately in wooden cages, with an average size of 1.0 × 0.5 × 1.0 m (length × breadth × height) and kept in a communal thatched room. They were fed boiled meat, milk (fresh or powdered), eggs, butter, corn soup and fruit juice. Although an assessment of the behavioural parameters of welfare were outwith the scope of this study, trapping methods, adaptation processes, housing condition, restraint and the techniques for musk extraction from the anal glands were stressful and injurious, and have important welfare implications. Approximately 20 g of musk was expressed from a single civet every 9-15 days. The civets often sustained injuries while being restrained during musk harvesting; 14% had swelling and bruising, 6.5% fractures and 11.2% had eye lesions. Cestodes were the most prevalent gastrointestinal parasites, followed by ancylostomes, ascarides and *Tricuris* spp. Skin lesions were identified in 19.6% of civets examined and an assortment of fleas and ticks including *Haemophysalis leachi*, *Rhipicephalus* and *Amblyoma* spp were found on the body. *Trypanosoma congolense* and *Babesia felis* were identified in blood smears taken from four animals. This study shows there is an urgent need to invest in research into improving the welfare, husbandry and health of civets, as well as providing educational programmes for those who farm these animals.

[104] FOREST FRAGMENTATION AS A RISK FACTOR FOR CANINE DISTEMPER VIRUS IN ARAUCANIA REGION IN CHILE

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Increased human population and transformation of original habitats to productive land have led to increased contact between domestic animals and wildlife, facilitating pathogen transmission. Canine distemper virus (CDV) is mainly maintained in domestic dog populations which can be source of infections to wild carnivores. The goal of this study was to assess whether forest fragmentation is a risk factor for canine distemper virus exposure. From November 2010 to April 2011 a questionnaire serosurvey was carried out in an area of 200 km^2 surrounding the Nahuelbuta National Park in the Araucanía region (38°27'S, 73° 05'W, 38° 27'S, 71°59' W) in Chile. Epidemiological questionnaires were applied to 130 household-owners. After the interview blood samples were obtained from 80 domestic dogs and then centrifuged and sera kept at -20ºC until analysis. After the interview coordinates of each household were recorded and then transferred to a GIS system (Arc GIS 9.3) to assess the native forest area surrounding each household. Serum samples to determine seropositivity to CDV were analyzed using an IgG ImmunoComb® test (Biogal, Israel). Multivariable logistic regression analysis indicate that after testing for 20 explanatory variables, the probability of detecting CDV seropositive dogs increase with age and with lower native forest area in a radius of 100 mt surrounding each household, suggesting an effect of fragmentation on CDV epidemiology.

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[105] SEROSURVEY OF CANINE DISTEMPER AND CANINE PARVOVIRUS IN WILD AND DOMESTIC CARNIVORES IN THE ARAUCANIA REGION IN CHILE

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Domestic dogs are recognised as the reservoir for many pathogens of wild carnivores including canine distemper virus (CDV) and canine parvovirus (CPV). Humans and their dogs are colonizing wild areas and threatening the conservation of endangered species. The goal of this study was to assess the exposure of CDV and CPV in free-ranging foxes surrounding protected areas and rural domestic dogs in an area of high priority for carnivore conservation. From November 2010 to March 2012 free-ranging foxes and rural domestic dogs were blood sampled in two areas the Araucanía region in Southern Chile. Overall twenty foxes of three species, the endangered Darwin fox (4), the culpeo fox (14) and chilla fox (2) were trapped. Additionally, 37 dogs were sampled. Blood sampled were centrifuged and sera kept at -20ºC until analysis. Serum samples to determine seropositivity to CDV and CPV were analyzed using an IgG ImmunoComb® test (Biogal, Israel). Chi-square and Fisher test were used for statistical analyses. No differences were found in the CDV and CPV seroprevalence between both sites for domestic dogs and foxes. Overall, the seroprevalence of CDV was 32% and 5%, and for CPV was 65% and 15% for dogs and foxes, respectively. Higher prevalence was found for both pathogens in domestic canids. The preliminary results of this study indicate that rural domestic dogs can be a potential reservoir for pathogens of conservation concern in southern Chile.

This study was funded by Fondecyt Nº 11100303.

[106] ENDOPARASITES OF PUMA (*P. CONCOLOR*), KODKOD (*L. GUIGNA*) AND FOXES (*PSEUDALOPEX* SPP.) IN THE NAHUELBUTA NATIONAL PARK IN CHILE

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Macroparasites can differentially affect wildlife species depending on ecological factors or host susceptibility, among others. The importance of parasites on the population dynamic of wildlife is only recently recognized. The goal of this study was to determine the biodiversity of endoparasites affecting the carnivore's mammal community in the Nahuelbuta National Park in central Chile. From September 2010 to February 2011 fresh fecal samples of three carnivores species inhabiting the Nahuelbuta National Park (37°47′ S, 72°59′ W) in central Chile, puma (*Puma concolor*), kodkod (*Leopardus guigna*) and foxes (*Pseudalopex spp.*) were collected and deposited in 70% ethanol. The coordinates of each fecal sample were recorded and then transferred to a GIS system (Arc GIS 9.3). Preliminarily, feces were assigned to each species by visual examination whilst further PCR analyses are in course. Flotation-sedimentation technique was used to assess coproprevalence of endoparasites of the three species. GLM analyses with Poisson errors were used to assess the effect of species, vegetation cover and type on parasite richness and abundance. Eighty four fecal samples were obtained, of which 56, 22 and 6 were visually assigned to puma, kodkod and foxes. None of the variables explained the parasite richness and carnivore species was the only variable that explained the parasite abundance, with felids showing higher parasite abundance than foxes.

This study was funded by Fondecyt Nº 11100303.

[107] COPROLOGICAL SURVEY OF ENDOPARASITES OF DARWIN FOX (*P. FULVIPES*) AND KODKOD (*L. GUIGNA*) IN CHILOÉ ISLAND IN SOUTHERN CHILE

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Macroparasites can induce loss of fitness in wildlife populations. In despite of this, knowledge of parasitism in endangered species is scarce. The goal of this study was to determine the species of endoparasites affecting two sympatric carnivore species of conservation concern in southern Chile. From January to February 2011 fresh fecal samples of the only two carnivores species inhabiting the Tantauco private park (43º 5’ N, 73º 6’ W) in the Chiloé Island, the endangered Darwin fox (*Pseudalopex fulvipes*) and the vulnerable kodkod (*Leopardus guigna*) were collected and deposited in 70% ethanol. Extraction and sequencing of mitochondrial DNA was done by PCR analysis for assigning each fecal sample to the corresponding carnivore species. Additionally, flotation-sedimentation technique was used to assess copropревalence of endoparasites of both species. Seventy nine fecal samples were obtained, of which 44 and 35 were from Darwin fox and kodkod, according to PCR analyses. Overall, 59% and 91% of Darwin fox and kodkod’s samples had at least one endoparasite species. Darwin fox was parasited by seven parasites and kodkod by eight. Finally, 41% and 15% of samples from Darwin foxes and kodkod were parasited by more than one species, respectively. This study is the first using PCR and parasitological analyses in these endangered species, showing differences in parasitism levels. Further ecological studies are needed to explain these differences and whether cross-infection occur.

[108] LYME DISEASE ECOLOGY IN BRITISH WILDLIFE

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Borrelia burgdorferi, the causal agent of Lyme borreliosis, is naturally maintained using *Ixodes* ticks as vectors and, primarily, wild-living vertebrates as hosts. Little is known about the relative importance of different UK wildlife species in this role; some evidence that the Eurasian badger (*Meles meles*) is a competent reservoir host for *B. burgdorferi* has been presented, and as this mustelid is present in the UK at densities higher than most other parts of its range, its role in the maintenance of the pathogen may be important here. We conducted a pilot study to determine the prevalence of *Borrelia* in ticks parasitising badgers in south-west England and in questing ticks within their territories. Three *Ixodes* species were recovered from badgers; *Ixodes ricinus* (18%), *I. hexagonus* (45%) and *I. canisuga* (15%) (22% not identified). We surveyed for *B. burgdorferi* DNA in these ticks and detected the pathogen only in *I. ricinus*. The prevalence of infection was 4.3% in adult and nymphal ticks collected from badgers and 5.1% in questing (*I. ricinus*) nymphs. Although *I.hexagonus* and *I.canisuga* appeared to be spatially evenly distributed in the study area, the distribution of *I.ricinus* appeared to be patchy. We also obtained some evidence that different *I. ricinus* patches were infected with different *B. burgdorferi* genospecies. Further work is warranted to better quantify the spatial heterogeneity of both *I. ricinus* and *B. burgdorferi* genospecies, and to expose its determinants.

[109] WILD CARNIVORES AS A SOURCE OF ZOONOTIC HELMINTHS IN NORTHERN PORTUGAL

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The Protected Landscape of Bertiandos and S. Pedro de Arcos Lagoons is located in the Northern Portugal, where a set of biogeographic factors interact to create a unique environment that includes in 346 ha the only Classified wetland in the Northern Portugal. The Area's vertebrate fauna is remarkable in its diversity, with 9 species of fish, 13 species of amphibians, 11 of reptiles, 41 of mammals and 144 of birds registered so far. Along this area, there are several footpaths that are daily crossed by humans that are nature enthusiasts or come for a walk in family. European genets (*Genetta genetta*) leave their feces on the top of the human relax places in this protected area. Some animal parasites may also infect humans. Environmental contamination by the feces should thus be considered a risk to public health. The aim of this study was to estimate the level of environmental contamination by feces with zoonotic parasitic forms in this area. For this purpose we collect 35 feces samples and performed qualitative coprology analysis. We found that 19 (54,29%) samples had zoonotic parasitic forms (*Ancilostomidae* and *Toxocaridae* eggs, both together in 3 samples). The *Toxocaridae* and *Ancilostomidae* parasites cause the *visceral* and *cutaneous larva migrans* respectively in humans. The nature of these zoonotic parasites suggests the need to implement preventive measures of environmental contamination accompanied by campaigns to promote health education in the community.

[110] A ONE HEALTH APPROACH INVESTIGATING GASTROINTESTINAL PARASITES AMONG URBAN COYOTES, DOMESTIC DOGS AND PEOPLE IN URBAN LANDSCAPES: THE CASE OF *ECHINOCOCCUS MULTILOCULARIS*, *GIARDIA* SPP. AND *CRYPTOSPORIDIUM* SPP. IN CITY PARKS OF CALGARY, ALBERTA, CANADA

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Urbanization is a worldwide phenomenon, with an increase of the interactions between humans, domestic animals (dogs and cats) and wildlife in city parks. Coyote (*Canis latrans*) is widely distributed and abundant in North America, and common in urban landscapes. In 2009, we started a research program to identify the potential for transmission of gastrointestinal (GI) parasite (i.e. *Giardia*, *Cryptosporidium*, and *Echinococcus* spp.) at the interface of coyotes, domestic dogs and people in city parks in Calgary, AB. We collected coyote carcasses (91) and coyote (N=248) and dog feces (N=494), and detected *Echinococcus multilocularis* in 15% of urban coyotes (25% in the rural surroundings). *Giardia* spp. was found in 20% of coyotes and 24.7% of dogs (with different strains, mostly not zoonotic) and *Cryptosporidium* spp. (14.7% in park-walked dogs). We also detect an association between GI infections in dogs and the frequency of off-leash activity, possibly related to the use of parks with high dog fecal contamination (3.1 kg of dog feces/ha left on the ground per week). By assessing the presence of *E. multilocularis* in intermediate hosts (rodents) and characterizing the strains of *Giardia* and *Cryptosporidium* spp. in coyotes, wild prey species, water sources and dogs, we aim to track potential routes of infection, and to assess the infection risk for people walking their dogs in city parks.

[111] *TOXOPLASMA GONDII* IN FINNISH WILDLIFE

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Toxoplasma gondii is a zoonotic parasite capable of causing disease and even killing its host. With serological methods, we have revealed that wildlife, semidomesticated animals, domestic animals, pet animals, and humans do encounter *T. gondii* in Finland. Moreover, *T. gondii* strains belonging to the endemic genotype II have been confirmed as an occasional cause of death of both wild and domestic hosts. The methods used were direct agglutination test and enzyme-linked fluorescent assay for detecting *T. gondii* –specific antibodies, and a multiplex-PCR-method with seven microsatellite markers for direct genetic characterization of *T. gondii*. Wild, free-ranging animals are part of the host range of *T. gondii*, but are they a risk factor for infections of humans and domestic animals, or victims thereof? It appears that the latter is mainly the case, as wildlife is more often infected in areas where human population is densest, indicating the crucial role of domestic cats in the epidemiology of *T. gondii*.

[112] DETECTION OF *LEISHMANIA* SP. IN SOUTH AMERICAN GREY FOXES IN PATAGONIA, ARGENTINA

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Blood samples were obtained from five South American grey foxes (*Pseudalopex griseus*) captured in “Bosques Petrificados” Natural Monument (Santa Cruz Province, Patagonia, Argentina; 47º 58’S, 67º 97’O), preserved in FTA cards, and analyzed for the presence of *Leishmania* spp. DNA by real-time PCR. All the foxes were positive for parasites belonging to the *L. donovani* complex. As far as we know, *Leishmania* spp. has never been found so far south in America. *Leishmania infantum* is believed to infect dogs and humans only in the northern provinces of Argentina and, according to the literature, the southernmost capture of a potential sand fly vector (*Lutzomyia oswaldoi*) was recorded 750 km northern to these cases, at 41ºS. This finding extends the distribution area of *L. infantum*/*chagasi* and highlights the relevance of wild carnivores as potential reservoirs of this parasite.

[113] SUSCEPTIBLES, SOURCES, AND SENTINELS OF COASTAL PATHOGEN POLLUTION

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Toxoplasma gondii infection in sea otters has raised awareness of coastal pathogen pollution. As felids are the only recognized shedding hosts, marine animal infection suggests land to sea transmission. We evaluated terrestrial environmental loading, as well as transmission dynamics in freshwater, estuarine, and marine systems. Oocyst shedding was higher in bobcats and unmanaged feral cats than other felids. Considering relative population sizes along with infection and shedding prevalences, domestic cats likely contribute more oocysts to the environment than mountain lions and bobcats. Molecular genotyping has allowed further exploration of host dynamics. Laboratory and field studies were also employed to evaluate the contribution of wetland loss to the flux of pathogens into coastal waters. Current levels of erosion of vegetated wetlands to mudflats increased pathogen flux greater than two orders of magnitude, while total degradation of wetlands increased transport up to six orders of magnitude. Pathogens were found to associate with and concentrate in aquatic macroaggregates, influencing waterborne transport and facilitating ingestion by invertebrate vectors that can transmit pathogens to susceptible hosts, including sea otters and humans. Continued development of coastal landscapes will likely change host population numbers and distribution, increase terrestrial pathogens in run-off, and alter disease dynamics at the human-animal-environment interface.

[114] ELUCIDATING THE RELATIONSHIP BETWEEN BOVINE TUBERCULOSIS IN CATTLE AND BADGERS IN ASTURIAS (NORTHERN SPAIN)

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Recent studies have identified Eurasian badgers (*Meles meles*) as possible reservoirs of *Mycobacterium bovis* infection for cattle in Asturias (Northern Spain). With the purpose of further elucidating the role of badgers in cattle tuberculosis (TB) breakdowns, we examined the epidemiological links between cattle and badgers. Culture and molecular typing data were available for cattle analyzed during the national TB eradication campaigns from 2008-2012. Additionally, results of 171 necropsied badgers (most of them road-kills) and of 60 badgers trapped in the same period from twenty setts located in the vicinity of TB-positive farms, are presented. *M. tuberculosis* complex strains were isolated from pooled tissues of 15 (8.77%) necropsied badgers. Ten were identified as *M. bovis*. We found three locations in Asturias where the same spoligotypes were shared between cattle and badgers. In these locations, TB-positive cattle herds and badgers lived in close contact, thereby increasing the risk of mutual *M. bovis* transmission. However, results are still not sufficient to decide if the badger could be a maintenance host of *M. bovis* in Asturias. Until a fuller picture of the role of badgers in the epidemiology of bovine TB in Northern Spain is available, measures to prevent or reduce contact between cattle and badgers would be sensible precautions to reduce the risk of disease transmission.

[115] LARGE-SCALE MOVEMENTS AND VISITATION NETWORKS OF BADGERS (*MELES MELES*) IN A BCG VACCINE FIELD TRIAL AREA: IMPLICATIONS FOR THE PROPAGATION OF BOVINE TUBERCULOSIS ACROSS A POPULATION?

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Effective vaccination of wild badger populations against *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB), is a desirable option for the eradication of bTB in cattle and wildlife. A large scale (755 km^2) field trial of bTB vaccine Bacille Calmette-Guérin in badgers is ongoing in Kilkenny, Ireland. As part of this trial, badgers have undergone a capture-recapture regime. We use these capture data to investigate badger movements and networks of sett (burrow) visitation. The mean geodesic distance between captures was 1.5km (SD 1.3). Over 55% of movements were >1km in length, which are considered long-distance (trans-territorial) for badgers. Frequent long-distance movements of badgers like these in medium density populations have not been recorded previously. Distances moved were significantly affected by the age and weight, but not sex. Mapping of these movements showed clusters of setts linked through badger visitation. We estimated that the mean size of these visitation networks were 5km^2 (SD 10; max. 50km^2). Many of these sett visitation networks are much larger than badger territories in similar habitats (0.5-1.3km^2). In the presence of an environmental reservoir of *M. bovis* around badger setts, these visitation networks could form transmission networks that would have implications for bTB disease dynamics. Future work will investigate if transmission networks are facilitated through sett visitation when the test status of badgers becomes available.

[116] ZOONOSIS = ZOOANTHROPONOSIS? RESISTANT BACTERIA IN URBAN RED FOXES (*VULPES VULPES*)

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The term zoonosis is frequently used for the transmission of pathogens from animals to humans, so actually describing zooanthroponosis. In urban areas wild animals live in close proximity to humans and companion animals and are able to gain their pathogens. The most frequently isolated bacterial pathogen from both humans and animals is *Escherichia coli*. At the same time *E. coli* is part of our autochthonous gut flora and one of the first bacteria to spread throughout the carcass during decomposition. This is why isolation of these bacteria from wild animals is often neglected. In our current study we investigated samples from urban red foxes from the Berlin city area for *E. coli*, resistant to 3rd generation cephalosporins. As wild animals are normally not treated with antibiotics this was an obvious selection criterion for bacteria of a possible human or pet origin. Samples from more than 200 foxes were taken from April 2010 to April 2012 and screened for resistant *E. coli*. The obtained isolates were investigated molecular biologically 1) for their phylogenetic relatedness amongst each other and in comparison to known pathogens from humans and other animals, and 2) for their pathogenicity by comparing virulence patterns. Our results show that foxes are carriers of potential pathogenic *E. coli* with close phylogenetic relatedness to those from humans and companion animals. Therefore, we cannot exclude that anthropozoonotic *E. coli* are involved in infections of wild animals.

[117] INTESTINAL HELMINTHS SHARED BETWEEN THE DOMESTIC DOGS (*CANIS FAMILIARIS* LINNAEUS, 1758) AND THE CRAB-EATING FOX (*CERDOCYON THOUS* LINNAEUS, 1766) IN THE SEMIARID REGION FROM NORTHEASTERN BRAZIL

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Helminthes are important to control hosts populations. In places where livestock and domestic animals are among wild animals, especially the generalist ones play an important approach to health. Canids are secondary consumers acting as connecting points of helminthes´ transmission. In northeastern Brazil, people are vulnerable by sanitary conditions, low risk perception and the overlap use of land by wildlife, livestock and humans. The main objective is to verify shared intestinal helminthes between wild and domestic dogs, by Sorensen´s similarity index, and the zoonotic ones. We used the Lutz´s method of spontaneous sedimentation in feces samples collected directly from the soil and observed in light microscopy at 400x, searching for helminthes eggs and larvae. We analyzed 40 samples from domestic dogs and 29 samples from *Cerdocyon thous*. The results show an 0,54 value for Sorensen´s index (SI), with nine morphospecies shared among wild and domestic canids: Trichuridae (2,89%) Ascarididae (7,24%), Trichostrongylidae (7,24%), *Spirocerca lupi* (8,69%), Physalopteridae (15,94%), *Toxocara* sp. (5,79%), Strongyloididae (8,69%), *Alaria* sp. (20,28%) and Ancylostomatidae (40,57%), in which the later four are zoonotic. The high SI shows a risk situation empowered by the approximation of *C. thous* to human dwellings and the dogs into natural and protected areas, mainly for hunting, and changes in ecosystem that favors the transmission of generalist parasites among susceptible hosts.

[118] *BRUCELLA PINNIPEDIALIS* HOODED SEAL (*CYSTOPHORA CRISTATA*) STRAIN INVADES HUMAN MACROPHAGES IN CULTURE

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Marine *Brucella* spp. have been isolated from and serologically indicated in pinnipeds (*B. pinnipedialis)* and cetaceans (*B. ceti)* from around the world. Although the zoonotic potential of marine mammal brucellae is largely unknown, reports of human disease exist. Studies of the mechanisms of bacterial intracellular invasion and multiplication involving the marine mammal *Brucella* spp. are however scarse. The present study aims to provide knowledge of the infective capacity of marine mammal brucellae measured by the ability to invade and replicate in professional phagocytes *in vitro*. Special emphasis is devoted to a *B. pinnipedialis* strain isolated from hooded seal. Human monocyte-like cells (THP-1) were challenged with bacteria at a MOI of 50 in a gentamicin protection assay. Following infection, the cells were lyzed at different time points and tryptic soy agar plates were inoculated and evaluated for the presence of colony forming units. Our results show that *B. pinnipedialis* is able to infect THP-1 cells; however they are slowly eliminated during the next 72-96 hours. These findings are confirmed by confocal microscopy using a polyclonal rabbit anti-*Brucella* antibody. Double staining shows that the intracellular *B. pinnipedialis* and the autophagic marker p62 reside in the same confocal plane. Further studies are indicated to investigate the intracellular pathways of *B. pinnipedialis* and gain more information of the zoonotic potential of this marine mammal strain.

[119] DOES THE WOLF HAVE A ROLE IN THE SPREADING OF *TRICHINELLA BRITOVI* IN ITALY?

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Trichinellosis is one of the most studied zoonosis and its causative agent *Trichinella spp*. is world wide distributed. Many reservoirs are reported for each species in different countries. In the studied Area, *T.britovi* and *T.pseudospiralis* are described, but only *T.britovi* in carnivores. We present the Dataset of Trichinella Surveillance in wildlife (Commission Regulation EC no.2075/2005) of Istituto Zooprofilattico of Umbria and Marche referred to the period January 2008- march 2012. In this Apennines Area of Central Italy, we found 6 wolves out of the 66 examined as well as 3 out of the 59313 wild boars analyzed. None of 603 tested foxes were found positive as well as others receptive species observed (62 raptors; 8 mustelids). The role of foxes as reservoir is well documented for *T.britovi*, however the spreading of Wolf population in Central Italy is discussed in order to the consequences for the surveillance of trichinellosis: the mean life of *T.britovi* in wolf (longer than in wild boar) and its wide home range, could represent a new epidemiological scenario for this zoonosis, in our Country.

[120] HOW TO ASSESS THE HEALTH STATUS OF A WILD ANIMAL POPULATION: NATIONWIDE WEB-BASED SYNDROMIC SURVEILLANCE OF WILDLIFE DISEASE AND MORTALITY

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In Norway, the National Health Surveillance Program for Cervids (HOP) became operative in 1998. As a part of this, the wildlife management in 62 - 65 selected municipalities in different parts of the country reported disease and mortality incidents in cervids with three months' intervals. The program gave valuable information about cause of death among cervids and allowed us to form an opinion on the incidence of disease and mortality not related to hunting or traffic accidents. However, commitment and compliance varied enormously among the municipalities and the data collection and registration was laborious. Based on this experience, the reporting scheme was abandon in 2007. However, we soon realized that without these reports, our contact with local managers was diminished and we lacked a source of information on fluctuations in disease incidence and mortality. As the Directorate of Nature Management in the same period started the web-based "National Cervid Register" containing a page where traffic accidents involving wildlife could be reported, we engaged in the development of a web-based reporting system of observations of wildlife disease. The interface allows the users to characterize the cause of debilitation/death as "disease, trauma etc" and further designate the findings to one of 15 categories. It is also possible to register the condition of the animal, observations on live animals and descriptions of the carcass site. All registrations are linked to a specified position on a map. We believe that this system will facilitate continuous monitoring of wildlife health and mortality, increase awareness among wildlife managers and enable rapid response in cases of disease outbreaks and situations where animal welfare is threatened.

[121] WHAT'S ON A TOOTH? DENTAL ENAMEL HYPOPLASIA IN CARIBOU AS A TOOL TO ASSESS POPULATION HEALTH

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Non-invasive, low-cost methods for understanding wildlife health are useful in informing wildlife management. We studied the presence of dental enamel hypoplasias (DEHs) in barrenground caribou and examined the utility of tracking these as a tool for monitoring population health. DEHs are developmental tooth defects associated with physiological stress events when enamel is deposited. A timeline of tooth enamel development was determined by radiographic examination of 48 mandibles from caribou aged 3- to 24-months-old. Mandibles from the Bluenose East (n= 56) and Bluenose West (n= 15) barrenground caribou herds in the Northwest Territories and Nunavut, Canada were examined for DEHs and 19.7% (14/71) were affected. Advantages to the technique include engaging local subsistence hunters in sample collection, relative ease of lesion observation, the lack of necessity for expensive equipment, the permanence of lesions on teeth, and the ability to place a time on the occurrence of the lesion. We concluded that DEHs do occur in barrenground caribou and tracking these over time may provide a tool to assess population dynamics in extant wildlife.

[122] BLUETONGUE AND BRUCELLOSIS IN SPRINGBOKS AND WILDEBEESTS IN THE ETOSHA NATIONAL PARK, NAMIBIA

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Little is known about the epidemiology and persistence of Brucellosis and Bluetongue in African wildlife. An opportunity to evaluate prevalences of these diseases in two wildlife species came up when, in May 2011, an animal diseases surveillance program was organized in the Etosha National Park, Namibia. In less than two months 200 springboks (*Antidorcas marsupialis*) and 50 blue wildebeests (*Connochaetes taurinus*) were sampled. Blood and serum samples were tested to detect BTV genome and antibodies by using Real-time RT-PCR and competitive ELISA, respectively. Sera were also tested for Brucella antibodies by the Rose Bengal Test (RBT) and the Complement Fixation Test (CFT). All the wildebeests tested (N=50) and 93% (170/182) of springboks showed BTV antibodies. BTV RNA was detected in 61/182 (34%) springboks and 1/50 (2%) wildebeests. No Brucella antibodies were detected either by RBT or by CFT. According to these results, it appears that BTV has circulated or is circulating widely in the Etosha National Park; more studies however are needed to establish the serotypes circulating and the exact epidemiological role played by these species for BT. Similarly, whether the apparent absence of *Brucella* antibodies found in the serum samples tested in this study depends on natural resistance or on the special condition present within a fenced National Park, also requires further investigations.

[123] DETECTION OF HAEMOPARASITES IN SPRINGBOKS AND WILDEBEESTS IN THE ETOSHA NATIONAL PARK, NAMIBIA

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Tick-borne diseases and trypanosomiasis affect over 80% of the world's cattle population and cause heavy livestock losses in Sub-Saharan Africa. There are no reliable recent estimates of the impact of these diseases and the role of wild ruminants, as potential reservoir hosts, has rarely been investigated. An opportunity to evaluate prevalences of these diseases in two wildlife species came up when, in May 2011, an animal diseases surveillance program was organized in the Etosha National Park, Namibia. The blood of 35 springboks and 10 wildebeests was therefore tested by Real-time PCR for: *Anaplasma centrale*, *Anaplasma marginale*, *Babesia bigemina*, *Babesia bovis*, *Babesia* spp, *Theileria annulata*, *Theileria mutans*, *Theileria parva*, *Theileria* spp, *Theileria taurotragi*, *Theileria velifera* and *Trypanozoon* subgenus. 90% (9/10) of wildebeests showed presence of *Theileria spp* DNA in their blood, but no other pathogen's genome was detected in tested animals. Sequence analysis of 4 out of 9 *Theileria* spp positives demonstrated the presence of a new *Theileria* recently detected in wildlife in South Africa. These preliminary data provide evidence that wild ruminants can be infected by unidentified piroplasms and that their distribution can be wider than previously suspected. Care should therefore be taken when translocating wild animals since the introduction of novel parasites into new areas could cause not only infection but even clinical disease in naïve wildlife and domestic animals.

[124] DETECTION OF PRION PROTEIN ASSOCIATED WITH CERVID CHRONIC WASTING DISEASE IN ENVIRONMENTAL SAMPLES

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy affecting North American cervids. The agent enters the environment from carcasses and shedding in feces, saliva, and urine where it can bind to soil and remain available for extended periods. Because the agent lacks nucleic acid, polymerase chain reaction cannot be used. Protein misfolding cyclic amplification (PMCA) exploits the ability of prion proteins to convert normal prion protein to a proteinase K-resistant form. Inclusion of Teflon® beads in the PMCA reaction (PMCAb) can increase the sensitivity and robustness for detecting hamster-adapted prions. We show that PMCAb with saponin dramatically increases sensitivity of detecting CWD without affecting specificity (i.e., no false positive results). Teflon® beads increased the robustness of the PMCA reaction, resulting in decreased variability of PMCAb. Three rounds of serial PMCAb allowed detection of CWD agent from a 6.7 × 10^{-13} dilution of 10% brain homogenate (1.3 fg of source brain) compared to Tg mice with detection from 10^{-6} dilutions. PMCAb has > 10^{5} fold higher sensitive than mouse bioassay. We also amplified CWD agent from white-tailed deer having *Prnp* alleles associated with reduced disease susceptibility. When combined with extraction and phosphotungstic acid precipitation, PMCAb can detect PrP^{TSE} in environmental samples. This technique holds promise for understanding the importance of direct and indirect transmission of CWD.

[125] PREVALENCE OF ANTIBODIES AGAINST PESTIVIRUSES IN WILD AND DOMESTIC RUMINANTS IN NORTH-EASTERN ITALY

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Pestivirus infection is considered a remarkable issue at the domestic-wildlife interface. Seroreactors have been previously detected within alpine chamois (*Rupicapra rupicapra*) and red deer (*Cervus elaphus*) populations in the study area and subsequently a BDV3 was identified for the first time in alpine chamois. A serological survey was implemented in order to define pestivirus related risk areas and possible interactions between domestic and wild ruminants. During the period 2009-2011, a total of 497 wildlife sera from 107 chamois, 72 red deer, 298 roe deer (*Capreolus capreolus*), 20 alpine ibex (*Capra ibex*) and 303 sera from 171 goats and 132 sheep were collected. All the samples were tested by an in house cELISA that specially detects antibodies against protein p80/125 common to BDV and BVDV strains. All cervids resulted negative. Sero-reactors were detected within chamois populations (6%) showing geographical clusters and within alpine ibex samples (45%). We found positive reactors also in domestic sympatric livestock: namely, 44% of ovine and 11% of caprine sera. We observed a seroprevalence decrease in chamois meta-populations that previously consistently showed high prevalence (50-70%). The reasons for this decrease are still unclear, although a possible explanation may originate from scabies-related mortality that could have modified virus and population dynamics. The highest seroprevalence in sheep and goats was detected in those same areas. Further insights will be provided by virus investigations that are ongoing.

[126] *DERMACENTOR RETICULATUS* AS VECTOR OF *ANAPLASMA PHAGOCYTOPHILUM*?

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As the role of wildlife is recognized as major in emerging diseases in humans as well in domestic animals, tick borne diseases are in this context a subject of growing concern. In Belgium, the most health implicated tick is *I. ricinus.* However, *D. reticulatus* seems to extend its geographical distribution more than previously thought, like in other parts of Europe. *D. reticulatus* can potentially or successfully transmit *Rickettsia sp, Francisella tularensis, Babesia canis, Coxiella burnetii* or TBEV, but was never related with *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. Indeed, only *I. ricinus* was recognized to carry these bacteria. Here we deal with a new potential vector species. We found on an adult red deer 35 ticks that were morphologically and genetically identified as *D. reticulatus*. We tested them for *A. phagocytophilum* by PCR and sequencing a part of the *msp2* gene and some of them were positive. One of them was enhanced by sequencing a part of the 16S RNA gene. To extend our knowledge about the variants that may be found in *D. reticulatus,* 108 additional specimens were collected by flagging and some of them were tested positive for *A. phagocytophilum* by Real Time PCR on the *msp2* gene. The positive ticks will be sequenced regarding a part of the 16S RNA gene to compare them with *A. phagocytophilum* isolated from other sources. This approach will enable us to learn more about the variants newly isolated from *D. reticulatus.*

[127] MYCOBACTERIA IN FREE-RANGE WILD ANIMALS IN SLOVENIA

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Different mycobacteria are capable of causing infections in a wide range of animals and in humans. Wild animal population was recognised as an important reservoir of mycobacteria. To determine the prevalence of mycobacteria, it is important that monitoring is directed at host population in which the infection can persist and may be transmitted among different species. A total of 214 different free-range wild animals were investigated for the presence of mycobacteria: red deer (n=69), roe deer (n=60), wild boar (n=31), fallow deer (n=23), wolf (n=11), chamois (n=8), badger (n=6), fox (n=4), stone marten (n=1) and polecat (n=1). Samples of liver and mandibular, mediastinal and mesenteric lymph nodes were Ziehl-Neelsen stained. Cultures were identified on the basis of colony morphology, GenoType Mycobacterium CM and AS Culture Identification Kits (Hain Lifescience, Germany) and sequencing of *16S rRNA* gene using mycobacteria-specific primer pair 285/264 (Kirschner et al., 1993). Mycobacteria were isolated from 33 (15.42%) samples. The most frequently detected species was *M. peregrinum* (n=6), followed by *M. avium* (n=5), *M. intracellulare* (n=3), *M. engbaekii* (n=3) and *M. celatum* (n=2). Single isolates were identified as *M. fortuitum*, *M. neoaurum*, *M. confluentis* and *M. nonchromogenicum.* Nine isolates were identified to genus level only (*Mycobacterium* sp.*).* Our data provide the first information on the prevalence of mycobacteria among different free-range wild animals in Slovenia.

[128] MOLECULAR AND SPATIAL EPIDEMIOLOGY OF TB IN WILD UNGULATES FROM CENTRAL SPAIN

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The Eurasian wild boar (*Sus scrofa*) and the red deer (*Cervus elaphus*) GG, maintain and transmit the agent and hamper the success in the control of TB in livestock. Multidisciplinary approaches are crucial to evaluate management, environmental and ecological factors determining the transmission and persistence of TB in wild populations. This study focuses on a high ungulate density area, composed by interspersed game ranges and natural protected areas in the Toledo Mountains (~1000 km^2, Southcentral Spain), with the existence of persistent TB infection. Wildlife and domestic livestock shared the same *M. bovis* strains, although some typical types occurred in a given species and/or management unit. Along the study period, we detected an increasing prevalence pattern in wild boar, reaching a peak at which most individuals became infected in both managed (fenced Estates) and unmanaged populations. Thereafter a decreasing trend synchronously occurred all over our study populations. A similar time-delayed pattern occurred for red deer, displaying lower TB prevalences than wild boar. We discuss this spatio-temporal pattern in relation to TB prevalence and incidence in local cattle. Field studies of wild boar behavior, evidenced movement patterns compatible with wild boar acting as a mobile TB infection source among ungulate populations and local livestock farms.

[129] SEROPREVALENCE AND RISK FACTORS ASSOCIATED TO *MYCOBACTERIUM BOVIS* IN WILD ARTIODACTYL SPECIES FROM SOUTHERN SPAIN, 2006-2010

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Additional epidemiological information regarding tuberculosis in wild ungulates, including affected species, prevalence, associated risk factors and appropriate diagnostic methods need to be obtained in Europe. A cross-sectional study was carried out on wild artiodactyl species, including Eurasian wild boar (*Sus scrofa*) red deer (*Cervus elaphus*), roe deer (*Capraelus capraelus*), fallow deer (*Dama dama*), Spanish ibex (*Capra pyrenaica hispanica*) and mouflon (*Ovis musimon*), in Spain to assess the seroprevalence against *Mycobacterium bovis* or cross-reacting members of the *Mycobcaterium tuberculosis complex*, and to provide information on associated risk factors. Previously, two in-house indirect enzyme linked immunosorbent assays (bPPD-ELISA and MPB83-ELISA) were developed using bPPD and MPB83 antigens. Positive reference sera were selected from infected animals confirmed by culture. The *M. bovis* isolates belonged to spoligotypes SB0121, SB0120, SB0295, SB0265 and SB0134. Two hundred and two out of 1367 (7.5%; 95% CI: 6.1-8.9) animals presented antibodies against *M. bovis* by both ELISAs. Significantly higher TB seroprevalence was observed in wild boar compared to the other species analyzed. Seropositivity against *M. bovis* was not found in any out of 460 Spanish ibex analyzed. The logistic regression model for wild boar indicated that the seropositivity to *M. bovis* was associated with age, location and year of sampling, while the age was the only risk factor associated with *M. bovis* seroprevalence in red deer and fallow deer.

[130] PREVALENCE OF PARASITIC INFECTIONS IN VARIOUS SPECIES OF WILD ANIMALS IN DINDER NATIONAL PARK

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The present investigation was carried in order to study the parasitic infections in various species of wild animals in Dinder National Park. The occurrence and prevalence of gastrointestinal parasites in 29 animals of five species belong to: Ostrich, Waterbuck, Warthog Reedbuck and Baffalo were determined by using standard qualitative and quantitative parasitological techniques .The overall infection rate of gastrointestinal parasite based on 29 samples examined for helminthes. Egg / oocyst of protozoa were found to be 82.4% percent. The parasitic egg / oocyst detected were of Coccidia, Schistsoma, Strongyle, Bluntidium, Moneizia, Fasciola, Trichuris. The most common detected parasite infection among animals was Coccidia (73 %) followed by Schistosoma (56.5%), Strongyle (22.7 %), Moneizia (17.6%), Fasciola (15%) and Trichuris (7.6%), while Bluntidium was founding in buffalo in 23% of the examined samples. Mixed infections were recorded in all of the wild animals examined.

[131] PREVALENCE OF GASTRO-INTESTINAL PARASITES IN GAZELLES (*GAZELLA SUBGUTTUROSA MARICA*) IN IRAQ

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This study was conducted to investigate types of gastro- intestinal parasites in adult Gazelles (Gazella subgutturosa marica) that prohibited in Al-dheabeah farm in desert of Al-anbar governorate-west of Iraq. 50 adult animals were included in this study to take fecal samples during winter season. The morbidity rate reached to 52%. Nematodes were the predominant that reported in 36% of infected animals while protozoa 12% and cestodes 4% of total infected animals. In this study the eggs of different types of parasites was reported in 26 animals distributed like the following: *Nematodirus spp*. in 8 animals, *Eimeria spp*. in 6 animals, *Chabertia spp*. in 3 animals, *Trichostrongylus spp*. in 3 animals, *Taeniidae spp*. in 2 animals, *Haemonchus spp*. in 2 animals and *Stronglyloides spp*. in 2 animals. The morbidity of Gastro-intestinal in gazelles in this farm it looks high because the owner depending irregular feeding of animals on indigenous plants like *citrullus colocynthis*. It concludes that gastro- intestinal is prevalent in Iraqi Gazelles in winter season.

[132] ANTIBODIES TO *NEOSPORA CANINUM* IN FREE-RANGING WILD UNGULATE POPULATIONS FROM THE FRIULI VENEZIA GIULIA REGION: NORTH EASTERN ITALY

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Neospora caninum natural infection was detected in several wild ruminants. Seroreactors have been recorded in wild suids, carnivores and lagomorphs. *N.caninum* is recognized as a major abortive agent in cattle in different countries including the study area of the Friuli Venezia Giulia Region. Main objective of this study was to determine the presence of *N.caninum* in local wild ungulate populations. A total of 382 sera were collected from 180 roe deer (*Capreolus capreolus*), 84 red deer (*Cervus elaphus*), 36 alpine chamois (*Rupicapra rupicapra*) and 82 wild boars (*Sus scrofa*). All the samples were tested by a competitive ELISA (VMRD® Inc., WA, USA). *N.caninum* antibodies were found in roe deer (6.6%), in red deer (2.3%) and in wild boar (2.4%). All chamois were negative (maximum possible prevalence 8%; 95%CI). The seroprevalence in cervids was consistent with previous surveys in Italian Alps, although it was much lower than in North American cervids. Negative outcomes observed in chamois differ from findings in Western and Central-Eastern Italian Alps. Seroprevalence in wild boar was much lower than reported in the Czech Republic, but higher than seroprevalence in Spain. Our results demonstrated the exposure of wild ungulates to *N.caninum* for the first time in the study area. Further investigations should deepen the epidemiological cycle of the parasite, overlaps between domestic and sylvatic cycle and the role of wild carnivores and lagomorphs.

[133] *BABESIA* IN WILD UNGULATES IN EUROPE: FROM ENZOOTIC CIRCULATION TO DISEASE

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Wild ungulates (roe deer, red deer, reindeer, chamois), are hosts of different *Babesia* species acquired through tick bites. In Europe, three main species are described: *B. capreoli*, *B. divergens* and *B. sp.* EU1, the two last being zoonotic. Reports of fatal cases are scarce, in contrast to frequent asymptomatic infection. In most reports, detection and characterization is based on molecular methods. Only the isolation of parasites by cultivation on red blood cells allows their full characterization (host range, pathogenicity, erythrocyte susceptibility tests). From an acute case of babesiosis in a red deer and from healthy roe deer in France, we have isolated *B. divergens* from the spleen, and on healthy red deer in Spain, we identified a new *Babesia* species, phylogenetically related to a *Babesia* pathogen for sheep in China (*B. sp.* Xinjiang) and giraffe in South Africa. In cell culture, this species was able to grow on sheep and cattle erythrocytes. Other unknown genotypes will most likely be detected in wild animals in the future. Clinical babesiosis appears generally when individuals are newly in contact with populations or locations where certain species of parasites are common without any noticeable impact (enzootic equilibrium). With global change, there are more and more occasions where this can happen. Thus surveillance must target causal agents of clinical disease, as well as commonly circulating species in multiple host species and geographical contexts.

[134] PRELIMINARY ANALYSIS OF SOME BIOCHEMICAL PARAMETERS IN BLOOD SERUM OF YOUNG WILD BOARS (*SUS SCROFA L.*) FROM FARMS IN BOSNIA AND HERZEGOVINA

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Wild boars are present in the country as game freely living in the nature, but in a controlled breeding as well for the purposes of training hunting dogs.Monitoring the health status of animals is important both from the clinical and from the epidemiological stand point. Studies of the health status of wildlife in Bosnia and Herzegovina are very rare, and we do not know any earlier information on the study of biochemical parameters of blood of these animals in our country. So our aim was not only to establish starting results but also to affirm similar research. The study was conducted on two farms with 23 and 16 young wild boars. The animals were in good general health condition and lived in a controlled, enclosed part of the natural habitat. After fixing and restraint of animals, blood was sampled from v..auricularis magna into vacutainers, without anticoagulant. The serum values of the following biochemical parameters were determined: potassium, sodium, calcium, urea, creatinine, alanine aminotransferase and alkaline phosphatase.The differences as regards activities of certain parameters were evidented among farms as well as in comparison with the results obtained by other authors.These and other studies aiming the protection of game health, health of domestic animals and humans, should continue in order to obtain more credible results.

[135] SEROPREVALENCE OF VIRAL AND BACTERIAL PATHOGENS IN WILD BOAR (*SUS SCROFA*) IN AUSTRIA

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Wild boar (*Sus scrofa*) populations have increased worldwide in the last decades leading to a higher potential contact rate within the host population as well as domestic pigs and humans. Many pathogens that cause disease in domestic pigs are also known to circulate in wild boar populations. Knowledge concerning the health status of the Austrian wild boar population is scarce. Therefore, we investigated the presence of antibodies against porcine parvovirus (PPV), porcine respiratory and reproductive syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV), Aujeszky's disease virus (ADV), classical swine fever virus (CSFV), *Leptospira spp.* and *Brucella suis* in sera of 48 juvenile wild boars from two hunting enclosures in eastern Austria and 70 juvenile wild boars imported from Hungary. Antibodies were detected against PPV (46.6%), PRRSV (2.5%), PCV2 (IgG 49.2%); (IgM 34.8%), SIV (2.5%), ADV (2.5%), *Leptospira spp.* (2.5%) and *Brucella suis* (9.3%). No antibodies against classical swine fever were found. A possible higher infection rate due to the potential increased density and spatial aggregation under artificial management conditions in the enclosures need to be considered. We conclude that wild boars in Central-eastern Europe could potentially act as a disease reservoir and host for a multitude of pathogens and further studies to evaluate the potential risk of transmission to domestic pigs are needed.

[136] OCCURENCE OF *SALMONELLA* SPP. AND ENTEROPATHOGENIC *YERSINIA* SPP. IN SWEDISH WILD BOAR (*SUS SCROFA*)

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The Swedish, as well as the European wild boar (*Sus scrofa*) population, is increasing in numbers, spreading continuously to new areas. Does this constitute a problem for humans and/or domestic animals? The estimated hunting bag in Sweden 2010-2011 was 60 000 wild boar. There are few previous studies on the presence of various presumptive, human pathogens in the Swedish wild boar population while European studies show that wild boar have the potential to harbour a wide range of pathogens that may cause serious illness among humans. Among these, *Salmonella* spp., *Yersinia (Y.) enterocolitica, Y. pseudotuberculosis* and E. *coli* O157:H7 are of particulate importance. Samples collected from the tonsils, ileocecal lymph nodes and feces from 88 wild boar were analysed using a combination of cultivation and PCR targeting these four pathogens. Results showed that 10%, 20% and 20 % of the sampled animals carried *Salmonella* spp., Y. *enterocolitica* and Y. *pseudotuberculosis* respectively, while no E. *coli* O157:H7 was found. In all, 34 (38,6%) individuals carried at least one of the pathogens and eight of these 34 carried two or three of the pathogens simultaneously. The three pathogens were found in a variable degree in all of the three sample types.
This indicates that wild boar may be a source of human infection. Moreover, the epidemiological significance of wild boar in the maintenance of the pathogens in pastures and spread to domestic animals is not clearly elucidated To evaluate these risks, further studies are needed.

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[137] TUBERCULOSIS IN WILD BOAR (*SUS SCROFA*) FROM ASTURIAS, A REGION OF NORTHERN SPAIN WITH ATLANTIC CLIMATE

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The relevance of wildlife reservoirs of the *Mycobacterium tuberculosis* complex (MTC) is increasing in Europe. Over 10 countries reported MTC infection in Eurasian wild boar (*Sus scrofa*), but this species is only defined as true reservoir in Mediterranean habitats of Portugal and Spain. Wild boars seropositive for MTC were recently detected in Asturias, a region of northern Spain with Atlantic climate. To assess the tuberculosis status of local wild boar, we collected tissue samples from 68 animals hunted in winter 2011/12. Mandibular, retropharyngeal, bronchial and mediastinal lymph nodes, lung and spleen were thoroughly inspected and further processed for histopathological and immunohistochemical (IHC) examination. Pooled tissue samples were submitted to culture, followed by molecular identification in case of mycobacterial growth. MTC-infection was confirmed by IHC in seven wild boar (10.3%, 95% CI 4.2-20.1), two of them showing positive culture. One culture positive animal displayed macroscopic lung lesions. Identification of spoligotypes is ongoing. We also found *Mycobacterium avium* complex in 9 wild boar. Actinomycosis, detected in further 9 animals, was correlated with the presence of macroscopic lesions (FET: P=0.001). Our results confirm the presence of MTC infection in wild boar from Asturias and suggest actinomycosis as an important differential diagnosis in this region. Future research should assess the potential role of wild boar as MTC reservoir in Asturias.

[138] THE SAME LOOK BUT DIFFERENT CONSEQUENCES: HYDATIC AND CYSTICERCUS DISEASES IN WILD BOARS

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Diseases shared with wildlife, particularly zoonosis, are of increasing concern in Europe. The family *Taeniidae* includes tapeworms of great importance. *Taenia hydatigena* and *Echinococcus granulosus*, share the same hosts, but among them, cystic echinococcosis is still a significant public health problem, especially in hunting communities, where sylvatic cycles are more important due to the infection of domestic dogs by the feeding of viscera of wild animals. We report two distinct parasitological diagnose for two very similar macroscopic lesions, in two different free-living wild boar hunted in the Northern of Portugal. Both were detected during *in loco* examination performed by qualified hunters and delivered to Sanitary Inspection Laboratory of the UTAD, and parasitological examination was performed. One of the cyst revealed to be *Cysticercus* spp. Total genomic DNA was extracted from the other, and the molecular methods revealed an *Equinococcus* spp.(G7). The macroscopic lesions of parasitic diseases may then be very similar, but the consequences of failure in their control can be very different concerning their zoonotic potential. This result underlines the importance of the hunters training, and the need for health education campaigns for this setting, increasing the detection of pathological processes and the implementation of corrective measures in order to reduce pathogens from the game production chain, from the environment and therefore for to reduce the human risk.

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[139] APPLICATION OF NEW BIOGEOGRAPHICAL TOOLS FOR MONITORING *TRICHINELLA* SP. INFECTION IN WILD BOAR (*SUS SCROFA*) POPULATIONS OF CENTRAL SPAIN

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In south-central Spain (SCS), the Eurasian wild boar harvest has increased in the last decades in association with more intensive management actions to increase hunting yields and consequent effects on wild boar health status. We investigated the spatial-temporal trend and the risk factors related to the prevalence of *Trichinella* sp infection in wild boar to obtain the annual probability of occurrence for these parasites in the Ciudad Real province of SCS. From 1998 to 2010, the mean prevalence for *Trichinella* sp by artificial digestion was 0.2% (0.17-0.23 95% CI) for the 95,070 examined wild boar. The presence of *Trichinella* sp infections in wild boar showed a decreasing trend during the study period, and was negatively related with fenced wild boar populations. Results revealed an absence of areas where *Trichinella* sp infection could be considered endemic, but also suggested that these parasites could occur sporadically in most of the study area. Risk maps based on biogeographical tools showed that even if the *Trichinella* sp endemic areas were very limited, most hunting estates presented favourable risk factor scores for these parasites at least during one of the studied hunting seasons. Thus, considering that human trichinellosis is still being reported yearly, application of new spatial epidemiology tools prove useful for risk factor assessment and for monitoring the parasite in wild boar populations over large spatial-temporal scales.

[140] BORDER DISEASE VIRUS IN PYRENEAN CHAMOIS: TWO STRATEGIES AND ONE AIM

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Pestiviruses are highly successful viruses found world-wide in several Artiodactyla species. "Hit and run" (HR) and "infect and persist" (IP) are the main evolutive strategies adopted by viruses to persist in animal populations. In pestiviruses, HR strategy is characterized either by transient or lethal infection, whereas IP strategy is characterized by specific immunotolerance to the virus, with little cost to the infected individuals. This mechanism of evading from the adaptive immune response is unique among persistent viral infections. Since 2001, a disease associated to a border disease virus has hit severely some populations of Pyrenean chamois (*Rupicapra pyrenaica*), while others have remained stable despite the infection has been detected. We compared the epidemiology of pestivirus infection in two areas. In the first one, in the Central Pyrenees, the infection is characterized by moderate to very high mortality, producing an epidemic wave and a significant increase of the seroprevalence that fits with the HR strategy. In the second, in the Eastern Pyrenees, the infection is characterized by a moderate to high seroprevalence, constant low viroprevalence and absence of mortality, which suggests an IP strategy. In addition, the effect of environmental, livestock and chamois population parameters in the two areas has been compared.

[141] VICIOUS CIRCLES ON ENDOPARASITE INTENSITY DO NOT MATCH WITH FOOD RESTRICTION IN PYRENEAN CHAMOIS

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Recent studies have suggested the existence of a strong relationship between host condition, host susceptibility and intensity of infection. This "trinity" results in "vicious circles", being hosts (i.e., individuals or populations) in poorest conditions, those that are more susceptible to infection, experiencing a higher pathogen load which further weakens its body condition. However, endoparasite intensities of infection (e.g., gastrointestinal nematodes and coccidian loads) in a free ranging population of Pyrenean chamois (*Rupicapra p. pyrenaica*) in the northeast Spain appeared to follow the inverse pattern: higher parasite intensity of infection in summer coinciding with the peak of primary productivity, whereas the lower in winter, when food shortage occurs. These cycles between food availability and intensity of infection are quite common in other free-ranging populations of ungulates including livestock, and suggest that we are probably using the wrong scale for detecting the apparition of "vicious circles". In this presentation, using data from a three-year endoparasite load survey in Pyrenean chamois and an experimental procedure with sheep, we will see how the relationship between different parasite species rather than the quantification of specific parasite loads would provide a more accurate detection of the existence of vicious circles in free-ranging host populations.

[142] SEASONAL SPREAD OF A PESTIVIRUS IN A STRUCTURED PYRENEAN CHAMOIS POPULATION: A MODELLING TOOL

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Recently, a Pestivirus emerged in populations of Pyrenean chamois (*Rupicapra r. pyrenaica*). The infection rapidly expanded across Pyrenees, leading to large epidemics with numerous deaths (as in Spain), or to infection persistence over several years (as in France, where a decline of population size was observed simultaneously). The virus can be transmitted both horizontally and vertically. Vertical transmission leads to abortion or to the birth of persistently infected (PI) animals with a short life expectancy. Horizontal transmission involves a complex dynamics because of a seasonal contact pattern. We aimed at better understanding the virus spread and its impact on the population dynamics. We developed an age-sex structured stochastic compartmental model of the virus spread accounting for seasonal population dynamics and contact pattern. Model outputs are the probability of virus persistence, prevalence and incidence over time, and losses due to abortions and disease-related deaths. A sensitivity analysis of the model showed a probability of virus persistence almost systematic for probable values of the parameters (+/-10%), and highlighted the main parameters influencing model outputs. These parameters represent potential control points of the biological system and require to be estimated precisely to ensure relevant model predictions. The model will be used to evaluate control strategies of Pestivirus spread and their impact on chamois population dynamics.

[143] MEASURING CHRONIC STRESS IN ROE DEER BRAIN: GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR MRNA EXPRESSION

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In the Netherlands, an estimated 60.000 roe deer are freely ranging. The various deer-populations are being exposed to different environmental conditions, some of which are more likely to induce prolonged stress than others. To gain a better understanding of their stress exposure, this study aims to develop a method to determine molecular markers for chronic stress in free-ranging roe deer. Chronic stress results in (mal) adaptations of the hypothalamic-pituitary-adrenal (HPA) axis in different species. Multiple studies revealed down-regulation of glucocorticoid and sometimes also mineralocorticoid receptors in the hippocampus of chronically stressed animals. These receptors are known to play a role in the negative feedback system of the HPA-axis and chronically stressed animals show a disruption of this feedback system. We hypothesize that chronic stress in roe deer results in similar changes in MR and GR mRNA levels. However, both the brain structure and the genome of the roe deer are unknown. Therefore, in this study we combined MRI scanning with histological staining on paraffin embedded tissue to develop an atlas of roe deer brain. Furthermore, we obtained MR and GR cDNA from roe deer to develop mRNA probes for MR and GR that allow the examination of MR and GR mRNA levels in the hippocampus of free-ranging roe deer using in situ hybridization. The development of molecular tools to evaluate chronic stress in roe deer is an important asset to wildlife welfare monitoring.

[144] SEROLOGIC FINDINGS IN ROE DEER IN FLANDERS (NORTHERN BELGIUM)

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Roe deer are the only wild living native cervid species in Flanders. Their population number has increased steadily in the last twenty years and is estimated at 20,000 with the highest densities in the eastern provinces. Contacts between wild and domestic ruminants are facilitated by the small-scale Flemish landscape structure. In order to detect circulation of human and animal pathogens in roe deer in Flanders, a preliminary screening was carried out from May 2008 to September 2011. Using different serologic methods, hunter-collected roe deer sera (n = 131) were examined for antibodies to twelve infectious agents (percent positives and suspects in brackets): *Mycobacterium avium* subsp. paratuberculosis (5.22 %; susp. 2.61 %), *Brucella abortus* (0 %), *Coxiella burnetii* (0.87 %), Leptospira sp. (2.54 %), *Chlamydia abortus (*1.65 %; susp. 1.65 %), *Anaplasma phagocytophilum* (56.1 %), Bovine Viral Diarrhoea virus (BVD) (1.85 %), Infectious Bovine Rhinotracheitis virus (IBR) (0 %), Bluetongue virus (BTV) (0%; susp. 3.48 %), Tick-borne Encephalitis virus (TBE) *(0 %), Toxoplasma gondii* (47.50 %) and *Neospora caninum* (6.72%). Like in Belgian cattle and southern Belgian cervids, paratuberculosis appears enzootic in Flemish roe deer. Except for *A.phagocytophilum ,T.gondii and N.caninum*, exposure to the other pathogens is low or non-existent. The quality of hunter-collected samples and the determination of the cut-off values used are important for the interpretation of the results.

[145] ROE DEER (*CAPREOLUS CAPREOLUS*) AS A SEROLOGICAL SENTINEL FOR TICK-BORNE ENCEPHALITIS VIRUS (TBEV) IN NORTH-EASTERN ITALY

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Roe deer (*Capreolus capreolus*) is the most abundant and the most widely distributed wild ruminant in North Eastern Italian Alps, where TBEV is one of the most important zoonotic agents present in the wild ecosystem. We studied roe deer as a suitable sentinel of TBEV at a local level, in consideration of ecological factors such as its small home- range. During the period 2006-2011, a total of 681 blood samples were collected from hunting preserves of 7 provinces including endemic and non-endemic enclosures. All the sera were tested for antibodies against TBEV by a cELISA. Overall percentage of seroreactors was 14% (94/681). Sero-prevalence locally ranged from 0% to 70%. Seropositive roe deer were constantly detected in endemic areas. Human incidence may underestimate TBEV distribution, due to the vaccination of risk categories and low TBEV circulation. Sheep and goats were evidenced as good sentinels also in consideration of the close relationship with human activities, but they are not as much distributed as roe deer. Serological surveys in roe deer populations may therefore be a useful screening for the presence of the virus in previously considered TBE-free areas. In this case the identification of possible new TBEV foci should be deepened by analyzing ticks and other sentinel species such as domestic ruminants and by testing the positive roe deer samples with other methods such as hemagglutination-inibition (HI) or plaque reduction neutralization test (PRNT) in order to confirm the results.

[146] CERVID HERPESVIRUS 1 (CVHV-1) INFECTION IN FREE-RANGING AND FARMED *CERVIDAE* IN FRANCE

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Cervid herpesvirus 1 (CvHV-1) belongs to the subfamily of alphaherpesviruses and is closely related to bovine herpesvirus 1 (BoHV-1). CvHV-1 infection is associated with conjunctivitis, purulent ocular discharge, uniform corneal opacity, and mucopurulent nasal discharge. This virus was described worldwide and specially in Northern countries of Europe (Scotland, Belgium..) with high seroprevalence. In France, it was only isolated from a farmed red deer in Northern France (Thiry and al, 2009), but CvHV-1 infection was not identified from free-ranging animals. The present study investigates the epidemiological situation of free-ranging animals in two French areas and also in a herd of red deer. The CvHV-1 infection was studied in free-ranging deer-like ruminants, in Champagne-Ardennes region and in "Vallée de l'Ubaye" area. The infection was also studied in a red deer livestock located in Saone-et-Loire department. Based on the antigenic homology between CvHV-1 and BoHV-1, cervid serum sample analysis was performed by using BoHV-1 glycoproteins B blocking ELISA. Cross-seroneutralisation assay was used to confirm that ELISA detected antibodies were specific to CvHV-1. Two CvHV-1 infected free-ranging animals, a red deer and a roe deer, were localized in Champagne-Ardennes. Within the studied livestock, 22 of 24 tested red deer were infected. Our results demonstrate, for the first time, the presence of CvHV-1 infected free-ranging *Cervidae*, in France.

[147] ANTIBODIES TO BHV-1 AND BVDV IN POLISH FREE-RANGING DEER

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Bovine herpesvirus type 1 (BHV-1) and bovine viral diarrhea virus (BVDV) are causative agents of highly important infectious diseases of cattle, namely infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD). The presence of these pathogens in free-ranging deer population is of high importance for IBR and BVD epidemiology. The goal of this work was to study the incidence of seroconversion to BHV-1 and BVDV in free-ranging deer species in Poland. The blood sampling was conducted over the period of two consecutive hunting seasons from November 2010 to January 2012, at 10 sites located in different regions of Poland. In total 578 serum samples were collected, and the number of animals sampled ranged from 29 to 105 per site. The sera were tested with commercial blocking ELISA assays for detecting BHV-1 and BVDV antibodies in domestic ruminant species. Overall 145 of animals tested (25%) were positive to BHV-1 antibodies and only 2 animals were positive to BVDV antibodies (0.35%). The proportion of seropositive deers from one site ranged from 0 to 50%. Both BVDV positive samples originated from one site. In our study we detected distinctive percentage of BHV-1 seropositve free-ranging deer in Poland. On the other hand BVDV seems to be rare. Further studies would be relevant to address the questions of factors that determine the BHV-1 seroprevalence level in populations, and what is the impact of high seroprevalence on the health of deer population.

[148] FREE-RANGING RED AND ROE DEER IN POLAND ARE INFECTED WITH CVHV-1 AND NOT BHV-1 AS EVIDENCED BY SERONEUTRALISATION ASSAY

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Bovine herpesvirus type 1 (BHV-1) is an important pathogen of cattle responsible for infectious bovine rhinotracheitis (IBR), the major viral disease of cattle. There are several ruminant alphaherpesviruses genetically and antigenically closely related to BHV-1 including cervid herpesvirus 1 (CvHV-1), that was isolated from ocular lesions, vaginal and nasal swabs from red deer. The serological cross-reactivity between BHV-1 and other closely related ruminant herpesviruses does exist, and because of the implications of BHV-1 infection in free-ranging deer for the epidemiology of IBR it is important to clarify the cause of potential serological cross relationships with BHV-1 in these animals. In our earlier study we have found deer in which seroconversion in blocking IBRgB ELISA was detected. The aim of this report is to present the results of seroneutralization analysis of the serum samples from 50 red and 9 roe deer against CvHV-1 and BHV-1 to identify the agent that caused the seroconversion. For this purpose BHV-1 (Iowa 4A) and CvHV-1 (Banffshire 82) strains have been used. We have found 32 serum samples (27 from red deer and 5 from roe deer) that neutralized both CvHV-1 and BHV-1. All positive sera had higher neutralizing titers against CvHV-1 than BHV-1. In summary, our results indicate that the Polish free-ranging deer population is infected with CvHV-1 and not with BHV-1. Also, it is the first report of roe deer infection with CvHV-1.

[149] SEROPREVALENCE OF *LEPTOSPIRA INTERROGANS* ANTIBODY IN WHITE-TAILED DEER FROM TWO SITES IN WISCONSIN

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White-tailed deer (*Odocoileus virginianus*) are the most economically and culturally important game species in Wisconsin. The Wisconsin Department of Natural Resources initiated a $2 million research endeavor in January – February 2011 to determine causes of mortality in fawn and adult male deer. To fit deer with radio-collars for mortality investigations, DNR biologists captured deer in box and Clover traps and via helicopter net – gun near Winter and Shiocton, WI. We collected blood samples from captured deer to evaluate exposure to numerous pathogens that affect deer and livestock. I will discuss exposure of deer to leptospirosis, a disease that can cause abortion in deer. Leptospirosis is a bacterial disease that infects primarily the kidneys of affected hosts. Bacteria are shed in urine that then contaminates the environment, including surface water. We tested blood serum for antibodies against *Leptospira interrogans* using microscopic agglutination. We used Fisher's exact tests to identify differences in seroprevalence by site and by gender. No male deer (0/44) were exposed to *Leptospira pomona* but 22.3% (21/91) of females were exposed (*P* = 0.003). Additionally, more females in Winter (16/49 = 32.6%) were exposed to *Leptospira pomona* than females in Shiocton (5/45 = 11.1%) but the Fisher's exact test was not significant (*P* = 0.053). At this time, we do not know how exposure to leptospirosis affects deer and we hope to evaluate exposure in relation to pregnancy, site, etc. in a multivariate analysis at the conclusion of this study.

[150] DETECTION OF *TOXOPLASMA GONDII* DNA IN EUROPEAN WILD RABBITS (*ORYCTOLAGUS CUNICULUS*) FROM PORTUGAL

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Infections with *T. gondii* in wildlife fauna may be a marker of environmental contamination with the parasite and an indicator of the potential transmission to human beings and domestic animals. The purpose of the present work was to detect *T. gondii* DNA in European wild rabbits (*Oryctolagus cuniculus*) from areas of Centre region of Portugal, and potential implications for public health. To our knowledge, this is the first study performed on *T. gondii* in European wild rabbits in Portugal. Heart and diaphragm samples were obtained from 28 rabbits slaughtered in hunter season from central Portugal. Nested PCR separately amplified the 5' and 3' ends of the surface antigen 2 (SAG2) gene. Four PCR were used to amplify the external and internal sequences of the first and second fragments of gene SAG2 with external primers SAG2.F4 and SAG2.R4 (first fragment) and SAG2.F3 and SAG2.R3 (second fragment), and the internal primers SAG2.F and SAG2.R2 (first fragment) and SAG2.F2 and SAG2.R (second fragment). *T. gondii* DNA was detected in 19 out of the 28 sampled animals, resulting in a prevalence of 67.85%. These reveal that *T. gondii* infection occurs in the Portuguese wild rabbit and therefore may pose a potential risk for humans if consumed raw or undercooked. This work highlights the importance of preventive measures that must be put into practice over the intermediate and the definitive hosts, in order to prevent infection by this zoonotic parasite and the conservation of wildlife.

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[151] HEALTH ASSESSMENT OF FREE-RANGING CAPYBARAS (*HYDROCHOREUS HYDROCHAERIS*) AT THE ALBERTO LÖFGREN STATE PARK – SÃO PAULO, BRAZIL

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Wild animals are known to play an important role in public health, as they may act as reservoirs for potentially zoonotic infectious agents. Capybaras (*Hydrochoerus hydrochaeris*), common Brazil, have been associated with the transmission of spotty fever (*Rickettsia rickettsii*), leptospirosis, leishmaniasis, rabies and Chagas disease, as well as enterobacterial, fungal and parasitic diseases. We examined free-ranging capybaras at the Alberto Löfgren State Park (São Paulo, Brazil) aiming to determine their health status and prevent potential zoonotic and environmental impacts.Thirty-one capybaras were physically and chemically restrained, identified with microships, and blood, rectal swabs, feces and hair samples were collected. Microcytic normochromic anemia and eosinophilia were observed in all animals; serum chemistry revealed mild hypoalbuminemia, considered suggestive of malnutrition. Serology for infection diseases was negative for all animals. *Salmonella* sp. was not isolated from the feces; however, *Escherichia coli* strains were retrieved from 19 animals (61.3%) and will be serotyped in the near future. Coproparasitology revealed *Protozoophaga* sp. (58%), *Strongyloides* spp (9.7%), *Vionella* spp (22.6%) and Ancylostomid eggs and larvae (9.7%). No dermatophytes were isolated from the hair samples, only molds. The studied animals were free from potentially zoonotic agents, however the State Park would benefit from implementing preventive sanitary measures to protect visitors.

[152] *LEPTOSPIRA* INFECTION AND HABITAT ASSOCIATIONS AMONG RODENT POPULATIONS IN MADAGASCAR: TRANSMISSION RISK OF HUMANS AND LIVESTOCK

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Leptospirosis has not been considered a health problem of increasing importance in Madagascar. The disease was first reported to Madagascar in 1954 through the diagnosis of a person never left Madagascar. Although serological surveys have suggested the presence of *Leptospira* in Madagascar for some time, we only obtained the first isolate in 2010. In Madagascar, the incidence of human infection and disease are largely unknown while many cases of fever are not identified according to a recent study. However, a serological study of livestock in southern Madagascar indicated fairly high infection rates. In spite of the increasing importance of this zoonosis in the ocean Indian, the role of wild reservoirs has recently been considered. The aim of this study was quantify spatial variation in prevalence of leptospira among small mammals in Madagascar and determine the relationships to bioclimatic zone, landscape and husbandry practices to investigate what factors influence exposure to *Leptospira* infections in small mammals and humans at a range of sites The identification and distribution of wild reservoir-hosts were carrying out in five districts in Madagascar and in four habitats: harbors, house, vegetation and in rice field irrigated. We tested 268 kidneys and 43 urine by real time PCR to reveal infection levels in small mammals. 268 animals were trapped of which rats (*Rattus rattus, Rattus norvegicus)*, mice (*Mus musculus)* and shrew *(Suncus murinus*). Eighty six animals were positive by PCR (34%). *Rattus norvegicus* had the highest rates positively and *Rattus rattus* had positives in all habitats. This study emphasizes the role of small mammals in the epidemiology of leptospirosis in Madagascar and may pose a disease threat to humans and livestock.

[153] AQUATIC RODENTS: WHICH ZOONOTIC THREAT?

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Nutria (*Myocastor coypus*) and muskrat (*Ondatra zibethicus*) are large semi-aquatic invasive rodents, naturalized throughout European countries. They are regarded as pests and can be infected with several pathogens transmissible to humans, livestock, and pets. In the 12 departments of Western France, 881 nutria and muskrats were sampled on 5 sites per department (river or pond). The sample design was built to estimate nutria and muskrats densities on each site and *Leptospira* sp. infection (respectively 20% and 35%) and exposition (44% and 51%), *Toxoplasma gondii* exposition (27% and 31%), tularemia infection (ongoing), alveolar echinococcosis infection (4 cases) and Q-fever exposition (ongoing). The first results of the study show that the sampled aquatic rodents are highly infected by zoonotic pathogens. The serological and infection data will be confronted to individual and environmental parameters to search for factors associated to prevalence of the studied zoonotic pathogens and thus characterize the infection in populations of aquatic rodents.

[154] PREVALENCE OF ZOONOTIC PATHOGENS IN MICROMAMMALS CAPTURED IN NATURAL AND RESIDENTIAL AREAS IN PERIURBAN BARCELONA (N.E. SPAIN)

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Micromammals are important reservoirs of zoonotic pathogens, and living in residential areas has been linked to higher risk of contact with some of them. We analyzed by real-time PCR blood and spleen samples of 187 *Apodemus sylvaticus*, 25 *Mus spretus*, 2 *M. musculus*, 18 *Rattus* spp., and16 *Crocidura russula* captured in spring and autumn, 2011, in four areas: two Natural Parks on the periphery of Barcelona (NE Spain), and two residential areas sited in the boundaries of these Parks. *Apodemus sylvaticus* was the dominant species both in natural and residential habitats. Overall prevalence was: *Ehrlichia/Anaplasma* spp. (E/A), 10.4%; *Rickettsia* spp., 10.8%; *Bartonella* spp., 37.2%; *Borrelia* spp., 8.2%; and *Babesia* spp., 0.4%. Mean number of pathogents per host was 0.57±0.77 (range 1-4), being higher in autumn (1.0) than in spring (0.2). Overall prevalence of *Borrelia* spp. was higher in autumn (18%) than in spring (1%). *Rickettsia* spp. and *Bartonella* spp. were also more frequently detected in *A. sylvaticus* captured in autumn (20% and 72%, respectively) than in spring (3% and 16%). When compared with the other host species, prevalence of E/A was significantly higher in *M. spretus* (32%), whereas, in autumn, prevalence of *Bartonella* spp. was higher in *A. sylvaticus* (72%), and prevalence of *Rickettsia* spp. was higher in *Rattus* spp. (57%). Prevalence of E/A was higher *M. spretus* captured in natural (50%) than in residential (0%) areas, and higher in juveniles (54%) than in adults (17%).

[155] LEPTOSPIROSIS EXPOSURE IN WORKERS INVOLVED IN RICE PRODUCTION IN JAPAN: AN EXAMPLE OF BBN MODEL

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We demonstrate the capacity of Bayesian Belief Nets (BBN) through a case study focused on describing exposure pathways for leptospirorsis. A review of the literature identified possible routes by which a person could be infected with leptospirorsis. In the second stage a BBN was constructed to describe one or more of the exposure routes. The final stage was to ‘solve’ the BBN with a series of different hypothetical input values. In Japan the most common routes for human infection related to contact with urine from infected animals either directly or through contact with contaminated soil or water. When using these values the probability an agricultural worker is exposed to leptospirorsis is 0.46% and the probability they develop clinical symptoms is 0.31%. When the soil temperature and water content in the soil are perfect for the growth the probability the worker develops clinical symptoms increases to 0.64%. The probability that the worker develops clinical symptoms will be even higher if the person has a wound (1.5%) and if the wound occurs at a time rats are known to be present in the field (2.34%).

[156] PLAGUE EPIDEMIOLOGY IN MADAGASCAR: SEASONAL CYCLES AND DISEASE RISK

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Madagascar has one of the highest incidences of plague in the world. Human plague cases are seasonal and patchily distributed. The zoonotic epidemiological cycle principally involves the black rat, *Rattus rattus*, and two species of flea, *Xenopsylla cheopis* and *Synopsyllus fonquerniei*. Whilst *X. cheopis* is the most common flea species on rodents found in houses, *S. fonquerniei* dominates rodent flea communities outside. Here we investigate the spatial and temporal patterns exhibited by flea and rat populations and the consequences for plague epidemiology and disease risk in humans. Over a period of 13 months, 6 sampling surveys were conducted. During each survey, rats and fleas were sampled from 4 villages (24 villages in total). Rats were trapped in 3 habitats: houses, sisal hedges and rice fields. Fleas were identified to species. Serological assays and rapid diagnostic tests that target the F1 antigen of *Y. pestis* were used to determine exposure rates in rats. Both rat and flea populations show distinct seasonal patterns. Rat numbers peak in early winter (July), just after the end of the rice harvest. *S. fonquerniei* numbers peak at the end of the winter (September), whilst *X. cheopis* are most abundant between November and March. As most human cases occur between October and March, both flea species appear important for transmission to humans. Models are being developed to examine the role of the two flea species in allowing plague persistence.

[157] BLACK-TAILED PRAIRIE DOGS AS RESERVOIRS FOR PLAGUE

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Three lines of evidence for black-tailed prairie dogs (BTPD) serving as reservoirs for plague have emerged in the past decade. Because prairie dogs suffer nearly 100% mortality from plague, other rodents were thought to serve as reservoirs, but research since 2000 failed to identify such reservoirs. 1. Mapping active prairie dog colonies on three National Grasslands since 1999 identified plague epizootics at one or more of these grasslands every year. At this large scale (radius 100 km) plague in BTPD was a constant presence. 2. Increased survivorship resulting from flea control and/or vaccinating black-footed ferrets against plague demonstrated that disease causing *Y. pestis* is present at some colonies, even when there are no obvious signs of an epizootic. 3. PCR analyses of fleas collected from BTPD burrows at colonies in Montana identified moderate rates of *Y. pestis* infection at colonies with no other evidence of plague. These observations, along with failure to identify reservoirs in other associated rodents, provide evidence that BTPD and/or their associated fleas are a reservoir for *Y. pestis* at appropriately large scales.

[158] MONITORING THE OCCURRENCE OF HELMINTHS IN WILDLIFE SINCE PRE-HISTORIC TIME USING FAECES AND COPROLITES

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The study of faeces is particularly interesting because its accessibility and low cost allows long-term studies on species. For 30 years the team of Paleoparasitological and Parasite Ecology Laboratories of the Oswaldo Cruz Foundation, in Brazil, study parasites in recent faeces and coprolites, making possible identify disruption distribution by climate changes on parasite assemblage, increased flow of parasites between wild and domestic species and eating habits over time and along global environmental changes. Using the Callen & Cameron (1960) rehydration and Lutz (1919) sedimentation methods it's possible to prepare material for classical parasitological analysis, as well for molecular analysis and electronic microscopy. The results using about 1,600 faeces' samples and 2,100 coprolites have allowed studies in dynamics of host-parasite relationship as the establishment of the semiarid Caatinga bioma reflected in a chronological gap, between 8,000 and 5,000 years, of *Trichuris* (Nematoda) abundance on its host, the *Kerodon rupestris*, a Caviidae rodent, and its current restoration; new contacts as a lizard's parasite (*Parapharyngodon*, Nematoda), common in pre-historic people, that still are a human dietary component in drought times and the growing biodiversity of helminthes fauna in secondary consumers, as wild and domestic dogs and cats, empowered by hunting, urbanization and large projects that promote workers migratory flow and ecosystem disturbance.

[159] BASE REFERENCE DATA FOR *LEISHMANIA INFANTUM* AND *TOXOPLASMA GONDII* IN MONTECRISTO ISLAND (ITALY), BEFORE ERADICATION OF THE BLACK RAT, *RATTUS RATTUS*

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Montecristo is an island of 1080 ha in the Tyrrhenian Sea and part of the Tuscan Archipelago (Italy). From 1971, when it was declared biogenetic reserve, it is totally devoted to nature conservation. It is among all Italian islands, the most isolated and distant from mainland. As part of a LIFE project (LIFE08 NAT/IT/00353 "Montecristo 2010") for eradication of invasive alien species, the population of black rat *Rattus rattus* was screened for presence of two parasite *Leishmania infantum* and *Toxoplasma gondii.* We captured 78 rats from four different areas of the island. Of these, we chose 43 individuals to be tested for *L. infantum*, and 22 for *T. gondii*, in order to have an even representation of al sampling sites. Two rats tested positive to a specific *L. infantum* PCR carried out on spleen, while all tested skeletal muscle samples resulted negative to T. gondii PCR. Considered the obtained results, we propose to expand our analysis to all available (already collected) samples. Black rat is currently being eradicated from the island. These preliminary data will serve as base reference to monitor how the epidemiology of these two parasites will change once the black rat will no longer occupy its ecological niche.

[160] MORPHOLOGICAL HETEROGENEITY OF MIXED TRYPANOSOME INFECTIONS: AN EXPLANATION FOR THE DECLINE OF THE BRUSH-TAILED BETTONG (*BETTONGIA PENICILLATA*) IN AUSTRALIA

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For the last 7 years the Brush-tailed Bettong (*Bettongia penicillata -alias*: Woylie) in Western Australia has been on the verge of extinction and is now restricted to four sub-populations. An extensive conservation effort involving government agencies, zoos and universities has had minimal influence, as the remaining woylies have recently experienced an alarming 93-95% reduction in numbers. The reasons for the decline are currently unknown. Our work involving the haemoparasites of the woylie shows that there are three morphologically distinct trypomastigotes from two different species. The smaller of the two species has a singular trypomastigote form and exhibits *Trypanosoma lewisi*-like reproduction in the blood during the subacute phase of infection. While the other larger species, *T.copemani* exhibits pleiomorphic trypomastigote forms. The two different phenotypes of *T.copemani* are distinguished primarily by the distance between the kinetoplast and nucleus. Results show that during the subacute phase of a mixed infection, the blood forms dominate for both species. They then appear to migrate from the blood and enter the chronic phase, where they infect the tissues. Each phenotype exhibits a predilection for certain tissues, where they can display *T.cruzi*-like pathology. Our study reveals that the trypanosomes may play a role in the decline of the woylie and highlights the need for extensive health checks at the time of translocation of endangered animals.

[161] MEASURING THE HEALTH OF URBANIZED SPECIES: THE WHITE IBIS (EUDOCIMUS ALBUS) AS A CASE STUDY

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The level of urbanization is projected to increase to 65% by 2025. Some species are taking advantage of this unprecedented growth of cities, with the field of urban ecology dedicated to understanding the shifts in foraging and behavior that these species undertake to adapt. In fact, shifts in foraging strategies and behavior are intimately tied to disease exposure and transmission and thus, understanding the health of these animals will challenge the previously established epidemiological paradigms. I will use the White Ibis (*Eudocimus albus*) in South Florida as a case study of a multifactorial approach to measuring their health soon after their relatively recent adaption to the urban environment, which has changed their exposure to pollution and peridomestic species, their shift in prey base and behavior. White Ibis are shedding Salmonella spp at a high prevalence and of serotypes known to match those reported in clinical cases in people in Florida. Additionally, they have a surprisingly high prevalence of antibodies against avian influenza and paramyxovirus, a high load of ectoparasites and are, in general, are in poor body condition. Yet, the bottom line still remains - what is their fitness?

[162] DO REGIONAL LAND-USE GRADIENTS AFFECT PREVALENCE OF MICROPARASITES AND CO-INFECTION RATES IN BIRDS?

EWDA Student Travel Grant

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Macro and microhabitat alterations that come with land use change have been proven to decrease bird species diversity and favor vector-borne diseases in general. Although hemoparasites are not considered an important selective pressure on birds, single or multiple infections with protistan parasites and parasitic nematodes may serve a regulatory function if the costs that parasitism incurs to individual birds are considered. Currently, there are no reports comparing prevalence, infection status, and land use influences of the Piedmont and Coastal Plains of Georgia, physiographic regions with different vector activity and land use gradients. This study determined the prevalence and diversity of hemoparasite infection and co-infection rates in over 50 species of passerine and non-passerine birds. Common resident and migratory birds were captured at three sites in the Piedmont region and three sites belonging to the Coastal Plains region in one year during all four seasons. Five species of hemoparasites were identified (from taxonomic orders Haemospororida, Trypanosomatida and microfilaria). Significant differences in seasonality, species and infection status and co-infection rates between the sites were found, suggesting effects of temporal, spatial and taxonomical drivers of infection.

[163] TISSUE MICRO-ARRAY AS A SCREENING TOOL TO INVESTIGATE THE ATTACHMENT PATTERN OF INFLUENZA A VIRUSES IN HUMAN AND AVIAN TISSUES

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Tissue microarray (TMA) technology enables studying a large set of tissues using only a small amount of reagent. In the present study, virus histochemistry was applied on TMA sections to explore the attachment patterns of 3 different influenza viruses (human H3N2, mallard H6N1, gull H16N3) in a wide spectrum of human, mallard (*Anas platyrynchos*), and gull (*Larus ridibundus*, *L. pipixcan*, *L. argentatus*) tissues. The proportion of cells with virus attachment was visually scored by two independent observers. The attachment patterns of the human and mallard viruses in human and mallard tissues were consistent with previously published results, therefore suggesting that TMA can be used as a screening tool revealing if a virus abundantly attaches to a particular tissue. The human virus attached to the human respiratory tract and the trachea of all investigated gull species. The mallard virus attached to the trachea and intestines of mallards as well as to human cornea and *L. pipixcan* trachea. Finally, the gull virus attached to the trachea and intestines of gulls (with some interspecies variations) and to mallard trachea. Interestingly, it also attached to human cornea, upper respiratory tract and mucinous salivary glands, therefore suggesting the need to investigate the possibility of H16 virus transmission to humans. Overall, these results confirm that patterns of virus attachment vary significantly between bird species and depend on the species from which the virus was isolated.

[164] A ONE HEALTH APPROACH TO UNDERSTANDING DISEASE ECOLOGY DYNAMICS AT POYANG LAKE, CHINA, AND ALONG THE EAST-ASIAN AUSTRALASIAN FLYWAY

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In the agroecological rice cropping wetland system of Poyang Lake, Jiangxi Province China, more than 26 million openly grazed domestic ducks, 3 million domestic geese, and 21 million chickens share habitat with thousands of farmed wild waterfowl, over a million over-wintering migratory waterfowl, millions of swine, and millions of people, ideal condition exists for a One Health approach to better characterize influenza and other disease transmission dynamics across sectors. Areas of expertise integrated into this project included wildlife ecology, veterinary epidemiology, ornithology, virology, anthropology, social sciences, public health, spatial mapping, and landscape ecology. Through a combination of radio-marking free ranging waterfowl, tracking movement of grazed farmed wild birds, conducting surveys at farmed wild bird and livestock farms, and conducting disease surveillance in both domestic ducks, farmed wild birds, and humans, we have been able to characterize the interface dynamics between the multiple sectors and understand the implications for disease transmission within the Poyang Lake wetland system, and along the East-Asian Australasian Flyway. Results from this integrated One Health approach to disease ecology in Poyang Lake will be presented.

[165] VIRULENCE OF LOW PATHOGENIC AVIAN INFLUENZA VIRUS INFECTION IN WILD BIRDS: AN EVALUATION

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Historically, low pathogenic avian influenza virus (LPAIV) was assumed to be avirulent for wild birds, but in recent years this assumption has been questioned. Knowledge of the level of virulence of LPAIV in wild birds is important to help us understand its epidemiology and evolution, and to assess how highly pathogenic avian influenza virus H5N1 would need to change to be able to be maintained in wild birds. Therefore, I performed a literature review to evaluate the virulence of LPAIV for wild birds. I evaluated 21 articles of both experimental and natural infections of anseriform and charadriiform birds infected with LPAIV from birds of those two orders. No studies reported mortality or prominent clinical signs from LPAIV infection. Subtle clinical effects were increased body temperature, reduced body weight, and reduced egg production. Infection of the respiratory tract was associated with mild inflammation, but infection of the digestive tract was not associated with any detectable lesions. Behavioural changes associated with infection were reduced feeding and fuelling rates, delayed migration, and reduced migration distance. This evaluation shows that LPAIV infection in wild birds, while not causing mortality or prominent clinical signs, may be associated with subtle clinical effects and behavioural changes. Future field and laboratory studies will require sophisticated methods to detect such effects and to determine whether they are actually caused by LPAIV infection.

[166] ECOLOGICAL DETERMINANTS OF AVIAN INFLUENZA INFECTION IN WATERFOWL ACROSS CANADA (2005-2011)

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Since Canada's Inter-Agency Wild Bird Influenza Survey was initiated in 2005, over 19,000 apparently healthy ducks have been tested for avian influenza virus (AIV). We explored generalized linear mixed models using this data set, to identify ecological and demographic factors associated with low pathogenic AIV infection in wild dabbling ducks (*Anas* spp.). We built separate sets of models for eastern and western Canada to test the relative roles of a range of factors because data from these two regions differed in species composition, sampling periods, and methods of population density estimation, Analyses of both Eastern and Western Canadian data subsets revealed higher AIV infection risk in hatch year birds and males. Species effect was apparent only in eastern Canada, with teal (*A. discors* and *carolinensis*) less likely to be infected compared to mallards. AIV infection risk increased with breeding population density, supporting the hypothesis of density dependent transmission. Precipitation and sampling date were important in both regions with different times of peak prevalence. There appeared to be a cyclic pattern of apparent prevalence of AIV over the years. Annual variation may be due to variation in environmental variables, and factors specific to waterfowl populations (e.g., AIV immunity). This study provides further insight into ecological and demographic factors associated with AIV infection in waterfowl in Canada.

[167] BIODIVERSITY, CLIMATE CHANGE AND DISEASE RISK MAPPING IN HUMANS AND MALAGASY WILD BIRDS

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We estimated risk of vector-borne disease to humans and biodiverse hotspots in Madagascar, by analysing and mapping two diseases with respect to climate: West Nile Virus and Avian Malaria, in comparison to biodiversity and human population density. West Nile Virus can cause high mortality among Corvids, other passerines and raptors, and human deaths, for example in the US. Avian Malaria can cause high bird mortality when strains are newly introduced into naive populations. Climate strongly influences disease, especially those that, like Avian Malaria and West Nile, are vector-transmitted and predominate in wildlife. While biodiversity may protect individual species against vector-borne disease, via the dilution effect, the effects of climate change on vector-borne disease (and on host species directly) may negatively impact animal biodiversity. Climate change may thus potentially cause a negative feedback of increased disease and biodiversity loss. Here, through combining and comparing risk maps of Avian Malaria and West Nile Virus in Madagascar, we demonstrate that climate change is expected to expose new regions of the country to heightened risks from vector-borne disease. Through comparison of these areas at risk with distribution of human and avian populations, we highlight populations which could be exposed to this negative feedback effect of disease and biodiversity loss and estimate the extent of this effect.

[168] LINEAGE 2 WEST NILE VIRUS IN CENTRAL AND EASTERN EUROPE

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During the history of West-Nile virus (WNV) circulation in the Old World, Lineage 2 WNV was never incriminated in outbreaks on the European continent until its emergence in Hungary in 2004. We documented the virtually continuous presence, circulation and spread of this particular WNV strain in Hungary since 2003, followed by its expansion to Austria in 2008, Greece in 2010, and recently Italy in 2011. Although high rates of clinical disease and mortality of wild bird species were never associated with European WNV outbreaks, WNV related clinical disease and mortality has been regularly detected by a semi-targeted passive surveillance in both free ranging and captive wild birds during the past decade. Since 2003 more than 50 cases of lethal WNV cases have been detected in goshawks (*A. gentilis*), which were the most reliable indicators of WNV circulation. Disease and mortality also occurred in other captive, non indigenous and indigenous bird species, as well as in free ranging red footed falcons (*F. vespertinus*) and sparrowhawks (*A. nisus*). Subclinical WNV infection was also detected in a wide range of bird species. The aim of this long term study is to identify the most potent amplifying, reservoir and sentinel host species, pinpoint the most likely migratory species implicated in the spread of WNV and provide baseline data for risk assessment, clarifying the potential of WNV for geographic spread and its impact on species conservation, wildlife, human and domestic animal health.

[169] FLAVIVIRUS CIRCULATION IN YELLOW-LEGGED GULLS (*LARUS MICHAHELLIS*) AND VECTORS IN MEDES ISLANDS (N.E. SPAIN)

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Zoonotic mosquito-borne flaviviruses (Family *Flaviviridae*) are increasingly detected in the world. We monitored the silent circulation of flaviviruses in chicks of yellow-legged gulls (*Larus michahellis*), soft ticks (*Ornithodoros maritimus*) and Culicidae mosquito species sampled in Medes Is. (northeastern Spain) from 2009 to 2011. This Mediterranean archipelago has a dense gull population and is neighboring a highly touristic area. Antibodies against flavivirus were found in 13 out of 256 (5.1%; $CI_{95\%}$: 2.4-7.8) gull sera tested using a cELISA (ID-Vet), which detects antibodies against an epitope common to Japanese Encephalitis virus complex. Seropositivity decreased from 6.7% in 2009 to 2.6% in 2011. All cELISA positive sera showed negative results by serum neutralization test against West Nile virus. The results indicate cross-reaction with other antigenically-related flaviviruses. In addition, thirty six tick pools and ten mosquito pools were analyzed by flavivirus RT-nested-PCR. Flavivirus RNA was detected in one tick pool of 4 ticks collected in 2011. Sequence analyses indicated a 95% similarity to the Meaban virus (a seabird tick virus) previously detected in France. Results are relevant in the context of wildlife monitoring of bird zoonoses, owing to the high densities and wide distribution and mobility of the gulls, which may pose serious risk of spill over to humans.

[170] BACTERIAL MICROFLORA OF GRIFFON VULTURE (*GYPS FULVUS*) IN THE PYRENEES (SPAIN)

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The current state of vulture populations is particularly sensitive as consequence of changes in livestock management and sanitary legislation. The Territorial Cooperation Program Spain-France-Andorra 2007-2013 (POCTEFA) is an operational framework that allows Pyrenean scale act in concert. The NECROPIR programme focuses on vulture species, to improve the knowledge of their biology, population trends, diseases and conservation. The importance of vultures as potential vectors of bacteria has gain interest in the last years. In 2011, a total of 75 griffon vultures (*Gyps fulvus*) were captured with cage-trap in two areas of the Pyrenees (Navarra and Catalonia, N Spain). To study the bacterial microflora carried by this species, swabs from the pharynx, nostrils and cloaca were obtained. The isolation was performed on MacConkey Agar (37℃ under aerobic, 24 hours), Columbia agar (37℃ in aerobic/anaerobic, 24-48 hours) and chocolate agar (37℃ in microaerophilic, 24-48 hours) and identification based on macroscopical and microscopical characteristics and biochemical tests using traditional or commercial multisubstrate galleries. A total of 95 bacteria species were isolated. Most common isolates were included in the genus *Aerococcus*, *Enterococcus*, *Escherichia*, *Clostridium*, *Lactococcus*, *Proteus*, *Staphylococcus* and *Streptococcus*. There was an asymmetric distribution of these genera and other minor ones between the two Pyrenean areas, suggesting specific spatial differences in the microbial population.

[171] SEROTYPING *CHLAMYDOPHILA PSITTACI* IN FERAL PIGEONS BY HIGH RESOLUTION MELTING ANALYSIS (HRM)

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Chlamydophila psittaci is a zoonotic pathogen currently classified into seven avian genotypes (A-F and recently described genotype, E/B) and two nonavian genotypes (M56 and WC). Despite each genotype is considered host specific, cross infections have been demonstrated. B genotype is usually found in pigeons whereas the A is naturally infecting some psittacine species. The last is considered with higher zoonotic potential. Due to the increasing population of the exotic monk parakeet (*Myiopsitta monachus*) in the city of Madrid during the last two decades, the introduction and spreading of genotype A to the autochthonous bird species could be possible. To evaluate this hypothesis, 44 fecal samples from feral pigeons (*Columba livia*), 34 from the city of Madrid and 10 from rural locations in the same province, tested as positive to *C. psittaci* by real time PCR were serotyped. For this purpose a previously described real time PCR with high resolution melting analysis (HRM) and further confirmation by sequencing was applied. The results obtained showed the presence of two serotypes in both urban and rural environments: B (64.7% urban *vs.* 75.0% rural) and E (35.3% urban *vs.* 25.0% rural). Thus, no evidence of genotype A was found. This work represents the first genotyping of a high number of positive samples obtained from feral pigeons.

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[172] THE ROLE OF BIRDS AS RESERVOIRS FOR *BORRELIA BURGDORFERI* S.L. IN WESTERN EUROPE: IS THERE A BURDEN OF CARRYING TICKS AND *BORRELIA*?

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Birds are important in the ecology of *Borrelia burgdorferi* sensu lato (s.l.), the etiologic agent of Lyme disease, because they are important hosts for vector tick immature stages and are known reservoirs for some *Borrelia* genospecies. The aim of our study was to assess the role of common passerine bird species as reservoirs for *B. burgdorferi* s.l. in Western Europe. We surveyed ticks and tissues (blood and skin) collected from birds in two enzootic areas in Portugal, where no information is available for birds as reservoirs for this etiologic agent and where *B. lusitaniae*, for which few reservoirs have been identified, is the dominant genospecies. Ground and shrub dwelling bird species were the most heavily parasitized by ticks. Infected ticks were found in 23 birds (2.9%), but only *Turdus* sp. harboured infected tick larvae. In one study area, *B. lusitaniae* was dominant in questing *Ixodes ricinus* but no ticks feeding on birds were infected with this genospecies, and *B. valaisiana* was dominant in *I. ricinus* larvae feeding on birds. In the other area, *I. frontalis* was the main tick species feeding on birds and was infected with *B. turdi*. Skin biopsies from *T. merula* were positive for *B. valaisiana* and *B. turdi*. This is the first report for *B. turdi* in Western Europe. Potential detrimental effects of tick parasitism and *Borrelia* infection on the birds' health state, evaluated through common clinical screening methods (plasma biochemistry and haematology) will also be discussed.

[173] PASSERINES AS SOURCES OF *SALMONELLA* INFECTION FOR MULTIPLE SPECIES

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An example of the ‘one health concept’ is that where disease at high prevalence in wild species which intimately share environments with humans and their animals, act as sources of the disease for those species. *Salmonella typhimurium* (Stm) DT 40 and 56 are associated with infection of wild passerines in the UK, particularly greenfinches (*Carduelis chloris*) and chaffinches (*Fringilla coelebs*). UK data suggests that salmonellosis in these birds is not infrequent. These birds use many habitats shared by farm animals, pets and pet owners. It has been queried whether the isolates of these Stm phage types in domesticated species may originate from infected passerines. To investigate this theory we performed profiles of isolates and compared these. Using pulsed field gel electrophoresis (PFGE) and variable number tandem repeat (VNTR) techniques we compared molecular profiles from wild passerines with those from cat, dog, horse, cattle, pig and chicken. The PFGE and VNTR data show that the *Salmonella* strain types cluster largely according to phage type and that there are closely related strain types from finch, dog, cat and chicken. This supports the hypothesis that wild passerines are a source of *Salmonella* infection for a range of domesticated species.

[174] PREVALENCE OF *SALMONELLA* IN PASSERIFORM BIRDS IN NORTHERN ITALY

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Background: Salmonella bacterial infection in wild birds can cause disease and death, mainly described in small passerines. Aclinical or sub-clinical infections are also common and wild birds can become healthy carriers of *Salmonella* spp. Aim of this study was to investigate the prevalence of *Salmonella* spp. in wild birds collected in Northern Italy. Methods: During the year 2011, 195 wild birds, belonging to the Passeriformes order (Corvus corone 88/195; Sturnus vulgaris 49/195; Pica pica 45/195; Turdus merula 6/195; Corvus monedula 3/195; Passer domesticus 1/195; Garrulus glandarius 1/195; Alauda arvensis 1/195), were necropsied at the Istituto Zooprofilattico Sperimentale of Lombardia and Emilia Romagna. A pooled sample (approximately 1g) of liver and intestine was analyzed using the method ISO 6579: 2002/Amd 1:2007. Results: Salmonella was isolated from 8 of 195 birds. The prevalence was 4,1%. Three serotypes were identified. Six strains of *Salmonella* Typhimurium were isolated from: Corvus corone (3/6), Corvus monedula (1/6), Garrulus glandarius (1/6), Pica pica (1/6). A strain of *Salmonella enterica* subspecies *enterica* 4,12: i:- and *Salmonella* Infantis were isolated from a Pica pica and a Sturnus vulgaris respectively. Salmonella isolation was never associated with typical macroscopic lesions indicative of Salmonellosis in birds. Conclusions: Salmonella spp. was isolated from 8 of 195 wild birds analyzed (4,1%). Our results showed a lesser prevalence if compared with earlier prevalence studies on the wild birds population and described in Spain (8,5%), Greece (8,6%), Ljubljana (5,7%) and Sweden (4,9%). Most of the isolates belonging to the serotype Typhimurium, considered to be one of the most frequently isolated from wild birds since 1990s. Continuous monitoring of the prevalence of *Salmonella* spp. infection in wild birds should be important to detect any future spillover to domestic animal or human population.

[175] MOLECULAR CHARACTERIZATION OF *SALMONELLA* TYPHIMURIUM ISOLATES FROM WILD BIRDS AND THEIR RELATIONSHIP WITH SALMONELLOSIS IN PIGS

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Salmonella Typhimurium (and its monophasic variant) isolates from wild birds (n=29) captured in pig farms with high prevalence of salmonellosis were compared by Pulse-Field Gel Electrophoresis (PFGE) with those strains isolated from faecal (n=36) and mesenteric lymph nodes (n=46) samples from pigs and faecal samples from small rodents (n=8). Eight PFGE clusters were discernible (90% similarity) among bird isolates. Three clusters (65% of the strains) corresponded mostly to samples from 3 farms, evidencing that same *Salmonella* isolates circulated among bird species within each of these farms In 3 clusters the isolates were collected at different locations and dates, indicating that these strains circulated freely among the wild bird population in the region. All but one (96%) of the *Salmonella* strains from birds were closely related (within the same cluster) to those from pig samples, and 17 (59%) to those from rodents. Among the 20 isolates from birds analysed for antimicrobial resistance (AR), 11 (55%) showed AR to at least one drug. The most common AR pattern was Aminopenicillins-Aminoglycosides-Sulfonamides-Tetracyclines (60%), similar to what was found in pig isolates (49%). These results show that *S*. Typhimurium and the monophasic variant are widespread in the area and wild birds have an important role on its maintenance among pig herds. Biosecurity measures aimed at keeping birds out of pig premises is of utmost importance for the control of this infection.

[176] WATERFOWL: POTENTIAL ENVIRONMENTAL RESERVOIRS OF THE CHYTRID FUNGUS *BATRACHOCHYTRIUM DENDROBATIDIS*

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Infections with *Batrachochytrium dendrobatidis*, the causal agent of chytridiomycosis, have been shown to play an important role in the decline of amphibians worldwide. Spread of the fungus is poorly understood. To investigate if bird movement could contribute to the spread of *B. dendrobatidis* in the environment, 397 wild geese in Belgium were screened for presence of *B. dendrobatidis* on their toes using real-time quantitative PCR (qPCR). In addition, chemotaxis towards, adhesion, survival after desiccation and proliferation of *B. dendrobatidis* on keratinous toe scales from waterfowl were examined *in vitro*. qPCR revealed that 76 geese (15%) were positive for *B. dendrobatidis*. Results of the *in vitro* tests showed that *B. dendrobatidis* is attracted to the keratinous toes of aquatic birds on which they can adhere and even proliferate. However, desiccation is poorly tolerated. This suggests waterfowl are potential environmental reservoirs for *B. dendrobatidis*.

[177] HELMINTH COMMUNITY OF AN ALPINE ROCK PARTRIDGE (*ALECTORIS GRÆCA*) POPULATION IN A DEMOGRAPHIC CRASH

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Alpine rock partridge (*Alectoris græca*) populations are drastically decreasing mainly due to habitat degradation and climate change. From 2003 to 2011, we investigate the helminth community of a rock partridge population from Lepontine Alps (Western Alps), showing a demographic crash in the last few years (2009-2010-2011), to analyse the helminth distribution within population in relation to different factors (sampling year, age, gender) and to disclose the parasites' role in this population trend. From 213 intestinal contents of hunted rock partridges we recorded *Ascaridia compar, Heterakis gallinarum* and *Aoncotheca caudinflata*: the most abundant species is *H. gallinarum* with a significant greater abundance in adults. A significant increase of abundance of all the three helminths was recorded in 2010. Juveniles and females were infected by the three helminths while in males we recorded *H. gallinarum* and *A. caudinflata* with lower abundances and prevalence. Data show a temporal association between the demographical population crash and the abundance peak of all the three helminths suggesting a possible health impact of parasites. Even if data did not show any effect of parasites on body weights, the known effects of these parasites on fecundity indicate the likely effect of parasites as a predisposing factor for the observed demographic crash and points out the importance of the health monitoring in wildlife.

[178] PHYLOGENETIC ANALYSIS OF HEMATOZOAN PARASITES CIRCULATING IN AMERICAN BLACK DUCKS

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Avian hematozoan parasites have a near worldwide distribution and although are frequently identified in dabbling ducks, molecular characterization of parasites from ducks is limited. For example, prevalence rates in the American black duck (*Anas rubripes*) is high (>20%), but no hematozoa have been genetically characterized. To understand the phylogenetic relationships of parasites in black ducks, we collected blood samples from 106 black ducks from January to March 2010 in four states, Connecticut (n=20), Maine (n=38), Maryland (n=43), and Rhode Island (n=5). Two PCR assays were run for three hematozoan genera of interest. A total of 69 (65%) and 66 (62%) were positive for *Haemoproteus*/*Plasmodium* and *Leucocytozoon*, respectively. A subset of positive samples was sequenced and phylogenetic analysis of 41 *Haemoproteus*, 31 *Leucocytozoon*, and one *Plasmodium* revealed two monophyletic groups of *Haemoproteus* and extensive genetic variation (~15% differences) among the *Leucocytozoon*. One black duck *Haemoproteus* group included two other *Haemoproteus* spp. from waterfowl while the other group included a *Haemoproteus* from a Galapagos penguin. Despite extensive variation among the *Leucocytozoon* sequences, all grouped into three monophyletic clades of other *Leucocytozoon* associated with waterfowl. However, because of high genetic variation, we believe that *L. simondi,* the only species in ducks, is a species-complex. Careful morphologic examination of distinct genetic groups is needed.

[179] EXPOSURE TO *TOXOPLASMA GONDII* IN CAPTIVE MAGELLANIC PENGUINS (*SPHENISCUS MAGELLANICUS*) IN BRAZIL

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Toxoplasma gondii is one of the most common protozoan parasite that infect humans and warm-blooded animals. Despite the wide variety of domestic and wild animals infected by this parasite, little is known about *T. gondii* in penguins. The goal of this study was to determine the occurrence of antibodies anti-*T. gondii* in Magellanic penguins (*Spheniscus magellanicus*) kept in captive. Twenty-two penguins (11 males and 11 females) rescued in beaches along Brazilian Southern Cost by Brazilian Authorities and kept in captive from one to four years were used in this study. All the penguins were from SABINA – Park School of Knowledgment in the city of Santo André, São Paulo State, Brazil. Serum samples were collected and used for antibodies determination by a Modified Agglutination Test (MAT) with a cut-off of 1:5. From the 22 examined animals, 2 females and 4 males (6 animals – 27.5%) presented antibodies against *T. gondii* at titres of 10 (2 penguins), 20 (1 penguin), 40 (2 penguins) and 160 (1 penguin). This is the first report of *T. gondii* antibodies in captive penguins in Brazil.

[180] GENETIC CHARACTERIZATION OF TRICHOMONAS GALLINAE STRAINS FROM WILD BIRDS OF THE EAST OF SPAIN

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Trichomonosis is one of the most important infectious diseases of the upper alimentary tract on birds as it has been shown in a recent epidemic focus on wildlife population in northern Europe[4], involving passerine and columbiform species. In order to monitor the prevalence of this emergent disease in our territory (East of Spain), we studied during four years (2005-2009) the presence of the parasite and the clinical presentation among raptor and columbiform wild birds. Strains were obtained from wild birds recovered in nestlings and Wildlife Recovery Centers and in animal health control campaigns on columbiform species carried out in the Valencian Community. Strains were isolated in TYM (Tripticase-Yeast-Maltose) medium and characterised by PCR and sequencied of the ITS1/5,8SrRNA/ITS2 and Fe-hydrogenase genes. Also, a RAPD analysis was carried out on 44 selected axenic samples, including samples from clinically ill and healthy animals. The results revealed a relationship between the genotype of the parasite and the clinical presentation or geographic origin of the animals. This fact was better demonstrated with the analysis of the RAPD, which clustered the samples according to the species, geographic distribution and, more important, the clinical presentation. These data reveals the genetic diversity of *Trichomonas gallinae*, indicating its capacity of adaptation to the host. To better understand the epidemiology of this disease, new studies about the interaction between the parasite and new hosts, the immune response and the expression of virulent factors should be done.

[181] OCCURRENCE OF *TRICHOMONAS GALLINAE* IN WILD BIRDS FROM REHABILITATION CENTERS IN THE CALGARY AREA

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Trichomonosis caused by *Trichomonas gallinae* is an emerging parasitic disease in Atlantic Provinces of Canada and in UK. This parasite primarily infects pigeons, has a number of strains of varying pathogenesis and is found on all continents except Antarctica. Spill-over of *T. gallinae* to birds such as finches and raptors can result in high mortality. Although few cases of Trichomonosis have been reported from finches in Alberta, not much is known about its prevalence in this province. As a part of the surveillance activities of Alberta-CCWHC, we undertook baseline surveillance for *T. gallinae* in wild birds admitted to local rehabilitation Centre. A total of 121 live/dead birds were examined. Oral swabs from live birds were cultured using modified Diamond's medium and the organisms were morphologically confirmed as *T. gallinae* using Giemsa's staining. Culture positives and crop/esophagus samples from dead birds were subjected to PCR amplification of ITS2 region and the sequence generated was compared to the known strains of *T. gallinae*. Overall, 23 birds were tested positive (19%) of which 22 were pigeons and one magpie. None of the raptors and finches was positive. All infected birds had the same strain, P107 (EU881911.1), which was found predominant in infecting raptors in Spain. Future studies are required to determine if *T. gallinae* is present in non-rehabilitation Centre wild birds in this area.

[182] POPULATION THREATS TO VULTURES LEADING THEM TO THEIR ENDANGERED STATUS

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Currently vulture population in India is at risk due to increasing human interference. This has lead to the extinction of many species and many are under threat and on the verge of extinction. Proper conservation efforts are needed to be planned in well organized manner for preventing this unwanted natural hazard. The great Indian Bustard (*Ardeotis nigriceps*) is on the verge of extinction due to Spread of agriculture, destruction of its habitat by over grazing by livestock, and indiscriminate shooting. Only less than 1000 birds exist till date worldwide. Diclofenac-Na has been proven to be responsible for the devastation of three species of Gyps vulture on the Indian subcontinent, and it is now regarded as one of the worst environmental contaminants in the recent past.

[183] REPORT OF WILD BIRDS SURVEY ON NORTHWESTERN ITALY RECOVERY CENTERS FOR WILD ANIMALS

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The monitoring of wild animals is an important concern for the conservative biology and for the health surveillance. The Recovery Centers for Wild Animals are shelter facilities for injured or sick wild animals and they give the possibility to set plans for wildlife pathologies control. The aim of this study is to perform a health monitoring of wild birds, hospitalized in the northwestern Italy Recovery Centers for Wild Animals (Piemonte, Liguria,Valle d'Aosta). In the period 2007-2011, a total of 221 wild birds, particularly raptors, crows and sparrows, were analyzed. Samples collected in the necropsy were tested for viral and bacterial infections: Avian Flu (AV), Newcastle Disease (ND) and West Nile Disease (WND), Salmonellosis, Chlamydiosis, with specific reference to *Chlamydophila psittaci*. Positivity for *Chlamydiaceae* (*Chlamydophila psittaci* negative result) was found in a *Tetrao tetrix*; sequence analysis performed on the isolates for omp A gene shows that it does not cluster with any known chlamydial species. Five cases of Salmonella infections (serovars *S.* Typhimurium and *S.* Typhimurium DT193; *S.* Suberu; *S.* Farsta and *S.* Rissen) are detected. No positive cases for AF, ND and WND. In conclusion, wild birds can contract and/or be a vehicle for infectious diseases and zoonosis. Therefore recovery centers guidelines are necessary for: animal and housing management, disinfection procedures and protection measures to prevent risks for other animals and humans.

[184] IDENTIFYING KEY CLINICAL SIGNS AND VALIDATING BODY CONDITION SCORES TO MINIMIZE DISEASE SPREAD AND MAXIMIZE INDIVIDUAL SURVIVAL DURING DESERT TORTOISE TRANSLOCATIONS

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The Mojave Desert tortoise (*Gopherus agassizii*) was listed by the United States Fish and Wildlife Service as Threatened in 1990. The recovery plan (http://www.fws.gov/nevada/desert_tortoise/dt_recovery_plan.html) documents disease, particularly upper respiratory tract disease caused by *Mycoplasma spp.*, as one of the causes for decline and an ongoing threat. Concerns remain as the population impacts and disease transmission of this and other infections are still being elucidated. Recent emphasis on renewable energy has resulted in a large increase in the number of proposed solar projects within the range of the desert tortoise. With these proposals come requests to remove tortoises from project sites and release them to other parts of the Mojave Desert. The key clinical signs used to make decisions on whether an individual should be translocated were identified by comparing clinical exam findings with post mortem findings to identify those signs that best predict disease outcomes. A body condition score (BCS) is also used as tortoises in good body condition are thought to have better immune function and be better equipped to withstand periodic bouts of disease than those in poor condition. The BCS is based on an evaluation of muscle and fat deposits in relation to skeletal features and estimates body energy reserves. We've attempted to validate BCS by comparing it to more objective measures such as condition index, liver weight, and biochemical parameters. The BCS has proven to be a reliable measure of overall health and can be easily monitored over time to allow for adaptive management interventions.

[185] HEALTH EVALUATION OF BOG TURTLES (*GLYPTEMYS MUHLENBERGII*)

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Bog turtles (*Glyptemys muhlenbergii*), the smallest turtles in North America, are critically endangered, mostly as a result of habitat alteration and over collection for the pet trade. Some bog turtle populations are known to consist of as few as twenty individuals, so loss of single animals for any reason can have negative consequences for as long as a generation. Due to health concerns and higher than usual numbers of reported mortalities, a project between the WCS, USFW, NYDEC and Nature Conservancy was undertaken to assess the health of bog turtles in the Northeastern USA. In April and May 2011, health assessments were performed on turtles at four different sites in Massachusetts and New York. A total of 45 animals underwent physical examination. Samples that were obtained during handling were: choanal and cloacal swabs nasal flush, blood, feces, and shell scrapings if shells appeared abnormal. Laboratory tests included: PCR for herpesvirus, ranavirus, and *Mycoplasma sp*; fecal culture; *Mycoplasma* serology; complete blood counts, hematology; plasma biochemicals; fecal exams; and analysis of shell scrapings. Only one animal was in poor physical condition; antibodies to *M. agassizi* were found in 3 turtles without external indicators of disease.

[186] HEALTH EVALUATION OF CAPTIVE RED-FOOTED TORTOISES *CHELONOIDIS CARBONARIA* AT THE GUARULHOS MUNICIPAL ZOO – SÃO PAULO, BRAZIL

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The red-footed tortoise (*Chelonoidis carbonaria*) is an omnivorous chelonian that may be found in the Northeast, Center-West, Southeast and South of Brazil. A variety of Gram-negative bacteria may be found in reptiles, such as *Salmonella* sp., *Citrobacter freundii, Pseudomonas* sp., *Klebsiella* sp., *Aeromonas* sp. and *Escherichia coli*. *Salmonella* sp. and *E. coli* are considered normal components of the intestinal microbiota of healthy tortoises, but may be highly invasive and virulent for humans. In this study, we examined the captive population of red-footed tortoises at the Guarulhos Municipal Zoo (São Paulo, Brazil), aiming to identify and prevent zoonotic pathogens. Twenty-two tortoises were physically restrained and blood, cloacal swabs and fecal samples were collected. Seven (33,3%) animals presented eosinophilia, basophilia 9(42,8%) and heterophilia 12(57,1%), suggesting on-going parasitic and/or bacterial disease. *Salmonella* sp. was not isolated, however *E. coli* was retrieved from all animals and strains will be serotyped in the near future; *C. freundii* (77.3%) and *Enterobacter cloacae* (17.6%) were also isolated. Coproparasitology revealed helminth eggs 2(9,52%). Unless the serotyping of *E. coli* strains demonstrates otherwise, the examined animals were free from zoonotic infections; however, the clinical findings suggest possible parasitic and/or bacterial disease.

[187] PRESENCE OF INCLUSION BODY DISEASE (IBD) IN A GROUP OF BOIDS IN COSTA RICA: A POTENTIAL RISK FOR DISEASE DISSEMINATION?

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Inclusion body disease (IBD) is a highly contagious infection; it was first reported in boid snakes in the USA in the 70s.Its geographic origin is unknown because it has so far only been detected in captive snakes. IBD is one of the most important diseases due to its fatal outcome on boa and python snakes. The disease is characterized by intra-cytoplasmic inclusions that occur in most internal organs of infected animals. The inclusions are aggregates of a unique protein (inclusion body disease protein, IBDP) observed mainly in the visceral epithelial and neuronal cells. It is suggested that the causal agent belongs to the family retroviridae, however the real agent is still unknown. In Costa Rica, this disease has not been confirmed or reported previously, although there are reports of symptoms and deaths among boas with clinical features suggesting that disease might be present. A total of 32 boids (Boa constricto, Corallus annulatus, Pythonmorulus and Python curtus morulus), kept in captivity, were submitted to the Pathology Service at the National University of Costa Rica, where a disease death investigation was performed. Our study covered a 20 year period and all the cases were studied histologically in order to look for the presence of intra-cytoplasmic inclusions in organs and other tissues. We estimated disease prevalence among the total sample size to be 37.5% (12/32). The species affected by IBD were Boa constrictor (9/12), Corallus annulatus (2/12) and Python morulus (1/12). A descriptive statistic analysis showed that central nervous system, liver, kidney; pancreas, spleen and testis are ideal tissues to search for inclusions, with 100% of presence in those organs, they were classified as good organs to find typical inclusions. Intestines, stomach, lungs (80-50%) and heart (12.5%) were classified respectively as average target organs to look for inclusions and the rest of tissue under analysis were classified as bad organs using the same criteria for all. Consistent with the histopathological findings we can report that IBD is present in boids in captive populations in Costa Rica. The positive cases are the most recent cases and were submitted between 2003 and 2006 which suggests that the disease could have been introduced in our country only recently. IBD-positive snakes could in many cases be sub clinically infected; therefore they could become potential spreaders. There are no regulations as to the release of captive or confiscated snakes in Costa Rica, and some local agencies release them into the wild without carrying out any pathogen test beforehand. This practice represents a risk of spreading this disease agent into susceptible free ranging boid populations in their natural habitat. In order to assess whether the free ranging boids have already been exposed to this agent, a field study should be performed.

[188] DETECTION OF ANTIBODIES AGAINST ADENOVIRUSES AND PARAMYXOVIRUSES IN NATIVE ARIZONA RATTLESNAKES

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Adenovirus infections are commonly found in snakes and it has been hypothesized that adenoviruses of the genus *Atadenovirus* may have co-evolved with their hosts. Paramyxovirus (PMV) infections are also common in snakes, particularly viperid snakes and all PMV detected in viperid snakes so far have been classified in the genus *Ferlavirus*. The prevalence of both viruses in wild populations is unknown. Blood samples were obtained from 100 wild-collected Arizona rattlesnakes. Antibodies against a snake atadenovirus were detected by neutralizations test. For screening for antibodies against ferlaviruses, isolates belonging to 4 different genotypes were used in hemagglutination inhibition tests. Relatively high antibody titers were detected against the atadenovirus in several snakes. Detection of antibodies against ferlaviruses depended on the virus used, and low antibody titers of 16 to 32 were detected in a few cases against individual viruses. This is the first report of testing multiple species of wild rattlesnakes of the genus *Crotalus* for antibodies against reptilian viruses.

[189] VENOM AND INTESTINAL BACTERIAL FLORA FROM VENOMOUS SNAKES IN CROATIA

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Reptiles can carry a wide range of bacteria in their gastrointestinal (GI) system and venom, with potential risk for human health. It was reported that these bacteria may additionally complicate the bite wounds. A total of 135 swabs were taken from the venom, oral cavity and cloaca of nose-horned viper, *Vipera ammodytes,* adder *Vipera berus* and meadow viper, *Vipera ursinii* from the wilderness of continental and coastal parts of Croatia. In meadow viper venom *Bacillus* sp., *E. coli, Streptococcus* sp., *Morganella morganii, Seratia* sp., *Staphylococcus* sp., *Pseudomonas luteola* and *Salmonella arizonae* were detected, listed by the frequency. In the adder venom only *Bacillus sp.* was detected while no bacteria were found in the nose-horned viper venom. Cloacal and oral cavity findings were similar in all viper species regarding the number of bacteria detected, with *E. coli* as predominant species in all of them. Also, the following bacteria were isolated: *Hafnia alvei, Burkholderia gladioli, Providencia retgeri, Salmonella arizonae, Salmonella* sp., *Proteus* sp., *Enterobacter cloacae, Acinetobacter* sp., *Pseudomonas fluorescens,* etc. The aim of this study was to identify potentially harmful bacteria from GI system and venom of snakes found in Croatia. Our study showed that the majority of bacteria from venom and GI system from venomous snakes belong to the Gram negative bacteria. This pilot study was the first one in Croatia and should be followed by further, larger scale investigations.

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[190] ZOONOTIC ENTEROBACTERIA IN A DENSE YELLOW-LEGGED GULL (*LARUS MICHAHELLIS*) COLONY IN THE WESTERN MEDITERRANEAN (SPAIN)

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Campylobacter and *Salmonella* are the leading cause of zoonotic bacterial enteric infections. Due to their scavenging feeding habits, some seagulls have often been reported as carriers of these zoonotic bacteria. A previous study in 2005 revealed the occurrence of *Campylobacter* and *Salmonella* in the Yellow-legged gull (*Larus michahellis*) colony in Medes Island (NE Spain), which has a dense bird population and is neighboring a highly touristic area. Here we further investigate the role of this gull colony as a reservoir of these enteropathogens with a broader sampling of gull chicks during three years (2009-2011). Cloacal swab samples were obtained from 270 gull chicks. No *Campylobacter* was isolated during any of the 3 years. However, *Salmonella* prevalence increased notably throughout the years: 8.7% (6/69), 39.1% (45/115) and 75.6% (65/86) in 2009, 2010 and 2011, respectively. A high diversity of *Salmonella* serovars was found, including some of public health importance, such as Typhimurium, Derby, Agona and Hadar. Also, the antimicrobial susceptibility of *Salmonella* isolates to a panel of 18 antimicrobials was tested. Most isolates were resistant to tetracycline, ampicillin, amoxycillin and streptomycin and some were resistant to 4 or more antimicrobials (multidrug resistant). The high *Salmonella* prevalence, the multiresistant isolates and the serious risk of spilling over to humans are of notable public health concern.

[191] PATHOLOGIC FINDINGS IN WATERFOWL NATURALLY INFECTED WITH DUCK VIRUS ENTERITIS IN SPAIN

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Duck virus enteritis (DVE) is an acute contagious herpesvirus infection that occurs naturally in birds of the family *Anatidae*. An outbreak of DVE affected an urban park duck population in the north of Spain including also wintering waterfowl and gulls. Detailed necropsy was carried out on five birds (four *Anatidae,* one *Laridae*). Macroscopic lesions included multiorganic congestion and vessel injection, petechiae in the mucosa of the proventriculus, ventriculus and large intestine, and small white scattered foci in the liver and the kidney. Microscopically, congestion and haemorrhages were generalized. Necrosis was seen in the spleen, liver, kidney, and intestine. In the intestine diffuse mononuclear infiltrates in the lamina propria and the muscular layer were characteristic. The brain showed marked gliosis, satellitosis, neuronophagocytosis, and neuronal necrosis. Acidophilic intranuclear inclusions were detected in hepatocytes, cardiomyocytes, renal tubular epithelial cells, intestinal crypt epithelial cells, pancreatic acinar cells, and inflammatory cells of different tissues. Sequence analysis of a DNA fragment obtained by consensus herpesvirus PCR showed 97% homology with published DVE sequences. Despite being a well known disease in waterfowl this is the first time that the disease is described in a member of the family *Laridae* and the second time that it is described in Spain. DVE should be a differential diagnosis for waterfowl mortality in Spanish wetlands.

[192] AVIAN INFLUENZA VIRUS SURVEILLANCE IN WILD BIRDS IN BRAZIL

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The spread of avian influenza virus (AIV) through wild aquatic birds has recently drawn much attention, particularly in face of highly pathogenic avian influenza viruses. From October 2008 to November 2010, 1.146 wild birds were sampled in Brazil. Oral and cloacal swabs were collected from each bird, and placed in cryotubes containing transport media. *Real time* RT-PCR revealed 4 positive samples for influenzavirus in one species, the Ruddy Turnstone *Arenaria interpres* (Charadriiformes), caught in Amazon region. Previous studies involving multiple shorebird species had also found *A. interpres* to present the highest AIV prevalence among shorebirds. The reasons for this are yet unknown; experimental infections would be needed to determine whether there are unique physiologic characteristics that explain the increased prevalence observed on these species in the field. Avian influenzavirus have been detected in migratory birds, especially in shorebirds, at numerous localities in North America. These birds arrive in Brazil through two flyways, Central and Pacific, and islands in the Brazilian Amazon forest are areas with large migratory confluence, a condition that might facilitate virus transmission. Our results thus emphasize that the surveillance and early detection of influenzavirus in Brazil is critical to assure the rapid implementation of control and prevention measures, focused on both public health and conservation of these birds and their environments.

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[193] H5 AVIAN INFLUENZA SPECIFIC ANTIBODIES IN MUTE SWANS FROM THE UNITED STATES

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Highly pathogenic avian influenza virus (HPAIV) H5N1 has been a major cause of mortality in wild birds from Africa, Asia, and Europe, but has yet to be detected in North America. Mute swans (*Cygnus olor*) have had high mortality in numerous HPAIV H5N1 outbreaks. In North America mute swans have had near exponential population growths over the last 3 decades. The goal of this study was to determine the antibody prevalence of avian influenza viruses (AIVs) in mute swans from the US, including antibodies specific to H5 AIVs. We obtained mute swan serum samples from Michigan and Rhode Island and analyzed the serum for nucleoprotein (NP) antibodies using a commercial blocking enzyme-linked immunosorbent assay (ELISA) and a commercial competition ELISA for H5 specific antibodies. We were able to detect NP antibodies in 212/269 (79%) and H5 specific antibodies in 136/269 (51%) of mute swans sampled. Experimental infection studies have shown birds that have been previously exposed to AIVs to have attenuated clinical signs associated with HPAIV H5N1 challenge. The high H5 specific antibody prevalence we detected in mute swans may act as a natural vaccine in these birds and prevent establishment of HPAIV H5N1 in North America because of flock immunity or permit the virus to circulate with the absence of high mortality. Further research is needed to understand how much protection is provided by previous exposure and what level of flock immunity is needed to prevent establishment of HPAIV H5N1 in North America.

[194] SIDE EFFECTS OF AVIAN INFLUENZA VIRUS SURVEILLANCE IN DUCKS SAMPLED IN FRANCE

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Surveillance of Avian Influenza Virus spread has lead to the sampling of hundreds of thousands birds worldwide. Samples are often cloacal swabs or/and blood. Their effects for sampled birds have not so far been evaluated. We here compared the fate of individuals from four duck species (Teal *Anas crecca*, Mallard *A. platyrhynchos*, Common Pochard *Aythya ferina* and Tufted Duck *A. fuligula*) depending on whether they were not sampled for AIV, were only swabbed or were swabbed and bled when captured and marked in France. No effect of AIV swabbing or swabbing and bleeding was recorded in Common Pochard and Tufted Ducks, which were equally likely to be re-observed alive or recovered dead during the year following ringing. A similar result was observed for Mallard dead recoveries (there were no marked Mallards which could be re-observed alive). Conversely, Teal were less frequently re-observed if swabbed (31.85% were so on average instead of 40.58 % if not sampled). The effect of bleeding was not tested in this species due to small sample size. Swabbed and non-swabbed Teal were re-observed at similar distances from their initial marking site, suggesting that swabbing may increase mortality (change in their behavior increasing predation risk, etc) rather than permanent emigration. Further survival analyses are needed to confirm this hypothesis. Until then, simple measures can be taken such as swabbing of feces collected from birds kept in individual boxes.

[195] ARE WASTEWATER TREATMENT WETLANDS SAFE FOR WATERBIRDS?

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In recent years, due to the loss of natural wetlands in the province of Ciudad Real (south-central Spain) waterbirds are increasingly dependent on wastewater treatment wetlands (WTW). However, the use of wastewater may increase the risk of avian botulism outbreaks. With this in mind we have studied the hypothesis that the presence of some avian enteropathogens in sewage may cause waterbirds mortalities and consequently start botulism outbreaks. To assess this possibility, between April 2010 and March 2011 we collected samples of sediment, water and waterbird faeces in three wetlands situated in Ciudad Real: two WTWs (Navaseca and Alcázar de San Juan) and one natural wetland (NW, Tablas de Daimiel National Park). We tested the samples for the presence of avian enteropathogens [Avian Pathogenic *Escherichia coli* (APEC), *Salmonella spp*., *Clostridium perfringens*] and *C. botulinum* following different culture and PCR protocols. The prevalence of *C. perfringens* was significantly higher in all the samples from WTWs than in those from the NW. APEC were significantly more prevalent in faeces from the two WTWs and in sediments from one of them than in the NW. Finally, the prevalence of *Salmonella spp.* and *C. botulinum* was low, but again it was higher in the WTWs. Moreover, in one of the WTWs (Navaseca) botulism outbreaks have occurred in 2010 and 2011. This high prevalence of avian enteropathogens observed in WTWs may be a determining factor in the occurrence of botulism outbreaks.

[196] INVESTIGATION OF RISK FACTORS FOR CAPTURE MYOPATHY IN SHOREBIRDS

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Capture myopathy (CM) is a debilitating syndrome seen in wild birds following capture and handling. Clinical signs include paralysis, pain and death. Damaged muscle tissue causes elevations of serum creatine kinase (CK) and aspartate aminotransferase (AST). Little is currently known about the risk factors for CM in birds, though capture method, age, sex and restraint time have been associated with CM in some peer-reviewed reports. We studied risk factors by capturing bar-tailed godwits (*Limosa lapponica*), red knots and great knots (*Calidris spp.*) by two methods of capture (mist-net and cannon-net) in 3 locations. We measured blood parameters immediately following capture (<20 minutes), and prior to release 1-6 hours later. Our data showed significant increases for muscle enzymes, reflecting muscle damage from capture and handling. We did not find significant differences due to sex, nor body mass in godwits. Species and location differences were found for potassium (K+) and uric acid (UA) measures. Significant differences were found for CK, AST, UA and K+ over time in all species. Capture method had a significant effect on the development of muscle damage in godwits.

[197] ONE HEALTH APPROACH TO *BABESIA* SPP. EPIDEMIOLOGY IN WILDLIFE AND DOMESTIC ANIMALS FROM NORTHWESTERN ITALY

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Babesia spp., infects a variety of mammals, including human, domestic animals and, wildlife. Recently new zoonotic *Babesia* species were described. Usually asymptomatic in wildlife, fatal cases are being reported in wild ungulates. There are also reports of changes occurring in tick-vector distribution. We therefore deemed necessary to assess *Babesia* prevalence in wildlife, cattle and dogs living in shared areas. Total genomic DNA was extracted from spleens of 799 wild animals and from blood of 145 dogs, and 465 cattle. Cattle sampled, are bred on alpine pastures during summer. Samples were tested by a *Babesia*/*Theileria* catch-all PCR. Resulting prevalence in wildlife is 3.35% (IC 95% 2.38-5.09). Single prevalence data for each wild species varies from 48.72% (IC 95% 32.71-64.97) in red deer, 1.62% (IC 95% 0.66-3.67) in roe deer, 1.16% (IC 95% 0.2-4.58) in wild boar, 0.5% (IC 95% 0.02-3.17) in fox. This is, to our knowledge, the first report in Italy of *Babesia* spp. in fox. In cattle prevalence is of 7.7% (IC 95% 5.6-10.7), while in dog, is 8.96% (IC 95% 5.05-5.14). Sequencing revealed the presence in wildlife of the zoonotic *Babesia divergens,* and of species shared with domestic livestock such as *B. bovis*, *B. bigemina.* In two positive cows, we isolated a parasite closely related to *Theileria equi*/*B. microti*. Further phylogenetic analysis are needed to establish the relationship and overlapping occurring between *Babesia* found in wildlife and those found in cattle and dogs.

[198] PARASITES IN PEOPLE LIVING IN PROXIMITY TO THE RANOMAFANA NATIONAL PARK ECOSYSTEM, MADAGASCAR: POTENTIAL FOR SPILLOVERS OF ANTHROPONOTIC PARASITIC DISEASES TO ENDANGERED LEMURS

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The increase in human-wildlife interactions, associated with the expansion of human settlements in the vicinity of pristine natural areas, can lead to an increased occurrence of pathogen spillovers and spillbacks. This "pathogen pollution" can be a threat to naïve wildlife populations. We investigated the occurrence of potentially zoonotic and anthroponotic parasites in human populations living in proximity to the Ranomafana National Park, Madagascar to assess the potential risks of parasites disease spillovers to neighboring endangered and endemic free-ranging lemurs. Fresh fecal samples were collected from human volunteers (n=168) from three villages located in the vicinity of the Ranomafana National Park. Each fecal sample was analyzed using a standard floatation technique for the detection of helminths and direct immunofluorescence (Merifluor Cryptosporidium/Giardia Kit®) for the detection of *Cryptosporidium parvum* and *Giardia lamblia*. Prevalences of *Ascaris* sp., *Trichuris* sp., *Ancylostoma* sp. and *Schistosoma* sp. were 46%, 44%, 2%, and 1%, respectively. *Giardia lamblia* and *Cryptosporidium parvum* were detected with prevalences of 27% and 14%, respectively. These parasites, which are known or are suspected to be potentially pathogenic in lemurids, may constitute a threat to endemic species of lemurs found within the park. Surveys for gastrointestinal parasites are currently being carried out in domestic animals, exotic rodents and lemurs in the Ranomafana ecosystem.

[199] SURVEY OF *SALMONELLA* SPP., EXTRA-INTESTINAL PATHOGENIC *ESCHERICHIA COLI* (EXPEC) AND SHIGA TOXIN-PRODUCING *E. COLI* (STEC) IN FREE-LIVING MAMMALS FROM THE CANTAREIRA STATE PARK, SÃO PAULO, SP, BRAZIL

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The Cantareira State Park (CEP) is considered the largest world's urban forest and was declared by UNESCO as part of the Biosphere Reserve. Surrounded by areas of high human population density it allows the interaction of wild populations with man and domestic animals. Pathogens with zoonotic potential may serve as health indicators within the One Health concept. The aim of this study was to assess extra-intestinal pathogenic *Escherichia coli* (ExPEC), Shiga toxin-producing *E. coli* (STEC) and *Salmonella* spp., as well, their antimicrobial susceptibility in wild animals captured in the CEP. Rectal swabs of *Nasua nasua*, *Didelphis marsupialis* and *Monodelphis domestica* were collected for bacterial isolation/identification and antimicrobial susceptibility test; survey of virulence genes and phylogenetic groups were conducted with *E. coli* strains by PCR. *Salmonella* spp. and STEC were not isolated. Of the 17 *E. coli* strains studied so far, respectively, 35% and 23% belonged to B2 and D phylogenetic groups. Four strains (24%) were positive for the *eae* gene, linked to diarrheagenic strains, and 16 (94%) had genes associated to ExPEC. Regarding the antimicrobial susceptibility, 24% of strains were multidrug resistant; 82% were resistant to β-lactams, 29% to sulfonamide, 18% to tetracycline, 12% to aminoglycosides and 6% to quinolones. The pathogenic potential of *E. coli* strains studied, associated with antimicrobial resistance, are suggestive of human disturbances on this natural area.

[200] EFFECTS OF STRESS AND ENVIRONMENTAL FACTORS ON PATTERNS OF INFECTION WITH GASTROINTESTINAL COMMENSALS AND PARASITES IN THE CRITICALLY ENDANGERED BLACK RHINOCEROS (*DICEROS BICORNIS BICORNIS*) IN ADDO ELEPHANT NATIONAL PARK, SOUTH AFRICA

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As part of a population health assessment of the critically endangered black rhinoceros (*Diceros bicornis bicornis*), 234 fecal samples were non-invasively collected from January 2008 to June 2011 from known individuals in two black rhino populations in Addo Elephant National Park (AENP), South Africa to examine the effects of stress and environmental factors on patterns of parasitism. The two sections of the park, Main Camp and Nyathi, vary with respect to important environmental factors including higher elephant density, dryer and colder climate, higher predator density, smaller habitat size, and a greater frequency of tourism at Main Camp. Samples were examined via fecal flotation and sedimentation as well as glucocorticoid analyses. Although patterns of parasitism did not differ among sites, Nyathi rhinos had significantly higher prevalence for the majority of commensal species and individual Nyathi rhinos were concurrently infected with significantly more species of commensals compared to Main Camp rhinos. Co-infection occurred significantly more than expected between *Oesophagostomum* sp. and *Necator* sp. as well as *Strongyloides* sp. and *Necator* sp. No relationships were apparent among stress level, total precipitation and parasite/commensal richness. These results demonstrate that commensal communities of herbivores can be sensitive to environmental variability even when parasites and host stress levels are constant.

[201] DETERMINATION OF THE NUMBERS OF NEMATODE SPECIES PRESENT IN THE STOMACHS OF KANGAROOS AND WALLABIES

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Kangaroos and wallabies harbour a diverse array of strongyloid nematodes in their sacculated forestomachs with 36 genera and 275 species known to date with a possible symbiotic relationship between worms and the host. Worm burdens in individual kangaroos can exceed 500,000. Determining the number of nematode species present in complex communities can be technically difficult due to the large size of the host organ, the large numbers of nematodes and the large number of closely related nematode species. This problem was investigated using species accumulation curves in 16 eastern grey kangaroos (*Macropus giganteus*), 15 red-necked wallabies (*Macropus rufogriseus*) and 6 swamp wallabies (*Wallabia bicolor*). Nematodes from a subsample of known size were identified individually to species and the number of nematodes needed to be counted before all of the species in the subsample were found was determined. In *M. giganteus* (mean worm burden 20,500), between 27 and 92 (mean 57) nematodes had to be examined before all present species (13) were recovered. In *M. rufogriseus* (mean worm burden 60,600), between 16 and 178 (mean 57) recovered all present species (8), while in W. bicolor (mean worm burden 16,300), 16-196 (mean 67) worms needed to be examined to cover all present species (15). It was concluded that a sample size of 200 nematodes from each animal would be sufficient to identify all parasite species present.

[202] TOXOPLASMOSIS EPIDEMIOLOGY IN CHILEAN PATAGONIA: CONSERVATION OF THE ENDANGERED SOUTHERN RIVER OTTER

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Increasing urbanization has important consequences for wildlife, including the possibility of a higher prevalence of disease in species closest to urban areas. *Toxoplasma gondii* affects a wide variety of hosts including southern river otter (*Lontra provocax*), an endangered species previously suggested as highly susceptible to this disease. Therefore, understanding the epidemiology of *T. gondii* in southern Chile is a priority for the conservation of the species. The goals of this study were to assess the seroprevalence of *Toxoplasma gondii* and describe the spatial distribution of positive individuals across an urban-rural gradient in the Patagonia landscape. Blood samples were collected from domestic dogs, cats, American mink and *L. provocax* in five locations with different degrees of urbanization. For the serosurvey we used a commercially available agglutination test. We detected *T. gondii* in 8 dogs, 3 cats, 8 mink and 5 southern river otter. Our results show a high seroprevalence of *Toxoplasma gondii* in populations of otters near urban areas. We conclude that positive domestic cats is a major risk factor for the seroprevalence in *L. provocax* and that alien American mink may act as a sentinel species along southern river otter distribution. Further research is necessary to evaluate the susceptibility and potential threat that this disease may represent in *L. provocax* populations to ensure the long lasting conservation of this endangered species.

[203] BOVINE TUBERCULOSIS INFECTION AND POPULATION DENSITY OF BADGERS (MELES MELES) IN TWO REGIONS IN FRANCE

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In France, surveillance programs were carried out to monitor the presence of bovine tuberculosis (bTB) in badgers (*Meles meles*) in two unrelated areas where cattle displayed high bTB prevalence (Côte d'Or, Dordogne/Charente). Diagnostic methods included post-mortem examination, PCR and/or culture. *M. bovis* was detected by culture in 49/878 (5.6%) in the Côte d'Or region. In the Dordogne/Charente region, the PCR prevalence reached 29/218 (13.3%), and badgers showed visible lesions more often. In each region, the same genotypes of *M. bov*is strains, defined by spoligotyping and MIRU-VNTR analysis, were isolated from wildlife and neighbouring cattle, demonstrating an epidemiological link between the two species. Studies are in progress to better understand the role that badgers may play in the multi-host system of *M. bovis* epidemiology. Among them, an estimation of badger population densities, based on the counts of main setts and evaluation of group sizes by using camera trapping on breeding setts have been implemented in both regions, using a systematic sampling. At the regional scale, first results show that badger densities reach much higher values in Côte d'Or than in Dordogne. Thus, badgers in Dordogne were more often infected and seem to live at lower density than in Côte d'Or. Other factors, including landscape and contact rate between the different *M. bovis* hosts are being analyzed to explain bTB transmission and persistence among badgers.

[204] FINE-SCALE ASSOCIATIONS BETWEEN MYCOBACTERIUM BOVIS MOLECULAR TYPES IN BADGERS AND CATTLE

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Bovine TB *(Mycobacterium bovis)* remains an important disease of livestock in the UK; one significant source of infection is the Eurasian badger (*Meles meles*). A long term study of an undisturbed wild badger population in south-west England provided a unique opportunity to study epidemiological patterns and processes. *M.bovis* isolates from this population have been routinely subjected to molecular typing; spoligotyping since 1990 and VNTR typing since 2000.This information was used to investigate epidemiological processes at high resolution within the socially structured badger population and amongst badgers and local cattle. We investigated spatio-temporal variation in the appearance of different molecular types of *M. bovis* in these two host populations at a local scale. We identified a single dominant *M. bovis* spoligotype common to both species and observed temporal associations between the two species in the appearance of uncommon spoligotypes. VNTR analysis confirmed the existence of a single dominant genotype and revealed further variation in strain diversity among the badger population. A greater diversity of spoligotypes was observed in the badger population than in cattle and all types found in cattle were also present in the badgers. The greater diversity of spoligotypes in badgers is consistent with *M. bovis* maintenance in badgers with occasional bovine TB transmission to cattle.

[205] HEPATITIS E VIRUS INFECTION OF WILD BOAR (*SUS SCROFA*) IN SOUTHERN BELGIUM

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Hepatitis E virus (HEV) infection is hyperendemic in developing countries and endemic in developed countries. Many evidences claimed for a zoonotic transmission involving strains from genotypes 3 and 4. The main putative animal reservoirs for HEV in Western Europe are pigs, wild boar (WB) and deer. This survey aimed to investigate the HEV apparent seroprevalence and viroprevalence in WB population in southern Belgium where this species is highly densely present with a global population estimated at more than 25000 animals. The aim of this survey was to provide quantitative data in order to investigate the role of WB in HEV transmission in Belgium. During the 2010 hunting season, 388 sera of WB older than 6 months, 69 sera and 61 livers of WB less than 6 months were sampled by the Surveillance Network in southern Belgium. A double sandwich antigen multispecies ELISA *(HEV ELISA 4.0v MP Biomedicals Asia Pacific Pte. Ltd., Singapore)* was used on the more than 6 months 388 sera and an apparent seroprevalence of 32% was obtained in WB older than 6 months. The viroprevalence survey was made with a nested RT-PCR amplifying a 348bp fragment after RNA extraction: 5/61 (8%) livers and 4/69 (6%) sera were positive. All the PCR fragments were sequenced and were specific of genotype 3. In conclusion, HEV is endemic in WB population in the investigated area. The apparent seroprevalence is high and only the genotype 3 was found. WB are thus a reservoir of genotype 3 HEV in Belgium.

[206] NOVEL ROTAVIRUSES IDENTIFIED IN BATS FROM NIGERIA

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Rotaviruses (RV) are very important etiologic agents of severe diarrheal disease in humans and animals. Of the 7 known RV serogroups, the serogroups A, B, and C have been identified in humans and animals while D, E, F and G have only been identified in animals. Although host species barriers may limit RV adaptation and circulation, gene segments from animal-origin RVs have been frequently identified in human RVs. In addition, direct transmissions of animal RVs to humans have been documented. In this study we report the presence of divergent RVs in bats from Nigeria. In total, 356 bats representing 7 genera and 9 species were collected during 2010- 2011 in (7 locations of) northern Nigeria. Fecal swabs (n=95) were screened for RV RNA using the pan-rotavirus RT-PCR assay; 18 (19%) were positive, including: *Eidolon helvum* (10), *Rhinolophous landeri* (2), *Nycteris macrotis* (1), *Chaerephon pumila* (3), *Epomops franqueti* (1) and *Lavia frons* (1). A phylogenetic tree based on a 200 bp fragment of the VP1 gene demonstrated that 14 sequences were related to RV A, 2 to RV B, and 2 to RV C. The partial VP1 gene sequence detected in a *Nycteris macrotis* bat shared 90% nucleotide identity with human RV A strain B10, and the RV sequence identified in a *Rhinolophus landeri* bat had 96% nucleotide identity to RV A 02V0002G3 from chickens. (Additional genetic characterization of these RVs is underway). This study suggests that bats may be a source of RV infection for other animals and humans.

[207] UNUSUAL MORTALITY EVENT OF YOUNG GREY SEALS (*HALICHOERUS GRYPUS*) ASSOCIATED WITH APICOMPLEXAN PROTOZOAL HEPATITIS IN NOVA SCOTIA, CANADA

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In March 2012, an unusual mortality event involving recently weaned young grey seals was reported on Hay Island, east of Cape Breton Island, Nova Scotia. All dead animals appeared in good body condition and had probably never yet gone in the water. The estimated mortality was approximately 400 pups out of an annual average production of 2,500 pups on this island. Ten animals were necropsied on site. All were in good body condition (sternal blubber thickness, 40-60 mm). Gross lesions were inconsistent and included irregular pulmonary congestion, mild lymphadenomegaly, and occasional petechiae in lymph nodes and thymus. Microscopic lesions were similar in all 10 animals, consisting of a marked, acute, multifocal, necrotizing hepatitis with intralesional protozoa. A few animals had a diffuse interstitial pneumonia of moderate intensity. On electron microscopy of the liver, the protozoa were identified as asexual stages of an Apicomplexan parasite with a basaloid nucleus, conoid apparatus and 3-5 rhoptries. Extensive survey of other whelping colonies of grey seals in the region suggested that this unusual mortality event was confined to Hay Island. Further investigation is planned for the next whelping season.

[208] MALFORMATIONS IN THE NEWBORN OF RECENTLY CAUGHT PREGNANT *BOTHROPOIDES JARARACA* (SERPENTES,VIPERIDAE)

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Organogenesis, the most complex stage in embryonic development, is particularly sensitive to exogenous agents, being therefore an important period for the occurrence of malformations, which may lead to newborn or premature death due to predation and/or diseases. This study describes the frequency and type of newborn malformations occurring in jararacas at the Butantan Institute (IB), Brazil. Free-ranging pregnant specimens of *Bothropoides jararaca* delivered to IB were kept incaptivity until the birth of their offspring. Newborns were weighed, measured and anatomically evaluated for the presence of malformations. After natural death or euthanasia, complete necropsy was performed. From 2007 to 2010, 113 jararacas gave birth to 1881 snakes, of which 1794 were studied. Congenital anomaly was observed in 66 (3.5%) newborns. Spine anomalies (kyphosis, lordosis and scoliosis) were the most common (48.5%) followed by fissure in the ventral midline (23.7%). Tail tip wound and prognathism were observed in 13.6% of the animals. Fusion of the ventral scales, eye malformations (microphtalmia and/or bufoftalmos) and bicephaly were seen in 10.6%, 6% and 3% of the animals, respectively. Internal organs were little affected and most alterations were related to cardiomegaly (7.5%), hepatomegaly (3%) and nephromegaly (1.5%). Complementary studies are necessary to properly understand the etiology and significance of these malformations. Acknowledgments: FAPESP, CNPQ, INCTTOX.

[209] PRELIMINARY CHARACTERISATION OF RESPIRATORY MICROORGANISMS IN THE LONG-FINNED PILOT WHALE, *GLOBICEPHALA MELAS*, FROM THE STRAIT OF GIBRALTAR, SPAIN

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Whilst epizootic outbreaks are increasingly being observed, regrettably, information on pathogen loads of cetaceans remains limited with most data from captive or stranded animals. These preliminary investigations aimed to characterise the respiratory microorganisms of free-living, long-finned pilot whale and to test a novel non-invasive technique in small cetaceans. To investigate the presence of eubacterial, fungal and metazoan DNA in blow collected from 20 pilot whales and 11 environment samples we used PCR for each taxonomic group. Positive samples were analysed for 11 respiratory microbes previously isolated from stranded marine mammals. All samples amplified eubacterial DNA. All blow samples contained fungal and metazoan DNA. We found mycobacteria and *Streptococcus equi* were most commonly detected in blow samples followed by S*taphylococcus* sp., *Streptococcus phocae*, unclassified streptococci and *Brucella* sp. *Staphylococcus* sp. and *Brucella* sp. were found in environment samples but at a significantly lower proportion than in blow samples. Our results demonstrate that it is possible to collect blow from free-living small cetaceans non-invasively and the technique could be used to establish baseline references to allow population health monitoring. The significance of β-haemolytic streptococci and mycobacteria in an apparently healthy, free-living population warrants assessment and as these pathogens are zoonotic, human health risks should be determined.

[210] SEARCHING FOR BLOOD PARASITES IN MAGELLANIC PENGUINS (*SPHENISCUS MAGELLANICUS*) ALONG THE PATAGONIAN COAST

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Magellanic penguins (*Spheniscus magellanicus*) are susceptible to blood parasites in captivity; it is not clear however whether these parasites infect the species' wild populations. From 24 to 27 January 2012 we studied 140 adult Magellanic penguins in the Argentinean Patagonia (n = 35 animals per colony): La Ernestina, Punta Tombo, Cabo Dos Bahías, and Caleta Malaspina (Bahía Bustamante). Blood was collected from the jugular vein and two thin blood smears were prepared, fixed in methanol and stained with Wright-Rosenfeld and Giemsa. A minimum 30000 erythrocytes per animal were examined under 1000x magnification. No blood parasites were observed. Sensitivity estimates of blood smear examination for the detection of avian blood parasites range from 52% to 81%. Blaker's Confidence Interval estimated that prevalence of blood parasites must have been ≤ 3.4% (test sensitivity = 75%). In a worst-case scenario (test sensitivity = 50%), prevalence must have been ≤ 5.1%; in a best-case scenario (test sensitivity = 90%), prevalence must have been ≤ 2.8%. Combining data from this and published studies, we found that to date 494 Magellanic penguins were examined and found to be negative in Patagonia; overall prevalence may be estimated to be below 1.0% (worst-case: 1.4%; best-case: 0.8%). The fact that no blood parasites have yet been observed in free-ranging penguins in Patagonia suggests that, if they do occur, prevalence must be considerably low and/or spatiotemporally heterogeneous.

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[211] *BATRACHOCHYTRIUM DENDROBATIDIS* IN LA AMISTAD INTERNATIONAL PARK: HAVE PRISTINE AND PROTECTED AREAS BEEN A REAL BARRIER FOR THIS PATHOGEN?

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Many amphibian population declines have been associated with chytridiomycosis, a skin disease caused by *Batrachochytrium dendrobatidis* (Bd). However, there is evidence to suggest that this pathogen could coexist with many species without causing disease. La Amistad International Park (PILA) (Costa Rica and Panama) is the largest protected area in Central America and one of the most important centers of biodiversity in the region. We conducted a survey for 10 communities of amphibians from PILA between 2007 and 2010 to assess the taxonomic and environmental factors that influence the distribution of *Bd* in anurans from the protected area. We examined 502 samples of 59 species and 11 families of anurans using quantitative PCR and histopathology. The pathogen was confirmed in a uniform distribution in all study sites with a prevalence of 20.12%. We found no dead specimens or any specimens with symptoms of disease. Prevalence and infection loads were high even for some difficult access sites. In a parallel study the oldest infected specimen of the region was determined by 1984, so we can say that PILA is endemic for *Bd*. We recommend detailed investigations focused on comparative experimental studies of susceptibility to *Bd* in frogs of middle and upper elevation areas in the Neotropics and improve understanding of the implications of the wide distribution of *Bd* in protected areas.

[212] EXPERIMENTAL INFECTION OF PREGNANT PYRENEAN CHAMOIS (*RUPICAPRA RUPICAPRA*) WITH BORDER DISEASE VIRUS SUBTYPE 4

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Border Disease Virus (BDV) has been shown to cause high mortality in Pyrenean chamois (*Rupicapra pyrenaica*) in the Pyrenean mountains. The aim of this study was to investigate the pathology induced by BDV in pregnant chamois through an experimental infection. Three females were inoculated during the second third of pregnancy with a BDV subtype 4 (BDV-4) strain. A control group was composed by a fourth pregnant chamois and one non-pregnant ewe. Pregnancy was unsuccessful in all infected animals. One suddenly died on 24 days post inoculation (dpi). The second animal had profuse diarrhea from 13 dpi to its death on 51 dpi. The third aborted on 46 dpi and was euthanized on 51 dpi. Viremia started on 4 dpi in all animals and remained positive until their death. Neutralizing antibodies against BDV-4 were detected from 12 dpi. Necropsies showed generalized lymphadenomegaly, associated in one case with disseminated petechial hemorrhages in the digestive tract. All tested organs (n=78) from inoculated adults and their fetuses were positive for viral RNA. The main histological lesions found were mild lymphohistiocytic encephalitis associated with moderate or moderately severe lymphoid depletion. Control animals remained negative for all virological, serological and post-mortem examinations. These results may explain the reported decrease in chamois populations in several areas and show that great care must be taken when interpreting infection status for wildlife studies in the field.

[213] DOMESTIC SHEEP AS A SOURCE OF *MYCOPLASMA CONJUNCTIVAE* FOR SOUTHERN CHAMOIS

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Mycoplasma conjunctivae is considered the main primary causative agent of infectious keratconjunctivitis (IKC), a disease affecting eyes of domestic and wild ruminants worldwide. Since interspecific transmission from domestic sheep to wild mountain ungulates has been demonstrated in the Swiss Alps, we have studied the presence of *M. conjunctivae* in domestic sheep flocks sympatric with both subspecies of southern chamois in Spain, *Rupicapra pyrenaica pyrenaica* in the Pyrenees and *Rupicapra pyrenaica parva* in the Cantabrian Mountains (CM). Conjunctival swabs of 141 sheep from 4 flocks in Cantabrian Mountains and 7 flocks in Eastern Pyrenees (EP) were collected randomly within a flock and at all year seasons during 2010 and 2011. Detection of *M. conjunctivae* was performed with a specific real time-PCR. Thirty-two of 141 domestic sheep (23%) tested positive in at least one eye. Prevalence of infection was higher in EP (26%; 29 of 111 chamois) than in CM (10%; 3 of 30 chamois), which correlates with higher IKC cases in southern chamois from Pyrenees. Mild clinical signs were observed in both study sites, but 100% of positive animals in CM (3/3) and 83% in EP (24/29) were asymptomatic sheep (84% in average). Seven sheep with IKC tested negative, pointing out to another infectious agent intervention. Our results show that *M. conjunctivae* is widespread and endemic in domestic sheep from Northern Spain, and supposes a potential risk of infection to southern chamois and other wild ungulates.

[214] DOES LANDSCAPE COMPOSITION INFLUENCE THE CIRCULATION OF PATHOGENS IN ROE DEER LIVING IN RURAL AREA?

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Historically, roe deer (*Capreolus capreolus*) is a forestry ruminant. However, range and density of populations have evolved these last decades, increasing their proximity with human activities. Those changes can impact the circulation of pathogens, especially because of higher indirect or direct contacts with domestic animals. Our hypothesis is that the exposition of roe deer to pathogens is structured by the local landscape composition of roe deer habitat. Especially, we expect a higher prevalence and diversity of parasites in roe deer inhabiting in open areas (meaning anthropogenic areas). Between 2008 and 2011, in south-western of France, 199 roe deer inhabiting in a heterogeneous landscape, ranging from totally closed (wooded) to open (cultivated) habitat, were sampled. Individual roe deer home ranges were collected using GPS collars. Seroprevalences of several pathogens were obtained using ELISA tests: *Toxoplasma gondii* 38% (95% confidence interval: [30-45]), *Chlamydophila abortus* 18% [12-24], *Coxiella brunetii* 9% [5-14] and *Infectious Bovine Rhinotracheiti* 17% [11-23]. We used logistic regressions and multivariate analyses to estimate the association between serology and landscape factors. Our first results show a major effect of the year of sampling and, in a lesser extent, of wood and meadow proportions of roe deer home range on the probability to be seropositive. These results are discussed in relation to the biological determinants of each pathogen.

[215] MERCURY IN LIVER OF TERRESTRIAL WILD BIRDS FROM WEST SPAIN: TEMPORAL VARIATION AND LEVELS DEPENDING ON THE SPECIES, LOCATION AND FEEDING HABITS

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Mercury (Hg) is one of the most dangerous nonessential metals emitted into environment. Wildlife may be exposed from a variety of environmental and human sources. The purpose of this study was to analyze its levels in liver of wild birds depending on the species, location, and feeding habits, and the variation over time. We analyzed a total of 118 samples, collected between 2001 and 2009, from 18 different species found dead in different places of Extremadura (Spain). Laboratory analysis was performed using an AMA254 Hg analyser after desiccation and homogenization of samples. Mercury was detected in liver samples from all species and remained stable over time. Attend to feeding habits, higher Hg levels were obtained in omnivorous, on the other hand scavenger have the lowest levels. The difference between this two groups was statistically significant ($p>0.001$). By species White Stork (*Ciconia ciconia*) had the higher Hg levels and Griffon Vulture (*Gyps fulvus*) the lowest being statistically significant this difference ($p> 0.001$). The higher Hg levels were obtained in agricultural and urban areas. In general, Hg levels seemed to be of little concern to wild birds, except for white storks (8 samples) and other individual samples with levels that could be associated to deleterious effects on the reproduction and behavior.

[216] RESCUE CENTERS AS SENTINELS OF WILDLIFE CONSERVATION USING SPATIAL EPIDEMIOLOGY

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The wildlife rescue centers (WRC) collect and provide care to the damaged wild animals, helping to their conservation and providing valuable information about their condition. This information represents an indicator of their habitat condition.

This study proposes the use of the gathered information in AMUS (WRC in Badajoz, Spain), as a sentinel of wildlife condition, identifying the most frequents causes and the time and location of the 1500 admitted animals (2009-2011). A descriptive statistical analysis and a retrospective spatiotemporal permutation analysis (SatScan) were employed. Birds represent the major admissions (96.51%) compared to reptiles (1.62%) and mammals (1.86%). Traumatisms, falls from the nest, orphans, trapped and run over animals are the main causes of admission. Spatiotemporal analysis showed 12 clusters mainly in April and May (75%) located in the western of the studied area (radius = 0-15 km), which enclosed fundamentally agricultural (66%) and forest/semi-natural areas (8%). These results show the great importance of WRC data which provide useful information in order to understand currently habitat condition. This information is being used to carry out analysis that would help to define specific corrective measures enabling to focus environmental conservation resources.

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[217] APPLICATION OF FLOW CYTOMETRY AND GAS CHROMATOGRAPHY TO STUDY FIBROPAPILLOMATOSIS IN *CHELONIA MYDAS* (TESTUDINES, CHELONIIDAE) IN BRAZIL AND THEIR CONTRIBUTIONS FOR CONSERVATION OF THIS SPECIES

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The fibropapillomatosis, disease characterized by benign skin tumors, is one of the greatest threats to the survival of green sea turtles (*Chelonia mydas*). Studies attribute the cause of the disease to the association of Chelonid fibropapilloma-associated herpesvirus, genetic and environmental factors like polychlorinated biphenyls (PCBs) and toxic potentially metals. This study aimed to assess the leukocytes function (phagocytosis and oxidative burst) by flow cytometry and investigate the PCBs presence in blood samples by gas chromatography (GC-MS/MS). Moreover, hemogram was carried out in the samples. Specimens of *C. mydas* were caught randomly or rescue in feeding areas: Ubatuba/SP (n=43), Almofala/CE (n=13), Vitória/ES (n=17) and Florianópolis/SC (n=3) and caught selectively in Fernando de Noronha/PE (n=17), a nesting and feeding area. PCBs (28, 52, 101, 118, 138, 153 and 180) were analyzed in the GC-MS/MS, operating in the Single Ion Monitoring mode. The preliminary results of the samples from Ubatuba/SP showed that the animals afflicted by the disease had a larger size and PCBs congeners monitored were detected. The statistical analyses showed that values of the hemogram parameters and in the flow cytometry between afflicted and non-afflicted turtles were similar ($p \geq 0.05$). The samples from the other areas of the study are still being analyzed which allows the expansion of the preliminary results and the comparison between the areas of occurrence of *C. mydas* in Brazil.

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[218] NOVEL METHODS FOR THE DETECTION OF TUBERCULOSIS INFECTION IN GREAT APES

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The introduction of novel infectious diseases has become a major threat to endangered primate populations. This is particularly true for habituated great ape populations, conditioned for close encounters with human observers, and there is ample evidence that exposure of primates to human pathogens, particularly those of respiratory origin, readily occurs. In an effort to sustain the health of habituated great ape populations, continued health monitoring of these populations is recommended. Unfortunately, health monitoring for some diseases is hampered by a paucity of sensitive, non-invasive diagnostic assays. Tuberculosis, a disease of high prevalence among humans in many African regions, is an example and poses a significant health risk for habituated great ape populations. The goal of this project was to validate fecal and urine biomarkers for the detection of *Mycobacterium tuberculosis* (M.tb) infection in non-human primates. An ELISA was developed to detect in urine lipoarabinomannan, a cell wall lipoglycan specific for pathogenic mycobacteria of the M.tb complex (MTC). A second set of ELISAs was also developed to detect fecal antibodies to highly antigenic proteins specific to members of the MTC: ESAT-6, Cfp10, and Ag85. Validation was carried out with the testing of known M.tb positive and negative macaques (*Macaca* spp.). Receiver operating characteristic analyses were used to assess the diagnostic accuracy of the ELISAs.

[219] CROSS-REACTIVITY TESTS OF KNOWN CELL MARKERS TO OBTAIN SPECIFIC ANTIBODIES FOR NON-MODEL SPECIES

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Wildlife or ecological immunology as a discipline, investigates immune processes and functions in free living populations under the influence of different environmental and ecological factors. The challenges of considerable genetic and environmental diversity, the lack of model-organisms with developed immunological tools and the lack of sequenced genomes impede the molecular and functional analysis of immune responses in wildlife species. Our group is working with different bat species (*Microchiroptera*) which are known important reservoirs for many emerging viruses, and act as natural hosts for zoonotic diseases. This project aims to establish methods that can transfer immunological investigations from the laboratory to the field with reproducible, single-time point measurements. Common laboratory methods are based on the use of monoclonal antibodies, and as an initial starting point we will test known lymphocyte markers for cross-reactivity via Western-Blot and Fluorescent Activated Cell Sorting (FACS). Based on these results we will purify sub-cell populations of B- and T-lymphocytes using antibody-dependent cell purification methods. Our goal is to obtain species specific monoclonal antibodies for different bat species and other wildlife using single B-cell technologies, where purified B-lymphocytes are essential.

[220] DENGUE VIRUS IN BATS FROM DIFFERENT LANDSCAPES IN SOUTHEASTERN MEXICO

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Dengue is the most common arboviral disease in southeastern Mexico. Some studies have shown molecular evidence suggesting the presence of the four serotypes of the Dengue Virus (DV) in groups like primates, marsupials, rodents and bats. However, little is known about their role in the transmission cycle in Neotropics. Recent studies have shown that bats are reported with DV in the Americas. Bats are ubiquitous, very diverse and some species are closely related with humans in disturbed landscapes. To identify DV in bats we captured them in a disturbed gradient from November 2010 to August 2011, in Campeche and Chiapas; both in southeastern Mexico. Spleen samples of 164 bats of the most abundant species (116 frugivores, 29 nectarivores, 7 insectivores, 2 hematophagous) were tested for DV serotypes 1-4 by nested PCR, using standard protocols. We found two frugivorous bats (*Artibeus* lituratus) positive to DV2 in Chiapas, one in a conserved site and one in a disturbed site. Also, we found four bats positive to DV2 in Campeche, two positive bats were found in conserved sites (*A. jamaicensis* and *Glossophaga soricina*) and two in disturbed sites (*A. lituratus* and G. *soricina*). It is possible that human activities are involving bats in epidemiological cycles where they do not participate, carrying pathogens where they did not previously exist, like dengue in conserved sites. The role of bats in the infection is still unknown. So there is a need of subsequent studies to determine it.

[221] CIRCULATING TESTOSTERONE AND VIRAL INFECTIONS IN NATURAL POPULATIONS OF DOMESTIC CATS

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Testosterone (T) influences physiological and behavioral traits linked to males' reproductive success. It is also suspected to impact their susceptibility and/or exposition to parasites, i.e., via immunosuppression and/or modulation of transmission-relevant behaviors. A positive correlation is often observed between T and infection risk and/or intensity; however contrasted patterns of association are reported. Given T modes of action and the diversity of host-parasite interactions, T should not act similarly on parasitism in all interactions. To reduce the effects due to host and context diversity, we studied several viruses in the same cat population: an aggressively transmitted virus, the Feline Immunodeficiency virus (FIV), and two viruses transmitted during friendly contacts, the Feline Calicivirus (FCV) and Herpesvirus (FHV). Results show that T has a strong effect on the probability of being positive to FIV, whereas its effect is significantly weaker on FCV and FHV. These findings demonstrate that T can differentially affect parasites of the same type (viruses). In our case, the observed difference was consistent with what was expected from a behavioral-mediated effect (increased aggressiveness). This supports the idea that T effect on infection risk is at least partially driven by behavioral mechanisms in our system, although further investigations (e.g., immunological measures) are required to confirm this hypothesis.

[222] MOLECULAR DETECTION OF NATURAL INFECTION BY SARCOCYSTIDAE COCCIDIAN IN NEOTROPICAL WILDLIFE FELIDS FROM BRAZIL: PRELIMINARY RESULTS

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Domestic cats are notably linked by transmission of *Toxoplasma gondii*, although wild cats are also hosts of these parasite; few studies prove its involvement in the epidemiology of the disease. Our goal is detect by molecular methods the presence of Sarcocystinae and Toxoplasmatinae in tissue samples from *Puma yagouaroundi*, *P. concolor*, *Leopardus geoffroyi*, *L. tigrinus*, *L. wiedii*, *L. colocolo* and *L. pardalis* and define the zoonotic potential of these hosts. The ITS-1 amplification by nested-PCR was performed in 364(63.9%) of the tissues collected from 94 individuals deposited in museums of natural sciences of state Rio Grande do Sul, Brazil. Positive samples for Toxoplasmatinae (43) and Sarcocystinae (30) were found. A RFLP with the enzymes *RsaI* and *TaqI* confirmed *T. gondii* in 10 samples (eye, tongue, hearth and muscle) from 10 different animals. Though the genotyping was not performed, is likely to be the participation of neotropical wild felids in a wildlife cycle transmission, since the material under study are from free-range animals in a region where previous studies demonstrate intense prevalence of toxoplasmosis. This study shows the usefulness of museums as a source of tissue for studying the natural history of diseases in wild animals without compromise of vulnerable species and describes for the first time the detection of *T. gondii* in eye muscles and vitreous humor of wild felids, remarking that the ocular form of *Toxoplasma* is one of the more pathogenic infection.

[223] PREVALENCE OF CANINE DISTEMPER VIRUS IN DOMESTIC DOGS, JAGUARS AND PUMAS ON THE BORDER OF THE CALAKMUL BIOSPHERE RESERVE, MEXICO

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The transmission of infectious diseases between domestic animals and wildlife is a conservation concern. Domestic dogs often act as reservoir species that can maintain infectious diseases in their populations and may transmit these diseases to wildlife. The risk of disease spillover from domestic dogs to wild carnivores has not been extensively investigated in Mexico. The goals of this study were to examine the disease exposure of canine distemper virus (CDV) in domestic dogs and free-ranging large felines captured and sampled near the Calakmul Biosphere Reserve in Southern Mexico and determine the risk factors associated to CDV seropositivity. We conducted a cross-sectional household questionnaire survey to obtain information on vaccination status and demographic data of dog populations in three local communities or ejidos in the surroundings of Calakmul. We used a microneutralisation test to determine serum antibodies to CDV in 98 domestic dogs sampled in the ejidos. Serum samples from 12 jaguars and 1 puma captured in previous years will be tested at Cornell University Veterinary Diagnostic Lab., Ithaca, NY. Dog population sizes and levels of exposure to CDV varied among ejidos, with a higher prevalence on the largest dog population. More than 90% of all dogs sampled had never been vaccinated against CDV and opportunities for direct contact with wildlife ocurr from hunting activities or predation of domestic animals. Our results demonstrate that domestic dogs may play an important role in CDV spillover to wild carnivores. Further results of distemper prevalence of jaguars and pumas will provide information on disease exposure and how a generalist pathogen like distemper is transmitted and maintained in these populations.

[224] SEROLOGICAL SURVEY OF PARVOVIRUS IN CARNIVORES, CHINA

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Parvovirus infections in carnivores may cause high fever, vomitting, leukopenia, diarrhea and severe dehydration and even death. However, parvovirus infection in wild animals in China is poorly known. The goals of this study were to conduct a serological survey to determine whether antibodies were present against parvovirus. A total of 416 serum samples from tigers, lions and foxes in different wildlife sanctuaries across China collected between 2002 and 2011 were used to measure the antibodies against parvovirus by serum neutralization (SN) assay and hemagglutination-inhitition (HI) assay. It was found that the antibody titers determined by SN were highly consistent with that to HI. Of the total 358 serum samples from tigers, 182 (51%) were found to be positive against feline parvovirus (FPV). However, only 2 (20%) of the 10 lions showed positive antibodies against FPV. Such lower positivity in the foxes was observed with 23% (11/48) against canine parvovirus (CPV). Our data demonstrate that parvovirus infection in the three wildlife animals have occurred across China, and the tigers have a higher seropositivity than lions and foxes. Further studies are indicated to determine the prevalence of this virus in free-ranging population of wildlife. Corresponding prophylaxis approaches should be advised to prevent the parvovirus outbreaks in wildlife animals.

[225] TICK IDENTIFICATION, BACTERIAL ANALYSIS AND COINFECTIONS OF *BORRELIA*, *ANAPLASMA*, AND *EHRLICHIA* FROM COYOTES (*CANIS LATRANS*) COLLECTED IN GEORGIA

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Coyotes (*Canis latrans*) are thought to be wildlife reservoirs for various tick-borne zoonotics. Among these are the bacterial agents for Lyme borreliosis, anaplasmosis and ehrlichiosis. Coyotes are known hosts for more than 6 species of tick. Among those parasitizing coyotes and known to be vectors for aforementioned zoonotics in this study are the black-legged tick (*Ixodes scapularis*) gulf coast tick (*Amblyomma maculatum*) & lone star tick (*Amblyomma americanum*). Investigations for the presence of vector-borne pathogens in nature and natural reservoirs are important in understanding disease cycles and epidemiology. Therefore the aims of this study are: 1) to determine what species of ticks are present (both morphologically and through PCR); 2) look at the literature and list what diseases these species are known to vector; 3) run 4DXsnap tests to determine what if any pathogens coyotes have been exposed to. Finally we will take this information and apply it to the big picture and phenomena of Lyme borreliosis. Preliminary results from 23 of 50 coyotes from Georgia have shown no infections of Lyme Disease, or Anaplasmosis, but Ehrlichia is present in low numbers, with heartworm being most prevalent. The two most prominent tick species recovered thus far are *Ixodes scapularis* and *Amblyomma americanum*. There is still a second phase of this project to be completed with more animals coming in at the moment.

[226] CHRONIC WASTING DISEASE AND CARNIVORES: A ONE-HEALTH APPROACH TO CONSERVATION

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Chronic wasting disease (CWD) is a progressive, fatal, transmissible prion disease first identified in captive mule deer in 1967 in Colorado, USA. Currently, the disease is known to infect mule deer, white-tailed deer, elk, and moose located in fourteen states and Canada. The method of disease transmission to naïve animals is still uncertain, although contact with contaminated environment is identified as a risk factor. There is no known way to contain or treat CWD. Although other transmissible spongiform encephalopathies (TSEs) infect humans and animals, CWD is currently only found in cervids. As a precaution, the Centers for Disease Control and Prevention (CDC) warns against exposure to CWD infected animals and meat products. The reintroduction of the wolf to the western U.S. in 1995 offered a unique opportunity to monitor changes to ecosystem health. The presence of wolves alters the behavior, population density, and productivity of its prey and other cohabitating species. In areas where CWD is present, wolves may target the weaker, infected animals. A literature review of the impact of wolf reintroduction and the history of chronic wasting disease in the United States identified areas of overlap between reintroduced wolf populations and CWD infected cervids. This provides an opportunity to study predator-prey interaction in CWD infected areas and apply the One Health approach to a critical disease and conservation issue.

[227] EPIDEMIOLOGY OF A NEW CANID ASSAMBLAGE AND THREATS TO THE ENDANGERED FUEGUINEAN CULPEO FOX (*PSEUDALOPEX CULPAEUS*) IN TIERRA DEL FUEGO, CHILE

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The Fuegian culpeo fox (*Pseudalopex culpaeus*) inhabits Tierra del Fuego and represents one of six subspecies, being the only one consider endangered due its declining population trend. It is mostly restricted to the southern beech *Nothofagus* forest in the south of Tierra del Fuego and over the past century, not only habitat loss and farming activities *per se* threaten culpeo, in fact shepherd dogs and the introduced chilla fox (*Pseudalopex griseus*) represents an additional challenge in the conservation of the species. We suggest that dogs associated to *per urban* areas, often in higher densities, might represent a source for pathogens as well as chilla foxes which are found in the steppe, connecting the north and south areas of the island. We conduct a research project which includes assessing the epidemiological threats for the Fuegian culpeo fox to respond to potential disease outbreaks. Working along with the community, besides outreach and education, we are conducting domestic dog sampling and performing captures of free ranging culpeos and chilla foxes. Using ELISA field diagnostic kits, we have detected the presence of antibodies against Distemper and Parvovirus, which we acknowledge as a major threat for this endangered population. We intend to use this information to have a better understanding of the threats that introduced canids might pose to culpeo populations and suggest from management measures, up to contingency plans in case the worst pathogenic scenario arises.

[228] *TOXOPLASMA GONDII* IN WILDLIFE RESERVOIRS – RED FOX (*VULPES VULPES*) AND RACCOON DOG (*NYCTEREUTES PROCYONOIDES*)

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Toxoplasma gondii is a common protozoan parasite of wildlife, domestic animals and human. However the role of wildlife in the epidemiology of *T. gondii* infections in livestock or humans is still under discussion. During 2011 heart samples were collected from 667 wild Canidae. Serosanguineous fluid was obtained upon thawing the hearts and screened with in-house ELISA for *T.gondii* –specific IgG antibodies and a protein extraction of *T. gondii* RH strain tachyzoites were used as the antigen. Antibodies were found in 51 of 344 (14.8%) of raccoon dogs (*Nyctereutes procyonoides*), 96 of 323 (29.7 %) of red foxes (*Vulpes vulpes*). A significant ($p<0.05$) difference of seroprevalence and red fox had 2.4 (CI 95% 1.6-3.6) times higher likelihood to be seropositive than raccoon dogs. These differences could be explained by different food habitat of those animals. Raccoon dogs are more omnivores but red fox – carnivores. Samples of heart of seropositive animals were digested in pepsin and prepared for further *T.gondii* isolation and genetic genotyping. Serological results indicated a widespread exposure to *T. gondii* among wild carnivores in Latvia.

[229] GEOGRAPHIC DISTRIBUTION AND ZOONOTIC POTENTIAL OF *ECHINOCOCCUS MULTILOCULARIS* IN WILDLIFE IN CANADA

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Alveolar Echinococcosis, caused by *Echinococcus multilocularis*, is an emerging public health concern worldwide. While human cases are rare in Canada, this parasite is increasing in prevalence and distribution in humans and wildlife in Europe and Asia. The need for strain determination in Canada is compounded by the occurrence of 2 Asian stains in Alaska and the discovery of a European strain in western Canada in 2009, which may have increased zoonotic potential or pathogenicity as compared to endemic North American strain(s). We characterized larval and adult cestodes at mitochondrial loci (NADH1 and 2, COB, and COX1) and microsatellite profiles at the NAK1 and EmsB loci. The strain in Arctic foxes (*Vulpes lagopus*) in mainland Nunavut was similar to that in Alaska (either N1 or Asian strains). The N2 strain (with a unique microsatellite profile) was identified in urban coyotes (*Canis latrans*) in Alberta and in deer mice (*Peromyscus maniculatus*) from rural Saskatchewan. Alveolar hydatid cysts were present in 6% of 700 deer mice, encompassing <5%-80% of the liver. 28% of positive deer mice were from one location, and prevalence was highest in hay fields as compared to native prairie and crop habitats. This suggests that this parasite has a focal distribution and unique genetic structure in the prairie provinces of Canada, which has significance for determining risks for public health at a changing wildlife-human interface.

[230] HELMINTHS OF RED FOXES (*VULPES VULPES*) FROM NORTH-WEST ITALY

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The fox is a potential reservoir of pathogens for wild and domestic animals and occasionally humans. A parasitological study on helminths of 130 red foxes from NW Italy (Imperia and Cuneo districts) was conducted from 2009 to 2012. The cardiopulmonary system, the stomach, the intestine, the urinary bladder, the liver and muscle samples were examined for adults, larvae and eggs detection. The prevalence of nematodes of the cardiorespiratory tract was: *Angiostrongylus vasorum* (75%), *Capillaria aerophila* (45%), *Crenosoma vulpis* (13%) and *Filaroides* spp. (3%). In the stomach *Spirocerca lupi* (22%) and *Physaloptera* spp. (3%) were found. Intestinal nematodes found were *Uncinaria stenocephala* (68%), *Toxascaris leonina* (28%), *Toxocara canis* (24%), *Molineus legerae* (24%), *Trichuris vulpis* (23%), *Aonchotheca putorii* (10%), and *Pterigodermatites affinis* (6%). About intestinal cestodes a high prevalence of *Mesocestoides* spp. (82%) was found, followed by Dilepididae (27%), *Taenia pisiformis* (3%) and *Taenia polyacantha* (2%). 59% of the examined bladders were positive for *Capillaria plica*. *Capillaria hepatica* was found in one fox liver: to our knowledge, this is a new host record for *C. hepatica*. *Echinococcus multilocularis*, *Trichinella* spp. and filarial nematodes were not found. A wide range of veterinary significant parasites were found, as all detected helminths can be directly or indirectly transmitted to dogs; the presence of zoonotic species, suggests potential public health risk.

[231] VACCINATION AND GOOD DOG MANAGEMENT PRACTICES AS A PRINCIPAL MEASURE TO PREVENT RABIES DISEASE IN AND AROUND THE VOLCANOES NATIONAL PARK, RWANDA, 2006-2009

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Human rabies is a fatal disease caused by the rabies virus and transmitted mainly by rabid dogs. Despite the existence of effective vaccines, 24,000 people die of rabies in Africa annually. We initiated an intervention in communities around the Volcanoes National Park to control rabies. From 2006 to 2009, we provided free rabies vaccination for dogs and educated dog owners and the general public on rabies prevention measures. We used a questionnaire to collect data on dog ownership, dog management practices, and dog owners' knowledge of clinical signs and symptoms of rabies in dogs. We analyzed the data using Epi- info software by calculating simple proportions. Dogs are kept for various reasons; guard (82%), hunting (12%), pet (7%), other (9%). From 2006 to 2009, 3,805 (88 %) dogs were vaccinated. Dogs' movement restriction outside the owner's compound improved from 57% to 78%. Again from 2006 to 2009, the proportion of dog owners with correct knowledge of the clinical signs and symptoms increased from 45% to 85%. Free rabies vaccination for dogs coupled with public education improved knowledge and practices that can reduce rabies. We recommend that the intervention be expanded to other areas to reduce the risk of rabies in Rwanda.

Key words: Vaccination, Rabies, dogs

[232] *ALARIA ALATA* MESOCERCARIAE IN TONGUES OF RACCOONS (*PROCYON LOTOR*) IN GERMANY

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Alaria alata is a parasitic trematode of carnivores from Europe. It needs two intermediate hosts to complete its life cycle and the addition of a paratenic host can occur at the mesocercarial stage. Alaria alata mesocercariae have been recently identified in wild boar meat from Europe and therefore, it is considered as a zoonotic agent. Previous histo-pathologic studies show the presence of unidentified mesocercariae within tongues of raccoons from Northern Germany. With the goal to identify this parasite species, tongue tissue samples of 105 raccoons were collected from a national park in Northern Germany and from Berlin metropolitan area. Histological examination and cryotome sections of frozen tongues were used to identify positive animals. Mesocercariae were successfully isolated from 11 animals. Via PCR assays, we tested Alaria alata mesocercariae in 10 of these 11 tongues. Out of these 10 positive animals, 9 were originated from the national park and one was collected in Berlin metropolitan area. Our results conclude that the raccoon can act as a paratenic host for A. alata in Germany. They highlight the possible role of the raccoon as a further paratenic host within A. alata related epidemiological studies and the effect introduced species like raccons can have in local parasites ecology.

[233] ALEUTIAN DISEASE IN WILD AMERICAN MINK (*NEOVISON VISON*) ON PRINCE EDWARD ISLAND

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Aleutian disease of mink, caused by a non-enveloped, single-stranded, DNA virus in the family *Parvoviridae*, is the most important production-limiting disease of the commercial mink industry. The disease causes fatal pneumonia in young mink as well as immunosuppression and chronic disease in older animals. In Prince Edward Island (PEI), Canada, the Provincial Department of Agriculture, Trapper's Association and mink breeding industry are concerned regarding the presence and potential impacts of Aleutian disease in wild mink (*Neovison vison*). The objectives of this study were to utilize polymerase chain reaction to detect Aleutian disease in PEI's wild mink population, and, if present, establish Aleutian disease's geographic distribution and prevalence in wild mink. Genotype determination as well as phenotypic analysis will be accomplished using viral capsid (VP2) nucleotide sequence data on PCR positive spleen samples. Twenty two wild mink were sampled during the 2010-2011 trapping season, and four animals testing positive giving an estimated prevalence of 0.18 (95% CI 0.053, 0.402). This prevalence is lower than those values reported from Atlantic Canada. This collaborative study will permit a better understanding of the epidemiology of Aleutian disease in PEI, leading to the development of specific management strategies to protect the health of provincial wild and domestic mink populations.

[234] CAUSES OF MORTALITY AND CHARACTERISATION OF MYCOBACTERIOSIS IN ADULT NEW ZEALAND SEA LIONS (*PHOCARCTOS HOOKERI*) AT ENDERBY ISLAND

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The New Zealand sea lion (*Phocarctos hookeri*) is endemic to New Zealand and is classified as threatened/in decline due to its declining population size and limited breeding range. To describe some of the present disease risks and therefore aid in conservation management of the species, this study investigated the causes of death in individual adult New Zealand sea lions. Samples were obtained from post mortems during annual expeditions to the sea lion breeding colonies on Enderby Island in the Auckland Islands, run from 1998 to 2012. Post mortems were performed on 71 non-pup individuals and 60 of these had samples taken for further diagnostic testing. The cause/s of death were determined using gross post mortem reports, histopathology and bacterial culture. Preliminary results show that 39% of individuals died from a range of infectious causes, 33% died from conspecific trauma, 4% died from interspecific trauma from sharks and 24% had an open diagnosis. Studies have shown that these levels of conspecific trauma are comparable to other pinniped populations. At histological examination, 20 out of 60 animals showed granulomatous lesions suspicious for mycobacterial disease and six of these individuals had visible ZN positive organisms. The lesions varied in severity, histological appearance and location, with the most common finding being a granulomatous bronchopneumonia. Samples of affected tissues have been sent for mycobacterial culture, results for which are pending. Positive cultures will have further VNTR (variable number tandem repeat) typing performed to speciate the organism involved. Due to the isolated range of this species, there is minimal human/livestock risk from the presence of mycobacterial disease in this population.

[235] PREVALENCE OF *SALMONELLA ENTERICA* AND *CAMPYLOBACTER* SPP. IN WILD-CAUGHT AND STRANDED NEONATAL AND JUVENILE GREY SEALS (*HALICHOERUS GRYPUS*) IN SCOTLAND

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Salmonella and *Campylobacter* spp. bacteria are zoonotic pathogens that have been isolated from marine mammal populations worldwide. However, their prevalence, type and significance remain poorly understood. Prevalence of these pathogens was assessed in faecal swabs from 200 neonatal and juvenile grey seals from Scottish coastal waters and evaluated with regards breeding colony substrate, spatio-temporal distribution, life stage and associated pathology. *Salmonella* and *Campylobacter* were isolated and identified using selective methods. Overall, the prevalence of Salmonella in grey seal pups was 21.1% with *Salmonella enterica* serovar Bovismorbificans and serovar Typhimurium being the only two serotypes isolated. *S.* Bovismorbificans was isolated from 18.9% of live seal pups with a significantly ($p<0.05$) higher prevalence in grey seal pups from pupping sites exposed to tidal seawater compared to pups that had not yet been exposed to seawater. Overall the prevalence of *Campylobacter* spp. was 42.2% in grey seal pups with *C. jejuni* and non-hippurate hydrolysing thermophilic *Campylobacter* sp. identified. Additional work is ongoing to correlate the presence of isolates with postmortem findings and to classify these isolates on a molecular level whilst examining their relationship with those found in the terrestrial ecosystem. This study also re-emphasises the zoonotic hazard and strict hygiene requirement when working with grey seals.

[236] EXPERIMENTAL INFECTION OF BALB/C MICE WITH *BRUCELLA PINNIPEDIALIS* FROM HOODED SEAL (*CYSTOPHORA CRISTATA*) AND CONCURRENT EXPOSURE TO PCB 153

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Brucella spp. have been isolated from and serologically indicated in marine mammals from most of the world. Especially high numbers of positive animals have been found in the declining Northeast Atlantic population of hooded seals. Persistent organic pollutants accumulate in the Arctic and studies indicate that exposure to PCBs lead to inadequate immune function and risk of disease development. PCB 153 is the congener found at the highest levels in the Arctic. The goal of this study was to evaluate the pathogenesis of *Brucella pinnipedialis* from hooded seal in the mouse model and whether the course of infection was changed by exposure to PCB 153. BALB/c mice were inoculated intra peritoneally with *B. pinnipedialis* or *Brucella suis* 1330 (positive control). Two of four groups á 20 mice were exposed to PCB 153 (588 ug/kg mice/day) in the food. Mice were killed at one, three, six and twelve weeks post infection (p.i.). The number of colony forming units (CFU) in spleen, liver and kidney was evaluated. *B. pinnipedialis* was able to establish an infection but at a much lower level than *B. suis* 1330. The infection with *B. pinnipedialis* was cleared from all organs within 12 weeks p.i. These results show that the hooded seal strain have a lower pathogenicity than *B. suis* 1330. This might indicate that *B. pinnipedialis* in the hooded seal may have little influence on the decline of the Northeast Atlantic population. Our study showed that exposure to PCB 153 did not alter the number of CFU.

[237] SKELETAL ABNORMALITIES IN HUMPBACK WHALES (*MEGAPTERA NOVAEANGLIAE*) STRANDED IN THE BRAZILIAN BREEDING GROUND

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Assessing the health of large whales in the wild is a difficult task since animals are often found dead, in variable decomposition state. The goal of this study was to investigate the occurrence of skeletal pathologic processes in humpback whales and evaluate their potential to compromise health. Bones of 49 humpback whales stranded along the Abrolhos Bank seashore and its adjacencies in Brazil (15°44’S; 38°53’W - 20°00’S; 40°09’W) were studied between 2002 and 2011. Twelve (24.5%) animals presented pathological changes in one or more bones. Inflammatory diseases were found in 10.2% (5/49) of whales involving the joint of ribs, radius and atlas of three whales, and have led to severe lesions on caudal vertebrae of two whales. Degenerative diseases were observed in 8.2% (4/49) of the animals in the vertebral column of three whales and humerus of one individual. Traumatic lesions were observed in 8.2% (4/49) of the animals. In one whale, the rib showed severe osteomyelitis, possibly resulting from the infection of multiple fractures. Developmental abnormalities found in 10.2% (5/49) of the individuals included fusion of first two ribs, fusion of spinal processes, and spina bifida. Chronic inflammatory lesions found in the axial skeleton may have restrained spinal mobility and had detrimental effects on the general health of the animals, contributing to stranding and death. To our knowledge, this is the first systematic study on skeletal lesions in stranded humpback whales.

[238] ASSESSMENT OF HERMATYPIC CORAL HEALTH BY DESCRIPTION OF MACROSCOPICAL AND HISTOLOGICAL LESIONS

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Coral diseases are almost worldwide distributed and are one the most important threats corals are facing nowadays. One of the problems associated with the study of coral diseases is the lack of consensus and ambiguity in the descriptions of lesions. A systematic and objective description of lesions at the gross and microscopic level is the first step to disentangle the disease dynamics and the interactions between host, pathogen and environment. In the Mexican Pacific there are no published data on coral health. This study focused on describing the morphological lesions present on *Pocillopora*. We visited four localities between November 2010 and September 2011, where we characterized the lesions according to three categories: loss of tissue, changes in tissue coloration and overgrowth by pigmented noncoraline material. Paired normal and lesion fragments were taken from affected colonies along with fragments from adjacent healthy colonies. Samples were histologically processed, stained with hematoxilin-eosin and Mallory. The tissue characteristics were described and compared between healthy and affected colonies. The main lesion seen microscopically was diffuse atrophy. Additionally, there was evidence of a drastic decrease in the number of dinoflagellate symbionts and the presence of bacterial aggregates inside the epitheliums; however bacterial aggregates were also seen in normal tissues. This is the first systematic study of pathology of coral disease in the Mexican Pacific.

[239] ENVIRONMENTAL FACTORS ASSOCIATED WITH THE SEROPREVALENCE OF *TOXOPLASMA GONDII* IN WILD BOARS (*SUS SCROFA*), FRANCE

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Understanding the dynamics of zoonotic diseases in their natural environment is essential for improving the management of between-host transmission and preventing human infection. Here, we address a key issue for the field of *Toxoplasma gondii* ecology, i.e. understanding environmental risk factors associated with the presence of this protozoan parasite. Wild boars (*Sus scrofa*) are frequently exposed to the parasite because of their burrowing behaviour and therefore appear as ideal indicators of the environmental risk of contracting toxoplasmosis. They also represent a public health issue because the consumption of undercooked meat may lead to human infection. In this study, we considered 938 wild boars sampled from 377 French 'communes' and found that overall seroprevalence was 23% (95% confidence interval: [22–24]). Using a Poisson generalized linear model, we found that seroprevalence in wild boars was positively explained by moderate winter temperatures and by the presence of European wild cats (*Felis silvestris*). These results may help explaining inter-regional variations in toxoplasmosis prevalence observed in many species including humans, and better identifying sources for human infection.

[240] STUDYING THE PATHWAYS OF EXCRETION OF AUJESZKY´S DISEASE VIRUS IN NATURALLY INFECTED WILD BOAR POPULATIONS

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Aujeszky's disease virus (ADV) continues increasing its geographic range in Spanish wild boar. However, knowledge on some basic features of ADV in wild boar is scarce. We aimed to identify the ways by which ADV is excreted by infected wild boar. We selected four wild boar hunting estates in south-central Spain where ADV is endemic and surveyed 85 wild boar in the 2010/2011 hunting season. From each wild boar we collected trigeminal and sacral ganglia, oropharyngeal tonsil, blood and nasal, oral, rectal and genital swabs. Tissues and swabs were analyzed by PCR to detect ADV DNA while sera were analyzed by ELISA. Global ADV seroprevalence was 59±11%. Females and juveniles had higher seroprevalence than males and adults, respectively. The average ADV DNA prevalence in swabs was 3±3.9% while the prevalence in tissues was 40±16.7%. The prevalence of PCR-positive tonsils was 28.6±9.7% while only 13.4±20% of the trigeminal ganglia were positive. 2 of the 21 animals where sacral ganglia were analyzed were positive (9.5±12.5%). One of these animals was positive only in sacral ganglion indicating that the venereal route is possible. Approximately the 30% of infections were recent while the 17% were latent and only 1% of animals have reactivated latent infections. The presence of ADV in secretions, and thus its possible excretion in endemic populations, is low during the hunting season. Further research is needed to determine the pathogenesis of ADV in wild boar and particular epidemiological features in naturally infected populations.

[241] SEROLOGICAL, MOLECULAR AND PATHOLOGICAL SURVEY OF PSEUDORABIES VIRUS IN WILD BOARS (*SUS SCROFA*) FROM CENTRAL ITALY

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During the hunting season 2010-2011, blood samples, nasal and genital swabs and tonsillar tissue samples were collected, in Tuscany, Central Italy, from 139 wild boars (39 piglets, 30 young and 70 adult subjects), to investigate pseudorabies virus (PRV) seroprevalence and viral DNA presence, using a commercial enzyme-linked immunosorbent assay (ELISA) and PCR, respectively. Immunohistochemistry with anti-PRV monoclonal antibodies was also performed on tonsillar tissue samples of selected serological- and PCR-positive subjects. Fourty-three of 139 (30.9%) subjects were PRV-seropositive, while a 194bp PRV specific product was amplified from 9 nasal swabs (6.5%) and 26 genital swabs (18.7%). PRV-positive PCR products were sequenced. Significantly higher seroprevalence was observed in piglets ($P<0.05$) and adult animals ($P<0.001$) when compared to young subjects. The viral DNA presence was significantly higher in genital swabs than in nasal ones ($P<0.001$). Piglets and adult subjects had higher percentage of virus-positive genital swabs than young (8.6% and 7.2%, respectively vs 2.9%). The results confirmed that PRV infection is endemic in wild boar population in the studied area. The presence of anti-PRV antibodies and of the virus in PRV-positive piglets could be related to a vertical viral transmission. The higher percentage of virus-positive genital swabs confirmed, in our field study, that in adult feral boars PRV is preferentially transmitted by the venereal route.

[242] CASE REPORT OF EXUDATIVE EPIDERMITIS IN A WILD BOAR PIGLET

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Exudative epidermitis is a porcine disease caused by *Staphylococcus hyicus*. The disease occurs as an acute infection in suckling and newly weaned piglets in which a generalized epidermitis may lead to dehydration and death. It produces important economic losses in severely affected herds. In this work, we describe for the first time a case of exudative epidermitis in wild boar. Affected animal was a male wild boar piglet about four months old belonging to a wild boar farm located in Toledo (Spain). The animal presented marked debility and dehydration until it died. After death, complete pathological, histopathological and microbiological studies were carried out. The main gross lesions found were necrotic areas in ears and tail with lack of tissue, ulceration in skin, mainly around the mouth, eyes, and near to hoofs. In addition, superficial lymph nodes were found swollen and lungs presented pneumonic foci. Microscopically, damaged skin areas showed exfoliation, formation of vesicles, acanthosis, perivascular inflammation and presence of gram-positive bacteria. These microorganisms were identified as *Staphilococus hyicus* subsp. *hyicus*. Lesions and clinical features found in the affected wild boar were similar to those described in domestic pigs. This disease classically affected piglets in hygiene-defective intensive breeding, thus intensification of breeding in wild boar farms would be at risk of *S. hyicus* infections for similar reasons.

[243] NONTUBERCULOUS MYCOBACTERIA IN WILD BOAR (*SUS SCROFA*) FROM THE SOUTH-WEST OF SPAIN

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Nontuberculous mycobacteria (NTM) are widely distributed in the environment, particularly in wet soil, marshland, rivers or streams. However, a large number of this species can cause serious illnesses in humans, particularly in immunocompromised patients. NTM are increasingly associated with infectious pulmonary disease and cutaneous disease in humans. Despite this, there is little information available regarding to the effects on wildlife of NTM. In this work, we present the more frequent NTM isolated in wild boar from the South-West Spain. A total of 213 NTM isolates were obtained from 1388 wild boar mandibular lymph-node sampled, between 2007 and 2011. All but 75 isolates were identified by PRA-*hsp65* (polymerase chain reaction and restriction analysis of a portion of *hsp65*gene) and a partial sequencing of the 16S rDNA was done to identify the rest of the isolates. Our results showed that *M. chelonae* was the most frequently NTM species isolated (131 isolates, 61.5%), followed by *M. avium* complex (24 isolates, 11.2%). *M. chelonae* is one of the most frequent rapidly growing NTM, that cause skin and soft tissue infections in humans, while *M. avium* complex bacteria are the most frequently NTM isolated from pulmonary samples from persons. These types of NTM may have a zoonotic risk to handling the carcasses of wild boars the elderly and immunocompromised persons.

[244] “SWINE AND THE CITY”: CLOSE CONTACT WITH ANTIMICROBIAL RESISTANCE

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Wild boars are becoming increasingly popular due to its urban habituation. In this work, 41 wild boars from the metropolitan area of Barcelona (Spain) were screened for antimicrobial resistance in indicative bacteria (*Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium*). We aimed to know whether host synanthropy influenced the probability that the isolates (n=72) showed phenotypic resistance to a set of antimicrobials. Thus, following a theoretic information approach based on Akaike’s Information Criterion we explored the effect of selected variables indicative for urban habituation (e.g. distance from the place where each wild boar was captured to the nearest building, distance to the nearest feeding point) and of the bacterial species on the probability of an isolate being resistant to any antimicrobial tested. We found that 10% of the *E.coli* isolates showed any phenotypic resistance, however, 50% of the *Enterococcus faecalis* and 95% of the *Enterococcus faecium* isolates showed any resistance. Probability of an isolate being resistant depended on the bacterial species (explained deviance = 47.58%), but models including also variables indicative for urban habituation had substantial support. As a conclusion, urban wild boars are carriers of antimicrobial resistant bacteria and thus the increasing presence of these animals in urban areas should be regarded as a public health concern.

[245] THE MORE COWS GRAZE THERE, THE MORE AND MOST DIVERSE *SALMONELLA* IN WILD BOARS

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Salmonella spp. is considered a true multi-host pathogen of relevant interest for public health. We aim to assess the effect of extensive cattle on *Salmonella* prevalence and serotype richness of co-habiting wild boars. 73 faecal samples were obtained from cattle, 157 from sympatric wild boars and 57 from wild boars from close cattle-free areas. *Salmonella enterica* was isolated according to the ISO Standard (ISO 6579, Annex D) and serotyped by Kauffmann-White scheme. For explaining *Salmonella* probability of infection in wild boar, we combined the variables host age and sex, cattle presence and cattle herd size and obtained several models. We performed a model selection following the theoretic information approach based on Akaike's Information Criterion. The prevalence of *Salmonella* in wild boars from cattle-free areas was 17.54 % (CI 95% 8.74-29.91) while their counterparts from cattle-grazed areas showed a higher prevalence: 35.67% (CI 95% 28.19-43.70). Prevalence in cattle was 21.92% (CI 95% 13.10-33.14). *Salmonella* probability of infection in wild boar increased with cattle herd size and decreased with host age (explained deviance= 11.56%). Serotype richness (corrected for sample size by bootstrapping) was higher in wild boars from cattle-grazed areas (probability=98.6%). Serotype Meleagridis, Anatum and Othmarschen were shared between cattle and sympatric wild boars. Our results show a spill-over of *Salmonella* between these species, being wild boar a potential reservoir.

[246] *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* AT THE INTERFACE BETWEEN FREE-RANGING ELK AND BEEF CATTLE IN SOUTHWESTERN ALBERTA

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Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease, a production limiting disease of domestic ruminants. The susceptibility of elk is increasingly being recognized in both farmed and wild animals. Through validation of diagnostic tools for elk, this project contributes to understanding how MAP may be indirectly transmitted between beef cattle and wild elk. Through capture and collaring efforts of free-ranging elk, 135 serum samples were obtained from 5 herds with intense contact with cattle, and 144 from 5 herds in areas with no cattle. In addition, fresh fecal samples were collected from distinct individuals from these 10 herds. The IDEXX MAP Antibody ELISA® kit was validated for elk serum. Panels of positive and negative samples were used to define a cutoff value and test performances. Fecal samples were processed by fecal culture and direct qPCR. The modified ELISA had a sensitivity and specificity of 82% and 99% respectively. 10.4% of serum samples tested positive. MAP could not be cultured however, direct qPCR on fecal samples indicated the presence of MAP in 7 of 20 seropositive samples. The level of interaction with cattle had no significant effect on the diagnostic outcome. Preliminary data indicate circulation of MAP in wild elk in southwestern Alberta irrespective of their interaction with cattle. It remains to be understood if elk may act as a reservoir host for cattle and what are the consequences for elk populations.

[247] *MYCOBACTERIUM AVIUM* SSP. *PARATUBERCULOSIS* IN WOOD BISON: DIAGNOSTIC OPTIMIZATION AND HERD-LEVEL INFECTION STATUS

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Translocation has been an essential tool for wood bison conservation, resulting in the successful re-establishment of a number of free-ranging herds. However, a risk of moving animals is the spread of infectious agents to new herds or locations. One pathogen of concern is *Mycobacterium avium* ssp. *paratuberculosis* (MAP), which in infected ruminants causes enteritis, chronic weight loss, and death. The objectives of this study were to: (i) optimize diagnostic tools for MAP infection in wood bison to determine herd-level infection status, and (ii) determine the genetic diversity of MAP among these populations using multi-target genotyping. We tested fecal samples from eight different herds in northern Canada (n=233) using direct PCR for three MAP-specific targets. Since previous attempts to culture MAP from PCR-positive wood bison samples were unsuccessful, we evaluated a panel of different culture conditions for their ability to support MAP growth from feces and tissues of animals that tested positive with direct PCR. Finally, a commercially available MAP ELISA kit was modified and validated for use with bison serum as an additional diagnostic tool. Direct fecal PCR results suggest that MAP is present in all eight herds tested. No association could be found between direct PCR results and seroconversion of individual bison. Results from the culture panel and preliminary genotyping results will be presented. This study will facilitate informed decisions for translocation.

[248] RELEVANCE OF SEASONAL DRIVERS FOR AN ONGOING OUTBREAK OF FOOT-AND-MOUTH DISEASE

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Foot-and-mouth disease (FMD) is contagious between cloven-hoofed animals and has huge economic impact on livestock industries. Recent outbreaks in Mediterranean regions as well as transmission experiments indicate that FMD may spread well in wildlife populations. We investigated the impact of two seasonal mechanisms on the duration of virus circulation: half-life of infectious material in the environment and host reproduction. Infection through contaminated environment may cause inter- and intra-species transmission of FMD. Temperature dependent decay of contamination in the environment is well demonstrated and induces seasonal variations in half-life. Reproduction of wild boar and deer exhibits strict seasonality with peaks in spring or early summer. We present a new method to model transmission of the infection between species and social groups that don't share physical contact, by making excretion and intake of infectious material explicit. We parameterized an individual-based multi-species FMD model with data on host ecology, FMDV epidemiology and temperature in the study region. We found seasonal decay limiting for circulation and spread of the infection, compared to constant mean half-live. Pulsed availability of susceptible offspring could partially compensate for the effect of seasonal half-life. The affected eco-region was found ideally equipped by its timing of host reproduction and temperatures for FMDV spread. Transition to an endemic situation was found unlikely.

[249] APPLYING MAXENT SPECIES DISTRIBUTION MODEL (SDM) TO *BABESIA* SPP. EPIDEMIOLOGY IN WILDLIFE

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SDM are numerical tools that link observations of species occurrence to environmental parameters. Traditionally, SDM focused on mammals or plants, seeking the causal drivers of species distribution or making prediction of distribution to unsampled sites. With these aims we target SDM on *Babesia* spp. rather than to its wild mammalian hosts. The Maxent model can be trained with multiple geographical and environmental predictors, chosen because ecologically relevant to the target species. As *Babesia* is an intraerythrocytic protozoa, its presence in the environment depends upon presence of vector-ticks, and of mammalian hosts. Their presence will be accounted as separate informative layers: density and richness of definitive hosts, and a satellite raster image of presence probability for ticks. Others, independent variables are: slope, roughness, exposure, temperature and, soil humidity. We consider as study/sampling area only Northwestern Italy. The limited extend allows the model to make better inference on unsampled sites. Parasite's presence data are obtained from a parallel molecular study on *Babesia* which is detected by PCR in: chamois, red deer, roe deer, wild boar, fox, and wolf. Preliminary results confirm the mathematical vigour of the priors, and the good fitness of the model. Current dataset is being expanded for a more robust output. Application will allow to extrapolate predicted data to future climatic settings and to climatically similar areas.

[250] POST-MORTEM EVALUATION OF GAZELLES WITH PLEUROPNEUMONIA AND LIMB LESIONS

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Necropsy was performed on 12 gazelles which died in 2009-2012 in a local zoo. Animals were coming from various origins, like wildlife or semi-wild conditions like national parks or zoos. Age range was 1-3 years old and 5 were male, 7 were female. Deaths occurred mostly following transportation to indoor enclosures in winter. Extremity wounds healing slowly were the only clinical observation. Interestingly, necropsies of 8 gazelles revealed; severe fibrinopurulent pleuropneumonia which was characterized with necrotic, spheroid, well demarcated lesions of the lungs and fibrinopurulent thoracal effusions. Granulation and scab formation of the limb wounds was remarkable. *P. auriginosa* in one of these eight gazelles, *A. pyogenes* in another was isolated from the lungs. Both of the isolated bacteria are opportunistic pathogens causing purulent and rarely systemic infections and rarely reported in gazelles. *A. pyogenes* is reported to cause necrotizing pneumonia, mandibular osteomyelitis, peritonitis and abscesses in various body parts of some captive wild ruminants, of which gazelles are rarely included. The immunosuppressive effect of the captivity, induction of pulmonary diseases due to population density and indoor housing is considered to play a role in the evaluated gazelles. It reporting this common phenomenon observed in 8 gazelles might be helpful for wildlife health researchers or rehabilitators. Further researches in order to prevent this problem should be investigated.

[251] BLOOD-ON-FILTER-PAPER FOR CARIBOU SEROLOGY: DIAGNOSTIC EFFICACY AND PERSPECTIVES OF POTENTIAL SAMPLERS IN THE NORTH

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Serological testing provides clues to the overall health of individuals and populations, but it is no easy task to collect and preserve conventional blood samples from caribou (*Rangifer tarandus* ssp.) and other wildlife. What if there was an easier, scientifically robust way to obtain and handle blood specimens in arctic field conditions? Could such a tool be adopted by subsistence hunters and others, and thus expand the scope of disease surveillance? We examined these questions for caribou blood collection on Nobuto filter-paper strips (FP). Initial experiments probed the efficacy of this simple, practical sampling technique in eight antibody assays, incorporating challenges that northern field personnel and subsistence hunters might face, namely, freezing and storage time. Concurrent with FP validation, the CircumArctic Rangifer Monitoring and Assessment Network implemented FP testing in an unprecedented initiative to obtain a "health snapshot" of circumpolar herds during International Polar Year 2007-09. Although experiments demonstrated the scientific value of FP samples for caribou serology, the question of acceptance by subsistence hunters as a mechanism for ongoing surveillance remained fundamental. We conducted 29 on-site interviews in remote, primarily aboriginal communities across the Canadian Arctic and documented positive attitudes towards hunter-based FP sampling as well as some challenges. Results of the efficacy studies and interviews will be presented.

[252] *COXIELLA BURNETII* IS THE MOST PROBABLE CAUSE OF ABORTION IN A SPANISH RED DEER (*CERVUS ELAPHUS*) FARM

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In spring 2011 an abortion outbreak took place in a red deer farm in southern Spain. To determine the cause of abortion we collected vaginal swabs and sera from 25 females in the farm; 12 of them had aborted. Sera were analyzed by ELISA to detect antibodies against *Chlamydophila abortus*, *Toxoplasma gondii*, *Neospora caninum* and *Coxiella burnetii*. Vaginal swabs were analyzed by PCR to detect the presence of DNA from any of these pathogens. Serological results showed antibody prevalences of 32.0±18.3% for *C. abortus*, 8.0±10.6% for *T. gondii*, 0±0.0% for *N. caninum* and 36.5±18.8% for *C. burnetii*. Seroprevalence differed between aborted and non-aborted animals only in the case of *C. burnetii* (50.0±28.3% and 23.0±23.0%, respectively) but remained similar for *C. abortus* (33.3±26.7% and 30.8±25.0%, respectively) and *T. gondii* (8.3±15.6% and 7.7±14.5%, respectively). Vaginal swabs were only positive for the presence of *C. burnetii* DNA, whose prevalence was 15.0±19.6%. Interestingly, the 40.0±30.3% of aborted females shed *C. burnetii* in vaginal secretions versus the 15.4±19.6% of the non-aborted females. Access to aborted material to properly diagnose the cause of abortion was impossible because deer are farmed in semi-extensive conditions. However, the higher seroprevalence of *C. burnetii* in the aborted group in addition to shedding of *C. burnetii* in vaginal swabs suggests Q fever is the most probable cause of abortion in the deer farm.

[253] Q FEVER IN FARMED AND FREE-LIVING RUMINANTS IN THE CZECH REPUBLIC

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Q fever is a highly contagious zoonosis caused by the obligate intracellular bacterium *Coxiella burnetii* (*C. burnetii*). This agent can infect many species of domesticated and free-living animals. Farm animals are the major reservoirs of infection, and transmission to humans is mainly accomplished through inhalation of contaminated aerosols. Free-living animals may be a reservoir, too. Passive monitoring focused on Q fever prevalence in cattle herds in the area of the Czech Republic was initiated last year, but the situation in free-living ruminants is unknown. Therefore the objective of this study was to detect specific antibodies against *C. burnetii* in farmed and free-living ruminants in the territory of the Czech Republic. The examination was performed in following ruminants species: Mouflon (*Ovis orientalis musimon*), bezoar goat (*Capra aegagrus aegagrus*), roe deer (*Capreolus capreolus*), sika deer (*Cervus nippon*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and pere david's deer (*Elaphurus davidianus*). A total of 275 blood serum or plasma samples collected in 2008 – 2011 were examined for the presence of specific antibodies against phase I and II *C. burnetii* by the commercial ELISA kit. One blood sample was tested dubious, the others were negative. On the base of our preliminary results we can conclude, that seroprevalence of Q fever in free-living and farmed ruminants is currently very low. Further studies in this field will follow.

[254] THE EFFECT OF NEMATODE INFECTION ON REINDEER FITNESS DURING THE ARCTIC WINTER

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Macroparasites have a central place in population ecology because of their potential to regulate host populations through effects on reproduction and/or survival. However, although nematodes are found in almost all wild vertebrate populations, few studies have investigated host-parasite relationships in the wild, mainly due to logistical difficulties in studying this relationship in free-ranging populations in the field. Svalbard reindeer provide a unique study system because experimental studies and capture re-capture of individual animals is tractable in the long-term, allowing manipulation of parasite loads. This study attempted to quantify the impact of a winter-transmitted nematode, *Marshallagia marshalli*, on Svalbard reindeer fitness by using a novel delayed-release anthelmintic bolus, designed to remove nematodes in October. The experiment was replicated over 5 years (2007-2011). Animals were recaptured in February and/or April and body mass and pregnancy status were compared between the control and the treated group. Preliminary results suggest that treated animals had a higher body mass in comparison to controls in more severe winters, and that the proportion of pregnant older animals was higher in the treated group compared to the control group. These results indicate that *M. marshalli* has a negative impact on Svalbard reindeer fitness, and could therefore have a role in regulating the population dynamics of the host. This is, to our knowledge, the first study to test the effects of over-winter parasitism on a free-ranging wild animal.

[255] GASTROINTESTINAL PARASITE DIVERSITY OF WEST GREENLAND CARIBOU AND POSSIBLE DRIVERS OF PARASITE COMMUNITY STRUCTURE

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Gastrointestinal (GI) parasitism in wild ungulates is linked to effects on growth and, in severe infections, death, yet few comprehensive studies on these GI communities have been undertaken. We determined GI parasite diversity for two populations of West Greenland caribou (*Rangifer tarandus groenlandicus*); Akia-Maniitsoq (AM) and Kangerlussuaq-Sisimiut (KS). Quantitative parasitological examinations were done on the feces, abomasa, and small intestines of adult females (n = 81) and calves (n = 15). GI nematodes were identified using morphology and DNA sequencing. Our results indicate core differences in GI fauna. The abomasal nematodes *Marshallagia marshalli* and *Teladorsagia* spp., were found only in KS, with *Ostertagia gruehneri* solely in AM. Nematodirine eggs were found in KS adult fecal samples and in calves from both populations, but absent from intestinal washes. The divergence in parasite communities may be driven by numerous factors, including climate, colonization history, and contact with introduced species. *Marshallagia marshalli* and *Teladorsagia* spp. are typical of muskoxen (*Ovibos moschatus*) and may reflect sympatry of KS caribou with a translocated population; whereas spill-over from introduced Norwegian reindeer (*R. t. tarandus*), may account for *O. gruehneri* in AM. Our results identify differences in parasite diversity between these herds and suggest a possible role of introduced ungulates in driving parasite communities in this region.

[256] A NEARCTIC PARASITE IN A PALEARCTIC HOST: *PARELAPHOSTRONGYLUS ANDERSONI* INFECTING SEMI-DOMESTICATED REINDEER IN ALASKA

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Parelaphostrongylus andersoni is muscle-dwelling protostrongylid nematode that infects caribou and white-tailed deer across North America, causing significant muscular and pulmonary pathology. The objective of the present study is to report the presence of the muscle-worm *P. andersoni* in a herd of semi-domesticated reindeer on Seward Peninsula, Alaska, USA. We collected fecal samples (n=44) from reindeer of the Kakarak herd which ranges on western Seward Peninsula, and has no historical direct contact with native Grant's caribou of the Western Arctic herd. Samples were processed using the modified beaker Baermann technique, and 22.7% (n=10) were positive for Protostrongylidae dorsal-spined larvae (DSL). Genomic DNA was extracted from individual DSL, and amplified by PCR using primers NC1 and NC2 targeting the ITS-2 region of ribosomal DNA. All DSL were identified as *P. andersoni.* One of the greatest impacts and concerns of reindeer establishment in North America was, and still is, the transfer of *Elaphostrongylus rangiferi* to sympatric caribou, as happened in Newfoundland culminating in disease outbreaks. Instead, the Nearctic protostrongylid nematode *P. andersoni* occurs in introduced semi-domesticated reindeer; a Palearctic host, and might be widespread among reindeer herds in Alaska, potentially causing deleterious impacts on infected animals and economically affecting commercial herding activities.

[257] VALIDATION OF PYROSEQUENCING^{TM} FOR ASSESSING PARASITE BIODIVERSITY IN WILD NORTH AMERICAN UNGULATES: A TOOL FOR MANAGEMENT AND CONSERVATION

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Protostrongylids are pathogenic parasites of wild ungulates, causing pulmonary and neuromuscular diseases. First-stage larvae (L1) shed in feces are morphologically distinguishable to two groups: one with a dorsal spine at the base of the tail, DSL (Muelleriinae, Varestrongylinae, and Elaphostrongylinae) and the other lacking this dorsal spine (Protostrongylinae). Larvae of the eight species that shed DSL in North American ungulates are morphologically indistinguishable, posing a challenge for studying these parasites. As a consequence, there is limited fine scale knowledge on host range and distributions for these nematodes. Pyrosequencing^{TM} is an innovative DNA sequencing technique that relies on detection of pyrophosphate release on nucleotide incorporation, and its use has been growing in parasitology. Our objective is to develop and validate the Pyrosequencing^{TM} for rapid and accurate species-level identification of protostrongylid nematodes infecting wild ungulates across North America. Larvae of all eight species, isolated from different hosts and geographic areas, were subjected to DNA extraction and PCR targeting the ITS-2 region of the nuclear ribosomal DNA. PCR products will be used for testing and optimizing Pyrosequencing^{TM} protocol. This technique will be applied in large scale studies on protostrongylid biodiversity and also as a tool for health assessment in management and conservation of wild ungulates.

[258] LONG-TERM PROGRESSION AND COLOSTRAL TRANSFER OF BTV ANTIBODIES AFTER BTV-8 VACCINATION OF SOUTH AMERICAN CAMELIDS

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Bluetongue (BT) is one of the most important diseases of domestic livestock. South American camelids (SAC) are also susceptible to BT disease and could play a role as virus reservoirs. Vaccination of susceptible species is regarded as the method of choice for BT control. This is the first study monitoring the long-term progression of BTV serotype 8 (BTV-8) antibodies after vaccination of SAC with any one of the inactivated BTV vaccines BLUEVAC®-8, BTVPUR® AlSap 8 and Zulvac® 8 Bovis. Two of the three used vaccines had never been evaluated in SAC at all. All SAC vaccinated twice with 1–2 ml of vaccine (n=46) were seropositive in an ELISA for the duration of the study (11–18 months), while only 79% (15 of 19) of SAC seroconverted after a single vaccination. Good safety of the studied vaccines was reported retrospectively by the study participants and by most of the owners of another 282 SAC from which no samples were collected. Interference of colostral BTV antibodies with immunity after vaccination and BTV infection has been reported for juvenile ruminants. To set a vaccination regimen for crias, it is important to know how long passive immunity persists. Therefore, the progression of maternally-derived BTV antibodies in crias born from vaccinated dams was analyzed the first time using ELISA. Colostral BTV-8 antibodies in crias were detectable for 2–7 months after birth. This study provides vaccination recommendations to initiate an appropriate BTV-8 antibody response in SAC.

[259] ANTIBODY RESPONSE AFTER BLUETONGUE VIRUS VACCINATION IN SPANISH IBEX (*CAPRA PYRENAICA*)

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Wild and domestic ruminants are susceptible to bluetongue virus (BTV) infection, which is transmitted by *Culicoides* midges and is the responsible of bluetongue (BT). As susceptible hosts, wild ruminant species can play a relevant role in the transmission and maintenance of BTV. Spanish ibex (*Capra pyrenaica*) is a wild caprine endemic to the Iberian Peninsula, with fragmented populations distributed mainly through the south and east. In the last decades, the species has been threatened by habitat alteration, human disturbance, competition with other domestic or wild ungulates and infectious and non-infectious diseases. In the present study, a total of 100 Spanish ibexes, ranging from zero to fourteen years old, were used for a longitudinal serological analysis after vaccination against BTV serotypes 1 and 8. Thirty ibexes were intramuscularly vaccinated with a single dose of inactivated BTV-1 vaccine and thirty two with BTV-8 vaccine. From the remaining forty ibexes, sixteen were left as non vaccinated controls, whereas the rest were born during the study and included as negative controls. All ibexes were seronegative and RT-qPCR negative before BTV vaccination. ELISA and serum neutralization test were performed on serum samples obtained at 0, 1, 4, 12 and 18 months post-vaccination (mpv). Non vaccinated ibexes remained seronegative throughout the study, while both BTV-1 and BTV-8 vaccinated groups seroconverted from 1 mpv. No BTV RNA was detected by RT-qPCR in any blood sample of the vaccinated or non vaccinated ibexes during the study period. In conclusion, one single inactivated BTV vaccine dose induced protective antibody levels in Spanish ibex for at least eighteen month after vaccination.

[260] BLUETONGUE IN MOUFLONS (*OVIS MUSIMON*) IN THE CZECH REPUBLIC (2008-2012)

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Bluetongue virus (serotype 8, BTV-8) which causes bluetongue (BT), an insect-borne disease of ruminants, was in the Czech Republic first detected in 2007 and by 2009 a total of 14 outbreaks were identified there, all in cattle herds. Since Nov. 2011, the whole area of the Czech Republic is officially BT free. Mouflons *(Ovis musimon)* are susceptible to BTV infection and like other wild ungulates could serve as possible BTV reservoir. In this paper we present results of our investigations of BT occurrence in farmed and free-living mouflons in our territory. From 2008 until early 2012, we obtained a total of 340 serum, plasma or whole blood samples of this species. All samples were examined for the presence of specific antibodies against BTV by commercial competitive ELISA kit (ID Vet, France) and 25 of them were also tested by Bluetongue Early Detection ELISA kit made by the same producer. Twelve selected samples were examined by the RT-nested PCR for the presence of BTV-8 RNA. Of all the tested samples were 2 samples collected in 2012 serologically positive – the 1st was obtained from three-year old mouflon, which could attract the infection years ago, and the 2nd one came from a yearling, which was repeatedly tested within 2 months with negative results both in serology and the RT-nested PCR. As well the other 13 samples were tested negative by the RT-nested PCR. We can conclude that at this time BT doesn´t represent any serious health problem in Czech mouflon´s population.

[261] *ANAPLASMA PHAGOCYTOPHILUM* INFECTION IN ROE DEER (*CAPREOLUS CAPREOLUS*) IN SOUTHERN BELGIUM

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Anaplasma phagocytophilum is a tick-borne pathogen of veterinary and human importance. Several genetic variants are reported within this species and they show differences in pathogenicity, host reservoirs and geographic distribution. Up to now, little is known about the epidemiological cycles of the different *A. phagocytophilum* strains and their reservoir hosts. In Belgium, roe deer (*Capreolus capreolus*) is the most common and widely distributed deer species and the major feeding host for *I. ricinus*. So, this study was carried out to determine the infection rate of *A. phagocytophilum* in roe deer in southern Belgium. Through an active surveillance program, 97 wild hunter-killed roe deer were sampled in autumn 2010 and screened for *A. phagocytophilum*. Geographic distribution of the samples covered 16 forest districts known to shelter wild roe deer in southern Belgium. The DNA was extracted from spleen and a 16S rRNA nested PCR was performed. The first PCR amplifies a fragment common to all *Anaplasma* and *Ehrlichia* species while the second is specific for the *16 S rRNA A. phagocytophilum* gene. A total of 83 of the 97 (85,6 %) spleen were tested positive for *A. phagocytophilum* DNA and all the forest districts harbored at least one positive roe deer. These preliminary results show that roe deer are largely infected by *A. phagocytophilum* in southern Belgium. In a second step, it will be useful to determine if genetic variants of *A. phagocytophilum* present in wild cervids are pathogenic strains for humans.

[262] *FASCIOLOIDES MAGNA* INFECTIONS IN MUSKOXEN (*OVIBOS MOSCHATUS*) FROM NORTHERN QUÉBEC, CANADA

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Following the introduction of 55 muskoxen (issued from 15 founding individuals) in the mid nineteen-seventies, the free-ranging population of muskoxen (*Ovibos moschatus*) of Northern Quebec, Canada, is now estimated to be over 1400 animals. Experimental harvesting of this population started in 2006 and provided a good opportunity to assess the health of this introduced population. Hepatic lesions associated with the presence of giant liver flukes (*Fascioloides magna*) were reported in the muskoxen harvested during the first year. To better characterize these parasitic infections, 54 muskoxen livers were obtained from 2007 to 2011 and evaluated for the presence of *F. magna* and associated hepatic lesions. Intact or fragmented *F. magna* were found in 43 of the 54 livers (79.6%) examined macroscopically, with infection intensities varying from 1 to 40 trematodes per liver. These parasites were often associated with extensive lesions such as hepatic necrosis, cholangiocellular hyperplasia, cavitations of the parenchyma with adult trematodes and eggs, and multifocal to confluent areas of hepatic inflammation and fibrosis. This is the first report of *F. magna* in muskoxen. The potential impact of these parasitic infections in this unusual host is unclear. However, the steady growth of this population does not suggest a significant impact at the population level. Semi-quantitative evaluations of the extent of the hepatic damage are currently underway.

[263] LAYING IN WAIT: HOW ONE PARASITE HAS ADAPTED TO A MIGRATORY HOST IN THE ARCTIC

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Migratory escape is one possible benefit of long-distance migration of host species. Host migration may limit exposure risk by separating the host from the infective stages of the parasite; however, the parasite population is likely to adapt to a migratory host in ways that will, in turn, optimize its transmission. Using the barrenground caribou/*Ostertagia gruehneri* system as an example, we address two questions regarding the migratory escape hypothesis: 1) how do seasonal migrations allow caribou to 'escape' parasitism by *O. gruehneri*, and 2) how has *O. gruehneri* adapted its transmission strategy to a seasonally absent host. Through long-distance migration, barrenground caribou escape parasitism by physically separating themselves from areas of high environmental contamination and by reducing exposure risk by delaying infection until the next year. Seasonal changes in the speed and directionality of migration, as well as changes to group size, may additionally reduce exposure risk through alterations to the effective host density. At the same time, *O. gruehneri* has adapted its transmission strategy through increased larval inhibition, longevity, overwinter survival, and extreme seasonality. Spatial strategies of escape by the host may be overcome with temporal strategies of transmission by the parasite; therefore, the effectiveness of migratory escape can only be assessed when considering the host and the parasite simultaneously.

[264] PARASITE DIVERSITY AND MORPHOLOGY VARIABILITY

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CERoPath project investigates the structures of murine rodent communities, and their interactions with pathogens and macroparasites in South Asia. In this context, our study tries to understand the various patterns of rodent host morphology in response to the parasite load. We expected a potential resource allocation trade off between anti-parasitic defenses (estimated by spleen weigth) and “expensive” tissue (i.e. brain or testes). This trade off may induce divergence (on this organs) between individuals, population or specie, subject to a range of parasite pressure. Three rodent species has been studied: *Bandicota indica*, *Maxomys surifer*, *Rattus exulans*. Each side of an individual skull picture was digitalized. A morphometric analysis was based on 2D landmark and superimposed Procrustes method, giving information on brain and skull variation of our samples. We used as well, respectively spleen weight and testes length (as “expensive” tissue measurements) as indicators of the investments in immunity versus reproduction (data acquired in previous studies). We expected a link between parasitic diversity and investments in immunity on the one hand and consequence on brain and testes morphology. This work, will allow us better understanding processes contributing to the host-parasite associations observed.

[265] SPATIAL DISTRIBUTION OF *TOXOPLASMA GONDII* IN RODENT SPECIES

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Toxoplasma gondii is a protozoan parasite able to infect all warm-blooded animals. Among the intermediate hosts of *T. gondii*, rodents may serve as indicators for assessing the occurrence and level of environmental contamination. To study the spatial distribution of *T. gondii*, we captured 715 rodents belonging to eight species located in a rural area in France. The study area comprised two villages and the surrounding landscapes mapped for type of soil and observations of cats. Rodents were examined for the presence of *T. gondii* using PCR, mice inoculation and agglutination test for antibodies. Prevalences varied between 1.5% and 28.4%. Using the spatial mapping of serological results, we searched for spatial autocorrelation between positive cases, and links with the presence of cats around the village or specific landscape elements. We expected higher infection rates near the village due to a high level of cats' presence than outside the village. However, the infection was distributed evenly in the whole study area and no determinant of the spatial distribution was found. This indicates that in rural areas, *T. gondii* infection of intermediate hosts may occur at a distance from villages. This may be due to cats contaminating larger areas than expected or wide oocysts diffusion. This study highlights the existence of a wide rural soil contamination that can represent a risk for veterinary and human health.

[266] DETECTING EMERGING AND RE-EMERGING ZOONOTIC PATHOGENS IN RODENT SPECIES USING MICROARRAYS

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Emerging and re-emerging diseases have the potential to have a significant impact on public health, animal health and the global economy. Rodents can act as reservoirs for many zoonoses, some of which are highly pathogenic. The aim of this project is to develop and evaluate a microarray for the detection and identification of a number of zoonotic pathogens transmissible by rodents. Samples from urban centres across Europe and from Vancouver, Canada will be screened for pathogens using this tool. During the screening of field samples, sequences from positive results will be compared to the strains in humans in the same location. Probes were designed from nucleotide sequences of multiple pathogens using a variety of probe design software packages; these probes were then printed onto Alere ArrayTube microarrays. Evaluation of the probes is being carried out using nucleic acid derived from cultured pathogens, experimentally-infected animals and from natural infected animals. Sequence-independent PCR is being used to amplify pathogen nucleic acid and the product is hybridised onto the microarrays. Preliminary results are promising. Some probes however, show cross-reactivity with nucleic acids from other pathogens; these will be excluded from the final array, before screening of field samples begins. Work will now focus on evaluating the remainder of the probes to select the best ones for the final array product which will be used to screen the rodent samples.

[267] LEPTOSPIROSIS IN EUROPEAN BEAVERS (*CASTOR FIBER*) FROM SWITZERLAND

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The European beaver (*Castor fiber*) was successfully reintroduced to Switzerland in the 19th century. After a decade of thorough, systematic pathological examinations which did not reveal serious health concerns, surveillance intensity was reduced to the investigation of suspect cases selected by field partners. In 2010 three emaciated beavers (#1, 2, 3) found dead over three weeks (August/September) along 110 km shore of the Aare river were submitted for pathological examination. Gross findings included obvious yellow mucous membranes, multifocal lung hemorrhages and enlarged kidneys with occasional loss of demarcation between medulla and cortex. Histologically, lesions included acute severe pulmonary alveolar hemorrhages (#1, 2, 3), severe tubulo-interstitial nephritis (#1, 3) and mild interstitial nephritis with severe fibrosis (#2). An infection caused by *Leptospira* sp. was suspected and PCR analysis was performed on either kidney (#1, 3) or lung (#2) samples. The presence of *Leptospira* sp. was confirmed in the kidneys but not in the lung. All findings were consistent with a systemic *Leptospira* sp. infection. To our knowledge, this is the first outbreak in Swiss beavers and the first description of acute pulmonary hemorrhages in infected beavers, similar to those reported in humans and dogs with leptospirosis. The detection of this outbreak indicates that health surveillance of the beaver population is worth continuing and that the current system is successful.

[268] THE EFFICACY OF ANTHELMINTIC DRUGS IN FREE-RANGING EASTERN GREY KANGAROOS

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Effective, broad-spectrum anthelmintics are valuable tools for biologists conducting manipulative field experiments to examine fitness and sub-clinical effects of parasites on wildlife hosts. Eastern grey kangaroos (*Macropus giganteus*) defaecate throughout their foraging areas and are highly susceptible to parasitism by gastrointestinal helminths. We conducted two experiments on free-ranging kangaroos (n=42) at an urban site in southern Australia, treating animals with the anthelmintic drugs moxidectin (s.c, 1mg/kg, 2mg/kg), ivermectin (s.c, 1mg/kg) and albendazole (oral, 3.8mg/kg). We also measured environmental infective larval patterns and seasonal changes in faecal egg counts in the population. After treating kangaroos, we monitored faecal egg counts over time and assessed efficacy using faecal egg count reduction tests. Surprisingly, moxidectin and ivermectin had low efficacy, with reductions of only 76% and 51% respectively. However, treatment with albendazole reduced faecal egg counts by 100% in all kangaroos, and egg counts remained low for up to 3 months. The results suggest that macrocyclic lactones, at recommended dose rates, are much less effective against nematodes in kangaroos than has been reported for domestic herbivores. It is unclear if this is due to the pharmacokinetics in the host or low susceptibility in some of the strongyloid nematodes of eastern grey kangaroos.

[269] ISOLATION OF *SALMONELLA ENTERICA* NEWPORT AND CARRAU SEROTYPES IN FREE-LIVING GIANT ARMADILLOS IN THE PANTANAL REGION OF THE STATE OF MATO GROSSO, BRAZIL

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Giant armadillo (*Priodontes maximus*) is a vulnerable species (IUCN, 2010). This species is naturally rare in its natural habitats, and its biology and health aspects are little known. In Brazil, it is found in the Pantanal region, where wildlife shares space with extensive cattle ranching. The objective of this study was to assess *Salmonella spp.* and pathogenic *Escherichia coli* and their resistance to antimicrobials in giant armadillos captured in the Pantanal region. Rectal swabs of eight adults were collected and microbiologically processed. *Salmonella enterica* strains were serotyped at a Reference Center and *E. coli* strains were assessed for virulence genes by PCR. The antimicrobial susceptibility followed international standardization. *Salmonella* Carrau serotype, multiresistant, and *Salmonella* Newport serotype were isolated from two animals. *E. coli* strains didn´t show the markers of virulence searched, but they were all resistant to β-lactams. *Salmonella* Newport serotype is an important human pathogen and its main host is cattle. Recently, serotype Carrau was associated to tegu lizards in Brazil, a species commonly present in the biome studied. The participation of armadillos in the maintenance of salmonella in the environment, as well as the importance of these bacteria for the health of this species deserves further studies. Antimicrobial resistance in bacteria isolated from free-living animals may be related to their environmental interaction with farm animals.

[270] COMPARATIVE ECOIMMUNOLOGY OF WILD RODENTS

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Ecoimmunology aims to understand the extrinsic and intrinsic factors leading to species specific differences in the immune system and how these changes contribute to disease susceptibility in various environments. Over the past decades, the focus has been on avian species but few studies have been undertaken with wild mammals. Rodents belong to the most diverse order of mammals (making up over 40% of the diversity), and occupy various habitats ranging from tropical to sub-Antarctic and from rural to urban environments. The aim of our research is to investigate the immune function of wild rodents inhabiting diverse environmental gradients, and to test whether biotic factors (social organization, contact rates, parasite loads, and diet) and abiotic factors (distribution, home range size, temperature changes, and precipitation) correlate with or influence the inter-specific differences. We will use existing methods and reagents applicable to laboratory rodents to measure both cellular mediated and soluble parts of the innate and adaptive immunity. The results will help us to understand the evolution of immune function from wild rodents in diverse habitats which considering their role as vectors for diseases, will promote knowledge relevant to public health.

[271] WING INJURY CAUSED BY BACKPACK HARNESSES FOR RADIO TRANSMITTERS IN TAKAHĒ (*PORPHYRIO HOCHSTETTERI*)

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The takahē, the world's largest flightless rail, is an iconic species endemic to New Zealand. Once believed to be extinct, the species now consists of a population of just over 200 adults that is intensively managed in the remaining native range and on several predator-free offshore islands. In these populations, radio-transmitters are commonly used, attached to a backpack harness to enable practical tracking in harsh environments. Radio transmitters are used worldwide in various avian species, however there has been little investigation in the literature into their adverse physical effects. Concerning evidence has been reported from cases seen at the New Zealand Wildlife Health Centre, of free-ranging takahē with soft tissue and bony injuries associated with backpack harness attachment. In a preliminary sample of archived post-mortem takahē specimens (*n*=26), 62.5% of the birds that had worn a transmitter harness (*n*=16) had some degree of injury to their wings. These injuries ranged from superficial patagial erosion to deforming deep bony erosions in the distal humeri. From this small sample, there is an apparent positive correlation between duration of harness attachment and severity of wing injury. Further testing is required, but preliminary results show that the current harness system is unsafe for takahē to wear for extended periods and may lead to severe wing damage. These results have implications for all avian species that wear backpack radio transmitter harnesses.

[272] SURVEILLANCE FOR VARIOUS PATHOGENS AND LEAD IN AMERICAN BLACK DUCKS (*ANAS RUBRIPES*) FROM THE NORTHEASTERN AND MID-ATLANTIC UNITED STATES

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American black ducks (*Anas rubripes*) are an important game species of waterfowl in North America, but populations have been declining since the 1950’s. This study aimed to determine prevalence of various pathogens in black ducks. We sampled 206 black ducks from wintering grounds in Connecticut (n=20), Maine (n=37), Maryland (n=43), Ohio (n=8), Rhode Island (n=6), Virginia (n=5), and breeding grounds in Maine (n=87). We tested blood lead levels and infection with hematozoan parasites, avian influenza viruses (AIVs), avian paramyxovirus viruses (APMVs), duck viral enteritis (DVE) virus, and *Pasteurella multocida*. The average blood lead level was 170 ppb with two ducks having levels >2000ppb. Some of these levels are high enough to cause clinical signs, although no sampled birds had obvious clinical signs. AIVs were isolated from 3% (6/206), APMV-1 from 3% (6/206), and APMV-4 from 0.5% (1/206). Antibodies to AIVs were common (41%). Infection with AIVs and APMVs were expected and occurred at low prevalences. Sequence results from 5 APMV-1 isolates, showed these viruses were low pathogenic viruses. *Haemoproteus/Plasmodium* and *Leucocytozoon* were detected in 72% and 52%, of birds, respectively. No duck was infected with DVE or *Pasteurella multocida,* both of which are known to cause mortality in ducks. Future research is needed to evaluate the affects these pathogens, especially hematozoa, may have on the fitness of black ducks.

[273] HOW DO THE *GYPS* SP. (*G. RUEPPELLII* AND *G. AFRICANUS*) OF EAST AFRICA COPE WITH IRREGULAR AND UNCERTAIN ACCESS TO FOOD?

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In East Africa live two species of Griffon Vultures; Rüppel's Griffon Vulture (*Gyps rueppellii*) and African White-backed Griffon Vulture (*G. africanus*). One of the apparently most important factors limiting the population of these birds is food supply. Griffon vultures nest for eight months of the year, and are during this period geographically tied down to the nesting place. The living biomass in the East-African protected areas Masai Mara and Serengeti consists mainly of migrating ungulate populations which causes an irregular and unsure food supply for the Griffon vultures. This literature review investigates how Griffon vultures secure a sustainable food supply by developing certain anatomic, physiologic and ethological characteristics. Griffon vultures are large, diurnal and have good sight. Long necks and flat feet are adaptations for an easier opening of carcasses and spending long periods of time on the ground. Griffon vultures also elicit social traits such as local enhancement, nesting in colonies, and monogamy which saves energy. Building flimsy nests and bathing every day decrease risk of disease. The literature concludes that while strategies for successful foraging and diminished risk of acquiring and spreading disease are costly energy-wise, they benefit the whole population which shows in the fact that the populations are stable and the number of successful reproductions and nestlings that reach adulthood is high.

[274] TAKAHĒ TRANSLOCATION NETWORKS AND PATHOGEN SPREAD

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New Zealand has had a history of native species extinctions since human colonisation and the introduction of exotic animals. Conservation efforts are focused on several unique species still classified as critically endangered. Consequently, intensive management and translocations have become increasingly common practice. Limited funds available for wildlife conservation place restrictions on disease-based research, therefore epidemiological guidance is required to maximise targeted disease surveillance for pathogens of significance to conservation efforts. Social network analysis has increased its place in the field of epidemiology and has been applied to several human and animal disease modeling situations. We collated translocation records of a highly managed endangered New Zealand flightless rail, the takahē (*Porphyrio hochstetteri)* and applied social network principles to analyse connectivity of the network. Common descriptors, measures of centrality and fragmentation impacts have identified key locations for targeting disease surveillance and prioritising control measures in the event of a disease outbreak. The ease of use of this technique and the visual representation of movements in a population can aid decision making when considering translocation proposals. Translocation network analysis can provide an informative tool for disease ecologists to model pathogen transmission pathways and increase our understanding of the epidemiology of pathogens within an ecosystem.

[275] HOST-PATHOGEN COEVOLUTION WITHIN A SYMPATRIC SPECIES COMPLEX

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The *Platycercus elegans* parrot species complex of Australia comprises five phenotypically and genetically distinct subspecies with a geographical range that encircles unsuitable habitat. Populations at the western end of this “ring” show striking clinal variation in phenotype. The eastern end consists of two terminal subspecies that hybridize. The proximate and ultimate causes for this extreme variability among interbreeding populations is unclear. This unique species complex makes an ideal natural laboratory to study host-virus coevolution. Our aim was to utilize this and test the hypothesis that parasite-host interactions contribute to the diversity within this species. We screened over 600 individuals throughout the *P. elegans* range, over eight years for Psittacine Circovirus (PCV). PCV is a rapidly evolving ssDNA virus which is threatening parrot populations worldwide. Prevalence and intensity varied markedly, with hybrids significantly less infected than their parental subspecies. Together with preliminary genotyping data for the pathogen and host, our study shows the potential importance of host/pathogen relationships for speciation.

[276] DO REGIONAL LAND-USE GRADIENTS AFFECT PREVALENCE OF MICROPARASITES AND CO-INFECTION RATES IN BIRDS?

EWDA Student Travel Grant

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Macro and microhabitat alterations that come with land use change have been proven to decrease bird species diversity and favor vector-borne diseases in general. Although hemoparasites are not considered an important selective pressure on birds, single or multiple infections with protistan parasites and parasitic nematodes may serve a regulatory function if the costs that parasitism incurs to individual birds are considered. Currently, there are no reports comparing prevalence, infection status, and land use influences of the Piedmont and Coastal Plains of Georgia, physiographic regions with different vector activity and land use gradients. This study determined the prevalence and diversity of hemoparasite infection and co-infection rates in over 50 species of passerine and non-passerine birds. Common resident and migratory birds were captured at three sites in the Piedmont region and three sites belonging to the Coastal Plains region in one year during all four seasons. Five species of hemoparasites were identified (from taxonomic orders Haemospororida, Trypanosomatida and microfilaria). Significant differences in seasonality, species and infection status and co-infection rates between the sites were found, suggesting effects of temporal, spatial and taxonomical drivers of infection.

[277] THE IMPLICATIONS OF CRYPTIC PARASITE DIVERSITY FOR PARASITE CONTROL IN WILDLIFE, USING AUSTRALASIAN COLUMBIDS AS AN EXAMPLE

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Parasite control is often employed in intensive wildlife management, especially for threatened species. Protozoa are typically identified using morphology, and management plans are developed based on assumptions about the identity of these organisms. However, due to constraints on phenotypic evolution, gross morphological variation may not occur in even deeply divergent protozoa, the result being that parasite diversity can remain hidden until genotyping is performed. This was recently observed with the discovery of a significant endemic *Trichomonas* radiation in Australasian pigeons and doves. These organisms were previously assumed to be *Trichomonas gallinae*, which is associated with the introduced domestic pigeon (*Columba livia*). The diversity and prevalence of apathogenic endemic *Trichomonas* species in Australasian columbids suggest the possibility of ecological trade-offs for their maintenance, and it is likely that they confer cross-protection against virulent strains of introduced *T. gallinae*. It would be prudent to manage *Trichomonas* holistically with their host rather than pursuing eradication in intensive conservation programs of columbids in Australasia. The surprising cryptic diversity and likely endemicity seen in *Trichomonas* almost certainly reflects the situation occurring in other parasite lineages in wildlife. This phenomenon demands the use of parasite genotyping as an essential tool in the management of wildlife health.

[278] PARASITOLOGICAL AND MICROBIOLOGICAL FINDINGS IN ROOKS (*CORVUS FRUGILEGUS*) FROM ITALY

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The rook is an omnivorous bird (Corvidae) with Eurasian distribution that migrates from central-east Europe to winter in the southern European countries. Our aim was to investigate the presence of endoparasites and antibiotic-resistant bacteria in rooks wintering in Italy. In February 2011, 145 rook faecal samples were collected in a roost in north Italy (San Benedetto Po, Mantua). Parasitological examinations were conducted at the the Dept. of Animal Pathology of Pisa University on frozen samples using a centrifugal flotation with zinc sulphate. Microbiological examinations were conducted at Brno University to identify fluoroquinolone-resistant bacteria from these faecal samples, as part of a study on antibiotic resistant bacteria in rooks wintering throughout Europe. DNA, isolated from bacterial colonies growing on MacConkey agar with ciprofloxacin, was tested by PCR for Plasmid-Mediated Quinolone Resistance (PMQR) genes. From parasitological examinations, 67.5 % of the samples were positive to at least one parasitic species; a high prevalence was found for *Capillaria* spp. (51.3%), followed by coccidian oocysts (10.3%), Spiruridae eggs (5.1%), *Trichostrongylus* spp. (2.6%) and *Syngamus* spp. (0.8%). Ciprofloxacin-resistant Enterobacteriaceae bacteria were isolated from 6% of the samples. One sample was positive for PMQR gene *qnrB* identified as a new variant *qnrB49*. Migrating rooks may spread parasites and antimicrobial resistant bacteria over long distances.

[279] ENDOPARASITISM IN WILD BIRDS HUNTED FOR SUBSISTANCE IN A COMMUNITY OF THE PERUVIAN AMAZON

EWDA Student Travel Grant

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The aim of this study was to identify endoparasites of free-ranging wild birds hunted for subsistence by people of the Community Nueva Esperanza, located in the North-eastern Peruvian Amazon. Fifty-one wild birds including *Mitu tuberosum, Pipile cumanensis, Penelope jacquacu*, *Ara ararauna, Psophia leucoptera*, *Ramphastos* sp., *Crypturellus undulatus* and *Tinamous* sp., and 7 domestic fowl were collected. The prevalence of parasitic infections in wild birds was high 76.5% (n=39), showing a high probability in wild bird populations to be infected by parasites in this place (p<0.05). Eight nematodes, one trematoda and one coccidia were identified in wild birds. Most frequent parasites were *Heterakis* sp. (51%) and *Tetrameres* sp. (35%). *Ascaridia* sp., *Tetrameres* sp., *Capillaria* sp., *Spirurida* gen.sp. and *Oxyspirura* sp. were identified both in wild birds and domestic fowl, suggesting a possible cross-transmission. Besides, a few lesions caused by these parasites are described. Nine parasites have new host record.

[280] PRESENCE OF ECTOPARASITES FROM FREE-LIVING BIRDS IN ATLANTIC FOREST IN BRAZIL

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A primary goal of conservation medicine is the pursuit of ecological health or, by extension, the health of ecosystems and their inhabitants. The Atlantic forest is the second largest forest in South America; this biome is rich in an avian biodiversity. Nevertheless, the adaptive function of many intriguing features of avian morphology, physiology, and behavior are still uncertain. Some of these features are thought to play a role in defense against harmful ectoparasites. Birds are plagued by an impressive diversity of ectoparasites and many of these could have severe negative effects on host fitness. From January to December 2010, 98 free-living birds were caught using mist nets in the Atlantic forest remnants in southeastern Brazil. All birds were identified with aluminum leg bands, weighed, and examined for body condition, molting, ectoparasites, age group and body lesions. The presence of the *Amblyomma longirostre* (nymph) ticks in two birds (*Tachyphonus coronatus*, *Turdus amauricolinus*) and *Amblyomma* sp (larvae) in three species (*Vireo olivaceus, Coereba flaveola* and *Sporophila falcirostris)* was observed. Five percent (5/98) of the captured birds presented ectoparasites. This fact suggests that this low occurence could be expected in a balanced area. On the other hand, the presence of *Amblyomma longirostre* needs attention, once this tick could transmit some *Rickettsia* species that are capable of causing diseases in humans.

[281] FLAVIVIRUS ANTIBODIES IN YELLOW-LEGGED GULL (*LARUS MICHAHELLIS*) EGGS IN THE WESTERN MEDITERRANEAN BASIN

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In recent years, an unexpected number of flaviviral zoonoses have emerged worldwide. The goal of this study is to help clarify flavivirus epidemiology by providing data about viral exposure in Western Mediterranean. We studied antibody prevalence against flaviviruses in the eggs of an abundant and widely distributed colonial waterbird, the yellow-legged gull (*Larus michahellis*). This species is interesting because previous studies have revealed that it is naturally infected by different flaviviruses. Samples were collected from 19 breeding colonies in Algeria, France, Spain and Tunisia. Samples were screened for antibodies against flavivirus by using a commercial ELISA kit. These analyses were followed by a neutralization test to determine if the antibodies were specific to West Nile virus, Usutu virus or tick-borne encephalitis. Over the three years of study, strong differences among colonies were detected. Across all colonies, the only positive eggs were found in the Medes colony (Spain); the colony demonstrated high prevalence levels over time. Out of all of these positive samples, only one presents antibodies against West Nile virus. No other sample is identified positive against Usutu virus or tick-borne encephalitis. The results of this broad-scale study suggest that gulls may be locally important in flavivirus circulation. However, we need to identify the specific virus associated with the detected antibodies in order to discuss possible public health implications.

[282] ANTIMICROBIAL RESISTANCE PROFILES IN *E.COLI* STRAINS ISOLATED FROM SYNANTHROPIC BIRDS IN SPAIN

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Antimicrobial resistances pose an emergent risk for environmental and public health, due to their widespread and high mobility capacities. Despite antimicrobial resistances have been studied in different hosts including wild birds, few works are available about synanthropic and urban birds. The present work describes the antimicrobial resistance profiles from 169 *E. coli* strains obtained from different bird species (feral pigeons, hybrid ducks, house sparrows, spotless starlings and white storks) from humanized environments (urban center, urban parks and cattle farms). Briefly, a total of 17 antimicrobials and/or combinations were tested by the diffusion method. An overall 33.73% of E. coli strains showed any resistance (60% of white storks, 50% of spotless starlings, 47.62% of hybrid ducks, 28.92% of feral pigeons and 14.81% of house sparrows). As opposite as expecting, synanthropic birds do not carry a high prevalence of antimicrobial resistance *E. coli* strains. However, some strains showed a high proportion of antimicrobial resistances, mainly found in spotted starlings. Due to the high distances that spotted starlings can cover, the role of this species may be relevant in order to spread antimicrobial resistances.

This work has been cofinanced by the agreement MARM-INIA CC08-C20 and the project CAM S2009/AGR-1489.

[283] BORRELIA GARINII AND FRANCISELLA TULARENSIS SUBSP. HOLARCTICA DETECTED IN MIGRATORY SHOREBIRDS IN PORTUGAL

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Migratory shorebirds use, among many, the East Atlantic Flyway that links breeding areas as north as Tundra habitats to aquatic wintering grounds in West Africa. As a consequence, they are potentially important in the spread of global zoonotic diseases transmitted by ticks, such as Lyme borreliosis and tularemia—two diseases previously detected in Portugal. In this study, we looked at the infection status of seven populations of shorebirds during their migration, breeding, or wintering in the Portuguese wetlands to access if they carry these pathogens and to discuss their potential risk in the Portuguese wetlands. A total of 212 migratory shorebirds captured in the Tagus and Sado estuaries; key staging and wintering sites in this flyway and important breeding areas for some species were analyzed for the presence of *Borrelia burgdorferi* sensu lato and *Francisella tularensis*. In the present study, *B. garinii* was identified in seven (3%) specimens (five black-tailed godwits *Limosa limosa*, one common redshank *Tringa totanus*, and one little stint *Calidris minuta*), whereas *F. tularensis* subsp. *holarctica* was identified in one (0.4%) little stint. To our knowledge, this is the first evidence that shorebirds that migrate through or winter in Portugal transport these pathogens, potentially contributing for their introduction along the flyway, including the Mediterranean region.

[284] *CAMPYLOBACTER* AND *SALMONELLA* PREVALENCE IN YOUNG GREATER FLAMINGOS (*PHOENICOPTERUS RUBER ROSEUS*) IN SPAIN

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Campylobacter and *Salmonella* are the leading cause of zoonotic bacterial enteric infections. Due to their great mobility, migrating birds can act as effective spreaders of pathogens through fecal contamination of the environment. A cross-sectional study was conducted to determine the prevalence and antimicrobial resistance of *Salmonella* spp and *Campylobacter* spp in the three most important greater flamingo (*Phoenicopterus ruber roseus*) colonies in Spain. A total of 327 chick flamingos (100 to 114 per colony) were sampled during the ringing operations on summer 2011. No *Salmonella* was isolated from chick flamingos, but similar *Campylobacter* prevalences, ranging from 14% to 15%, were found in the three colonies. All *Campylobacter* isolates were identified as *C. coli*. Susceptibility to seven antimicrobials was tested, and resistance was only detected to nalidixic acid and/or ciprofloxacin in three isolates from one of the sampled colonies. Contact with the parents or other sympatric species, food and water are possible transmission routes implicated in *Campylobacter* spp infection in chick flamingos. Results indicate that greater flamingos can act as carriers of *Campylobacter* spp but not of *Salmonella* spp and as a migrating species, may contribute to the widespread of this zoonotic agent. Also, they can be of certain importance in the spread of antimicrobial resistant bacteria, which may be relevant for public health.

[285] CORONAVIRUS IN MAGELLANIC PENGUINS (*SPHENISCUS MAGELLANICUS*) IN BRAZIL

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Coronavirus infections in avian species may cause Infectious Bronchitis (IB), which has an important impact on poultry industry due to morbidity and losses in reproductive efficiency. IB infection usually presents clinical signs and pathological alterations involving the respiratory, genitourinary and digestive systems, however systemic disease is not uncommon. Prevalence of antibodies against Infectious Bronchitis Virus has already been described in free-ranging rockhopper penguins (*Eudyptes chrysocome*), but so far there is no report of clinical disease in penguins. The aim of this study was to determine the occurrence of IB viruses in recently-caught and captive Magellanic penguins (*Spheniscus magellanicus*). We used RT-PCR to detect coronavirus in cloacal swabs of 24 penguins undergoing rehabilitation from oil fouling and 32 wild-born captive penguins, and in macerated kidney samples of 65 penguins found dead on beaches or deceased during rehabilitation, in a total 121 samples. Coronavirus was successfully detected in 10% (13/121) animals: 5 swabs from live penguins (1 from captivity and 4 from rehabilitation) and 8 macerated kidney samples from deceased penguins (3 from beach carcasses and 5 from rehabilitation). Further studies are recommended to determine the prevalence and potential impacts of this virus in free-living populations of this migratory bird.

[286] HOW MANY SAMPLES ARE NECESSARY TO DETECT BLOOD PARASITES IN MAGELLANIC PENGUIN BREEDING COLONIES?

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Blood parasites have not yet been reported in free-ranging Magellanic penguins (*Spheniscus magellanicus*). There is however great concern on whether these parasites, known to be highly pathogenic for captive penguins, could pose a significant conservation threat. We used a probability formula to calculate the sample sizes required to achieve 95% flock sensitivity under different population size scenarios (50, 700, 5000, 35000, 400000 individuals), test sensitivity (40%, 50%, 60%, 70%, 80%), test specificity (99%), and expected parasite prevalence (0.5%, 1%, 5%, 15%, 85%). Expected prevalence was the most critical parameter, as the required sample sizes could range from 8 individuals (85% prevalence) to 250 individuals (0.5% prevalence) for a given population and diagnostic test. Test sensitivity influenced the required sample sizes but to a relatively small extent (in the worst case, an increase of 44 individuals would suffice to compensate for poor test sensitivity). Population size had a negligible effect for population size ≥ 700 individuals, which constitute the majority of Magellanic penguin colonies. In all scenarios, 250 animals would suffice to state that blood parasites did not occur at prevalences ≥ 0.5% in a penguin population, even if diagnostic tests performed poorly. It is likely that *Plasmodium* distribution is highly heterogeneous across fine spatial and temporal scales, and sampling should be stratified at breeding colonies with mixed landscapes.

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[287] EXPERIMENTAL EXPOSURE OF VARANIDS TO AN ENVIRONMENTALLY RELEVANT MIXTURE OF POLLUTANTS: THE ORGANOPHOSPHATE PESTICIDE CHLORPYRIFOS-ETHYL THREATENS HERPETOFAUNA

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The present study aimed at understanding absorption and accumulation of environmental contaminants in varanids and how far they are affected. The usefulness of non destructive sampling was also evaluated. 26 experimental savannah monitors (*Varanus exanthematicus*) were orally exposed during 6 months to a mixture of lead, DDT and chlorpyrifos-ethyl (CPF), or to the vehicle only. Proportionally to their body mass, exposed monitors received the same environmentally relevant dose. Individuals surviving contamination were euthanized after 4 or 6 months of treatment. Several tissues were analysed for lead by atomic absorption spectrophotometry and for DDT and CPF by gas chromatography. Exposed monitors readily absorbed all three pollutants; CPF killed 10 individuals but only lead and DDT (plus its main metabolites) accumulated. Clear correlations were identified between the quantity of lead or DDT administered during the whole study and their final concentrations in tissues. The use of tail clips and skin samples as non-destructive indicators of poisoning by lead and organochlorine pesticides (respectively) are recommended. Although varanids withstand lead and DDT contamination without conspicuous effects, our results show CPF can be lethal at very low doses and emphasize the importance of considering all taxa in impact assessment studies, including reptiles. This work also strongly suggests that monitors should be used as relevant indicators of environmental pollution.

[288] SPOTTED FEVER GROUP RICKETTSIA SPECIES AND *ANAPLASMA PHAGOCYTOPHILUM* IN LIZARD TICKS, ALGERIA

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Zoonotic pathogens, which reside in animal reservoir species and may at times spill over into human populations, are emerging at an unprecedented rate. Among these pathogens, several vector-borne pathogens have garnered considerable attention for the toll they exact on human health, which a growing body of evidence indicates may be exacerbated by anthropogenic environmental change .A rigorous understanding of the transmission dynamics of pathogens from infected wildlife hosts to vector organisms is critical to explorations of the ecology of vector-borne diseases. For this occasion we did our research about identification of different pathogens coming from wildlife. Our study was conducted between 2008 and 2009 covers about the existence of agent pathogens from Lizards' Ticks in the Park National El-Kala. 422 Nymphs and Larval of *Ixodes ricinus* were collected from 184 lizards (*Psammodromus algirus, Lacerta pater, Podarcis hispanica vaucheri*) in altitude forest in North-East of Algeria. Among the three species of Lacertidae studied, *Psammodromus algirus* (the most important species in the park) were the lizard who occur the highest intensity and prevalence of ticks (*Ixodes ricinus*), and the highest prevalence of infected ticks too. *Rickettsia helvetica*, *Rickettsia monacencis*, and *Anaplasma phagocytophilum* were detected in 185 lizards' ticks using a MassTag multiplex Polymerase Chain Reaction assay (PCR). These findings provide insights regarding the distributions of potentially pathogenic and emerging tick-borne agents, and raise the possibility of agents transmitted to the human by ticks where the reservoir can be our biological model. According to the number of rickettsia transmitted by ticks, our results could not be ignored (especially since our research is pioneering in the sense that it was for the first time that Rickettsia were detected in ticks from lizards in Algeria). This would lead us to assume a movement of diseases and vectors in an area without borders, which enables man to face a risk of contamination, especially those related to wildlife.

[289] COMBINED HEMATOLOGY AND IMMUNOLOGY: A BREAKTHROUGH TO ASSAY HEALTH STATUS OF STRANDED SEA TURTLES

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The loggerhead sea turtle is considered endangered by the IUCN and its populations have declined worldwide in consequence of human interactions, environmental pollution and various pathogens. Hematology and plasma biochemistry are among the most essential diagnostics to facilitate specific medical management and treatment that stranded sea turtles require. Immune testing provides an additional diagnostic tool to better understand the competence of the immune system in stranded animals and to help in their management during rehabilitation. The goals of this study were to assay stranded sea turtles' immune functions, once every two weeks over two months, by measuring changes in innate and adaptive immune functions, to correlate them to hematologic parameters and to the clinical improvement. Six studied sea turtles were categorized into control, trauma and debilitated animals. Hematologic parameters, including PCV and leukocytes demonstrated improving trends during rehabilitation. Simultaneously, lymphocyte proliferation and phagocytosis differed significantly among animals and over time, which indicates varying levels of adaptive and innate immune function involvement during rehabilitation. Natural killer cell cytotoxicity of immature loggerheads was similar to values obtained of healthy immature captive loggerheads. Large number of sea turtles for each different stranding category is indicated to determine the exact immune function trends during rehabilitation.

[290] TEMPORAL CHANGES IN THE PREVALENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN TEMPERATE MIXED SPECIES AMPHIBIAN ASSEMBLAGES

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Batrachochytrium dendrobatidis (*Bd*) is an amphibian pathogen that has received global recognition as a fundamental driver underpinning amphibian declines worldwide. Heralded by the Amphibian Conservation Action Plan as 'the worst infectious disease ever recorded among vertebrates' this extraordinary organism is known to infect amphibians of over 450 species and can be found in almost every habitat in which it has been sought. Taking into consideration, also, that amphibians are frequently to be found within mixed species communities, this presents an enormous diversity of ecological systems within which to study the epidemiology of this pathogen. In 2010 we undertook an intensive regime of sampling at 8 mixed-species breeding sites in the UK, all of which had a history of infection with *Bd*. The primary aim was to gain a greater understanding of *Bd* dynamics at system level, but with a wider goal of identifying trends which might be more generally applicable. Specifically, our objectives were (1) to identify differences in infection prevalence between UK species and (2) to identify differences in infection prevalence through time. To achieve this, each of the 8 selected sites was surveyed on 5 separate occasions during the breeding season (March-June). At each time point, the surveyor was tasked with catching and sampling the maximum number of metamorphosed amphibians as was possible, within a 6 hour time frame. Samples for *Bd* were collected by swabbing the skin with a dry-tipped rayon swab and were subsequently analysed using rtPCR. In total, over 2,500 samples were collected, representing all 6 UK native species and 2 introduced species of amphibian. Primary analyses suggest a number of significant results, including both temporal and species components of *Bd* infection. The final results of this study will be presented and their implications for *Bd* surveys of temperate, mixed species amphibian assemblages will be discussed.

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[291] ENHANCING ECOLOGICAL CONNECTIVITY IN THE ALPS: A CATCH-22 SITUATION IN RESPECT TO DISEASE SPREAD?

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Conservation of the Alpine biodiversity during the past 100 years has been driven by a “protected areas” approach - isolated reserves, separated from the rest of the Alpine space. However, in today's increasingly human-dominated landscapes and in the face of global climate change this approach is being revised. Conservation efforts aim at preserving and restoring a permeable landscape-matrix where the movement of flora and fauna is not hampered by barriers through the implementation of ecological networks. Conventions, such as the CBD, the Alpine Convention and directives like the “Habitat Directive”(92/43/EEC) and “Water Framework directive”(2000/60/EC), emphasize the importance of the ecological networks. Additionally, numerous EU-funded projects and initiatives (e.g. Green Infrastructure Initiative) strive to enhance ecological continuity across the Alps and Europe. While landscape-level connectivity clearly benefits biodiversity it inherently facilitates the exchange and movement of pathogens. For example an enhanced continuum from western Austria towards Switzerland will quite possibly accelerate the spread of tuberculosis in wildlife. It appears essential that the notions of potential disease emergence hotspots and pathogen dispersal are firmly integrated into these European programs. A clear understanding of the interaction of pathogens and enhanced ecological connectivity appears critical to human health and economic well-being in the Alps and beyond.

[292] WILDLIFE POPULATION MONITORING AS A KEY COMPONENT OF WILDLIFE DISEASE SURVEILLANCE

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Wildlife disease monitoring consists in the systematic recording of epidemiological data, with no other specific purpose than detecting temporal trends. Ideally, disease data should integrate with data on host abundance and distribution but unfortunately, this information is often lacking or fragmentary. As a noteworthy exception to this role, we present the example of Aragon, a 47,719 km^2 large region in Spain, where wildlife species were monitored for the last 20 years. Data on kilometric abundance indices were recorded in 63 localities with a total of 176,951 km covered. Hares showed a global decreasing trend. The 1998 hare abundance peak coincided with a peak of hare-linked human tularemia cases. The European wild rabbit recovered over the studied period after an historical decline due to myxomatosis and the more recent rabbit hemorrhagic disease in 1988-89. Canids such as stray dogs and foxes experienced a significant decline, possibly due to the combined effects of closing rubbish dumps and improved domestic ruminant carcass disposal after the BSE crisis. Contrary, Eurasian badgers (a suitable host for *Mycobacterium bovis*) increased significantly in abundance and distribution. Three ungulates showed variable time trends. This can affect animal health through effects on vector or host availability. In summary, the monitoring scheme allowed identifying short and long-term population trends that significantly improve our understanding of wildlife disease dynamics.

[293] A ZOO-BASED WILDLIFE SURVEILLANCE SYSTEM FOR AUSTRALIA

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Australian zoos play an important role in caring for native wildlife. Every year, thousands of free-ranging wildlife cases are submitted to Australian zoos for treatment, rehabilitation and, where possible, release. Nine of the major zoos in Australia see an estimated 17,000 wildlife cases each year, ranging from amphibians to reptiles, birds, marsupials and other mammals. Wildlife surveillance provides valuable information on existing, new and emerging diseases which may have significance to human and domestic animal health, global trade and biodiversity conservation. In 2010, the Australian Wildlife Health Network and the Zoo and Aquarium Association established a pilot project to investigate the usefulness of zoo-based wildlife data to national surveillance. Vets from six participating zoos selected wildlife cases from six categories of interest and entered data into an electronic database for 12 months. Vets were provided with training on appropriate case selection and data entry. An independent review determined the pilot project captured significant data, which expanded the range of species and locations covered by the Australian wildlife disease surveillance program. It is recommended that the program be supported to continue long term.

[294] SET-UP OF THE EPIDEMOLOGICAL WATCH PROGRAMME FOR WILDLIFE IN ANDALUSIA (SPAIN)

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Based on what was established in the Regulation for Hunting Organization, the Regional Environmental Ministry of the Regional Andalusian Government set up in 2009 the Epidemiological Watch Programme for the Wildlife in Andalusia (PVE), whose objective is to carry out the monitoring of the state of health of wild species, detect the appearance of diseases, determine disease prevalence, and establish together with the Regional Ministries of Agriculture, Fishing and Health, the pertinent intervention measures, either for disease prevention, fighting or control. The Programme has 15 specific protocols for species or groups of species, including the following game species: red deer, fallow deer, roe deer, wild boar, rabbit, red-legged partridge, Spanish ibex, mouflon and Barbary sheep. The official laboratories for the analysis and diagnosis of the diseases which are the objective of the study are: the Cordoba Laboratory for Animal Production and Health (Regional Agricultural and Fishing Ministry), the Campanillas, Malaga Laboratory for Animal Production and Health (Regional Agricultural and Fishing Ministry), the Central laboratory for Animal Health of the Environmental, Rural Life and Marine Ministry in Algete (Madrid), and the Center for the Analysis and Diagnosis of Wildlife (CAD, Andalusian Regional Government – AMA, Agencia de Medio Ambiente y Agua). Since it was set up in September 2009, different samples have been taken from 3264 animals (576 red deer, 147 fallow deer, 104 roe deer, 758 wild boar, 716 rabbits, 456 red-legged partridges, 398 Spanish ibex, 70 mouflons and 39 Barbary sheep), and 100% of the planned analysis have been carried out by February 2012.

[295] TRADING IN PATHOGENS: WILDLIFE MARKETS AND DISEASE EMERGENCE

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The wildlife trade has contributed to the emergence of a suite of diseases including SARS, plague, and monkeypox virus. Animal movement, crowding, and dietary and behavioral changes are conditions inherent to the wildlife trade that align with known drivers of disease emergence. These types of trade and market conditions alter natural contact rates and transmission pathways among individual animals, species, and the commensal and pathogenic organisms they carry, thus artificially creating new opportunities for both pathogen evolution and pathogen range expansion (geographic and host species). Pathogen evolution and persistence within the trade is not well understood, but it is generally perceived as less important than pathogen range expansion, as basic ecology and evolutionary principles dictate that pathogens should not kill their hosts prior to infecting other individuals. The problem with that perception is that it does not take into account the turnover rate of new potential hosts in the wildlife trade, which may provide a compensatory mechanism to allow for higher prevalence and virulence of pathogens. Here we modify a classic SIR compartment model to show that turnover rates in market settings alter disease prevalence and select for more pathogenic viruses. We found that the prevalence of both background viruses and introduced, more pathogenic, viruses increased during market simulations with turnover rate of less than a month. High turnover rates also allowed these invading viruses to expand into niches that were occupied by the background viruses at low turnover rates. Our results suggest turnover in the wildlife trade disrupts patterns of pathogen evolution and persistence and may be a strong driver of zoonotic disease emergence.

[296] BOVINE TUBERCULOSIS SURVEILLANCE IN WILDIFE (FRANCE)

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Bovine tuberculosis (TB) is a priority disease in France in terms of economic impact on the farming industry. France holds the TB free status according to EU standards, but in recent years new clusters of outbreaks have occurred in several geographical areas where wild ungulates and badgers were also found infected, whereas some aspects of their epidemiological role are still poorly understood and under discussion. The French Ministry for Agriculture and the main institutions involved in wildlife health launched in September 2011 a national surveillance program for TB in wildlife, named Sylvatub. The main goal of the program is to estimate the prevalence of TB in wildlife. It relies on a combination of several passive and active surveillance protocols which are applied according to the estimated risk levels in each department or area of the country. Passive surveillance is based on (i) detailed pathological game inspection, (ii) the SAGIR network (monitoring of dead or dying animals), and (iii) the TB detection of road-killed badgers. Active surveillance on deer, wild boars and badgers is implemented in delimited geographical areas considered at high risk. The poster will present the different implemented monitoring methods, the functioning of the system and some intermediate results (data collection and analysis are still in progress).

[297] NEW BAITS FOR NEW TIMES: MIXTURES OF XENOBIOTICS ARE POISONING WILD ANIMALS IN S.W. SPAIN

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Poisoning of wildlife is a widespread environmental problem, not only in Spain but also in the whole European Union. All wildlife species have been potential victims of accidental or deliberate poisoning across SW Spain during the last years, according to the experience of the Toxicology Group of Cáceres, thus rendering criminal poisoning a serious threat for animals and ecosystems. During many years, aldicarb constituted the most important pesticide to be used in baits for wildlife, according to how easily it was to obtain it for agricultural purposes. Nevertheless, when aldicarb was banned by the EU on environmental grounds, a general evolution in bait was observed, carbofuran being the most abundant pesticide. Similarly, in 2008 the EU agreed to ban carbofuran insecticide, and so the composition of baits in SW Spain evoluted. Even if carbamate-based baits are still detected, new mixtures have appeared, indicating a general knowledge of the toxic effect of pesticides to animals: rodenticides (bromadiolone+brodifacoum), organophosphorus (fenthion + chlorpyrifos or dimethoate, diazinon + chlorfenvinphos), insecticides with barbiturates (chlorpyrifos + phenobarbital), or even three different families of insecticide compounds (metomile + chlorpyrifos + endosulphan). This evolution must be taken in account, in order to prevent future incidents of wildlife poisoning, as those mixtures has an increased toxic potential at lower doses, rendering chemical analysis of great interest.

[298] DISEASE INVESTIGATIONS IN EUROPEAN WILD CATS (*FELIS SILVESTRIS SILVESTRIS*) FROM GERMANY

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In Germany the European wild cat (*Felis silvestris silvestris*) was almost brought to extinction in the early 20th century and still today it is considered a very rare animal species. Despite short-term increase in population numbers in a long term trend they will be threatened by marked population decrease particularly as suitable habitats are missing. To improve the national situation of the European wild cat an action plan is currently set into place to provide migration corridors to connect the fragmented populations. One of these populations is located in the southern Harz Mountain area in the center of Germany. Forty-four of mostly road-killed wild cats from this region were collected between 2006 and 2011. Carcasses were investigated for macroscopic and microscopic pathological lesions and tissue samples were collected for microbiology investigations. Virology examinations of organ tissues included feline calicivirus, feline coronavirus, feline immunodeficiency virus, feline herpesvirus, feline leukemia virus and feline parvovirus. Moreover, tissue samples from almost half of the animals were also examined for the occurrence of *Toxoplasma gondii*. Additionally, bacteriology investigations were performed on tissue samples with suspicious macroscopic lesions.

[299] METAGENOMIC ANALYSIS OF THE VIRAL FLORA OF PINE MARTEN AND EUROPEAN BADGER FECES

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Emerging pathogens are a major threat to public health, with animals - particulary wild animals—being an important source of emerging infections. Viruses such as severe acute respiratory syndrome (SARS) coronavirus and pandemic H1N1 (2009) influenza A virus were of zoonotic origin and caused severe epidemics among humans. Therefore, it is important, next to surveillance of known pathogens, to determine virus diversity in wildlife. In this study, next-generation sequencing was used to gain insight in the fecal viral populations from wild pine martens (*Martes martes*) and European badgers (*Meles meles*) in The Netherlands. Rectal swabs of four pine martens and three badgers were processed by large scale molecular virus screening, based on host nucleic acid depletion, viral nucleic acid isolation, sequence-independent amplification and next-generation sequencing. We demonstrated a new anellovirus and bocavirus species in the feces of pine martens and a new circovirus-like virus and geminivirus-related DNA virus in a European badger. Additionally, sequences with homology to viruses from the families Paramyxo- and Picornaviridae were detected. The discovery of new viruses in the feces of wildlife emphasizes the expansion of our knowledge of virus diversity in animal reservoirs. Furthermore, we provide a relatively simple, unselective technology to identify new viral species.

[300] EXPERIMENTAL INFECTION OF STRIPED SKUNKS (*MEPHITIS MEPHITIS*) WITH LOW PATHOGENIC AVIAN INFLUENZA VIRUS

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Striped skunks (*Mephitis mephitis*) are commonly associated with agricultural operations and suburban areas in the U.S. and are also susceptible to infection with some influenza A viruses. However, the viral shedding capability of this peridomestic mammal and its potential role in influenza A virus ecology is largely undetermined. In this study, striped skunks were experimentally infected with an H4N6 avian influenza virus (AIV) and monitored for 20 days post infection (DPI). All exposed skunks shed large quantities of viral RNA, as detected by real-time RT-PCR, from nasal washes and oral swabs. Following necropsy on 20 DPI, low quantities of viral RNA were detected in select tissue samples. All treatment animals yielded evidence of a serological response by 20 DPI. Overall, these results indicate that striped skunks have the potential to shed large quantities of viral RNA through the oral and nasal routes following exposure to an AIV. The peridomestic nature of these animals, the duration of shedding observed, and their presence in aquatic habitats, poultry operations, and human habitations could potentially influence influenza A virus epidemiology in key situations.

[301] ELIMINATION OF FOCAL RABIES OUTBREAKS: OPTIMISATION AND ECONOMICS

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In Western Europe we are unique in that classical rabies has now been eliminated with the use of oral vaccine baits targeted at the local reservoir, the red fox, and terrestrial rabies elimination now seems a possibility in many areas. The (re)introduction of rabies into rabies-free areas will however remain a risk, particularly where the local host population is at a relatively high density. Here I examine the UK situation, where we have been free of terrestrial rabies for about a century, but have maintained a continuous contingency plan. The UK situation is now much more similar to Western Europe and other rabies-free areas, with a relatively high host density. I use mathematical models to look at the choice of control method (culling, vaccination, or vaccine plus fertility control) and highlight the differences between them in terms of efficacy and logistics. I then present results from a simulation model that looks at the economics of rabies control in a single host species in both rural and urban areas of southern England as a model for any rabies-free area. The work results in two or more rabies control strategies, there efficacy (mean time to achieve disease control) and their cost. This work highlights the need for a planned and rapid response to an outbreak of wildlife rabies and an understanding of the approach required to determine the optimum strategy for rabies control, and the costs of the different options.

[302] SQUAMOUS CELL CARCINOMA IN TWO LIONS FROM THE SERENGETI NATIONAL PARK, TANZANIA

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Squamous cell carcinomas (SCC) are common neoplasms of domestic cats (*Felis cattus*) but rarely reported in free-living exotic felid species. We describe and discuss two cases of SCC in wild female lions (*Panthero leo*) from monitored populations in the Serengeti National Park, northern Tanzania. In both cases, the neoplastic lesions presented initially as minor proliferative nodules at the muco-cutaneous junction of the lip and progressed to become severe, ulcerative and destructive lesions which markedly extended into surrounding tissues. A pathological fracture of the underlying mandible was observed in one case. On microscopic examination, the tissues showed histopathological changes diagnostic of squamous cell carcinoma including expansion of the epidermis and invasion of the dermis with trabeculae and nests of poorly differentiated neoplastic epithelial cells, keratin pearl formation and marked epidermal ulceration accompanied by a severe suppurative inflammatory response. Solar elastosis was not a feature in either case. To the authors' knowledge, this is the first report of SCC in free-living lions. In domestic cats, risk factors include prolonged UV exposure, pale skin pigmentation or sparse hair coat at affected sites. SCC has also been reported as a FIV-associated neoplasm in cats. In this population, FIV is widespread but no direct link between SCC and FIV has yet been determined. SCC should be considered as an important differential diagnosis of persistent or progressive ulcerative skin lesions in large felid species in free-ranging or captive populations. Further research will be required to determine whether FIV infection is an important co-factor for the development of SCC in this particular species.

[303] ENTEROPATHOGENS OF JUVENILE EURASIAN BADGERS (*MELES MELES*): DIAGNOSIS, TREATMENT AND CONTROL

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Adult Eurasian badgers (*Meles meles*) are commonly presented to veterinarians in the United Kingdom. Orphaned badger cubs are less common patients and require specialist rearing and rehabilitation. Enteric disease is frequently encountered in these young animals. A number of potential enteropathogens have been identified in Eurasian badgers in Great Britain and rehabilitated cubs provide further opportunity to study the clinical, microbiological and, when mortality occurs despite veterinary treatment, histopathological findings associated with enteric disease. Over a three years period, causes of enteric disease were investigated in badger cubs at a wildlife rescue centre. Coccidiosis was the most common clinical problem. *Salmonella* species were frequently isolated, most often *S. Agama*, however clinical salmonellosis was not a significant problem. Helminths were only seen as subclinical infections. Chronic diarrhoea and mortality resulted from infection with *Giardia* species. Acute diarrhoea and deaths occurred in cubs with dual parvovirus and orthoreovirus infection. These are the first occasions in which parvovirus, orthoreovirus and *Giardia spp.* have been identified in Eurasian badgers. Treatment protocols for coccidiosis and *Giardia* were successfully implemented in clinical cases. Preventative measures now include; vaccination on arrival against parvovirus, screening of faecal samples for worm eggs, coccidia and *Giardia*, and improved cleaning and disinfection of pens.

[304] FIRST REPORT OF *AELUROSTRONGYLUS FALCIFORMIS* LUNG WORM IN SWEDISH BADGERS (*MELES MELES*)

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In 2009, the first cases of *Aelurostrongylus falciformis* (AF) were reported in Swedish Eurasian badgers (*Meles meles*). The aim of this study was to gather knowledge of the incidence rate and geographical spread of AF in wild badgers in Sweden, and to evaluate pathological changes and the impact of infection on the overall health status of the infected animals. Lungs and feces from 44 badgers killed in traffic or shot by hunters were collected in three counties; Skåne, Östergötland and Uppland. Adult nematodes collected from the terminal airways, although impossible to remove in their entirety from the parenchyma, were identified as AF by their location, the larvae present in the females as well as their morphological appearance (width of the adult parasite and accessory spicule). L1 larvae isolated from the parenchyma by lung tissue scrapings further supported the identification. Baermann test was done on the fecal samples to determine the presence of L1 larvae. Of 44 examined badgers 64% were found to be positive for AF. The prevalence of infected animals in the three counties ranged between 36 and 70%. With a high incidence of infected animals in all three counties it is assumed that AF is likely to be present in badgers throughout most of their geographic distribution in Sweden. Pathological lung lesions in association with the parasites were found to be minor and the expected impact on the animals' overall health status therefore minimal.

[305] INFECTIOUS PATHOGENS AND RESISTANCE TO DISEASES RELATED TO URSIDS: ARE MICROPARASITES A FACTOR IN THE URSID THREATENED SPECIES MANAGEMENT PLANS?

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The Carnivora comprises 15 families and they are identified as one of the mammal groups most threatened by infectious agents. However, in the case of Ursids different authors suggested that members of this family have a high degree of resistance to infectious diseases and therefore they couldn't be relevant for their management. In order to verify the relationship between microparasites (virus, protozoa, bacteria) and susceptibility to infection and clinical disease in Ursids a review has been conducted. Were found reports showing susceptibility to infection by 43 different pathogens and disease by at least 65 clinical reports and 20 pathogens, being viruses most associated with clinical cases. Despite these cases were mostly individual and no wild populations have been affected, it is very important to take into account for ex situ and translocation management programs. Thus, biosecurity and preventive plans may be established for selected microparasites as an important issue for bears captive populations and translocation programs. In conclusion, further studies about the relationship of infectious pathogens and Ursid family may be conducted.

[306] PHOTOMICROGRAPHY AND MORPHOMETRY OF *UNCINARIA* SP. (NEMATODA: ANCYLOSTOMATIDAE) FROM BEARS IN WESTERN CANADA: NEW SPECIES OR UNDESCRIBED MORPHOTYPE?

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Hookworms (family Ancylostomatidae) are blood-sucking nematodes that live in the small intestine of a wide range of wild and domestic mammals, including humans. Species of the genus *Uncinaria*, as members of the family, fasten themselves to the intestinal walls of the host, causing anaemia, haemorrhage and hypoproteinemia. Since October 2010, black (*Ursus americanus*) and grizzly (*Ursus arctos horribilis*) bear carcasses from western Canada have been collected and necropsied to determine the cause of death. Digestive tracts have been analysed; isolated parasites (*Uncinaria* sp. and *Baylisascaris transfuga*) have been identified on morphology and archived in 70% ethanol. Anatomical characteristics of hookworms parasitizing the two bear species revealed inconsistencies with the current taxonomic classification of this parasite genus in black and grizzly bears from different parts of North America. Images and measurements seem to suggest either the existence of more species of *Uncinaria* within bears, or the possibility of just one widely distributed species with different host races and morphofunctional adaptations. These data are not sufficient to discern whether the different *Uncinaria* types are the same species. Further studies combining parasite morphology with molecular biology are required to elucidate the taxonomy of *Uncinaria* spp. in bears from western Canada.

[307] CONTROL OF FOX RABIES IN NORTH-EASTERN ITALY

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A rabies epizootic, spreading from Italy's eastern border, has been occurring in the fox population of North-Eastern Italy since October 2008. From October 2008 to February 2011, a total of 287 cases were detected. Six oral rabies vaccination (ORV) campaigns were implemented from December 2009 to November 2011, by aerial distribution of baits. After each campaign, 4-6 foxes/100 km^2 were culled, and their immune response was determined by fluorescent antibody virus neutralization test. Moreover, to approximately estimate the fox population distribution and trend, hunting indexes were calculated for each ORV monitoring period and in each regular hunting season, and the index of kilometric abundance (IKA) was calculated by spring night counts (using data from the 2010-2011 red deer censuses). In emergency ORV campaigns, 77%, 69%, 46% and 78% of the tested foxes were considered immunized (antibody titre ≥ 0.5 IU/ml). In the first ordinary ORV campaign, 75% of the tested foxes were immunized. The ORV campaigns led to a progressive reduction in rabies incidence, and no new cases have been detected since February 2011. Given the level of immunized foxes and the absence of new cases in the last 12 months, eradication may be expected after the next scheduled ORV campaigns in 2012. Considering fox population dynamics, a positive trend was observed in both hunting indexes and IKA. Population data collection is still in progress to validate the use of the above data.

[308] A RABIES OUTBREAK IN POLAR FOX AND REINDEER IN THE SVALBARD ARCHIPELAGO

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In 2011 several polar foxes (*Vulpes lagopus*) and Svalbard reindeer (*Rangifer tarandus platyrhynchus*) on the Arctic archipelago of Svalbard (77 to 80˚N) were diagnosed with rabies. Some of the polar foxes showed classical clinical signs of the furious form of rabies and attacked dogs, people and cars. The reindeers, however, showed less specific signs of disease affecting the central nervous system. Some animals were found recumbent, but seemed otherwise alert and apparently normal. When people approached them, they were unable to rise due to limb paralysis, mimicking the clinical signs of trauma to the spinal cord. Others were found with signs of trauma to their head (external haemorrhages and wounds on their head), pressed their head against the ground or showed signs of severe cerebral lesions like ophistotonus, loss of consciousness and spasms. We are currently collecting serum and saliva from wild, appararently healthy Svalbard reindeer. Our aim is to investigate if reindeer may be infected by rabies virus and have virus in their saliva without showing clinical signs. Knowledge of 1) the wide spectrum of clinical appearances of reindeer with rabies and 2) to which degree apparently healthy animals may be infected, is important, both for future management of rabies outbreak and for establishing adequate preventive measurements for hunters and other people that get in contact with reindeer.

[309] MANAGEMENT OF WILD IBERIAN LYNX (*LYNX PARDINUS*) SHOWING CLINICAL LESIONS

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During the currently ongoing Iberian lynx conservation LIFE project in Andalusia (LIFE10 NAT/ES/000570; “Recovering the Iberian lynx former distribution in Spain and Portugal, 2011-2016”) 32 sanitary check-ups have been performed in the wild Iberian lynx population. Most of them were routine sanitary evaluations included into an epidemiological surveillance program (specifically focused on Feline Leukemia Virus and/or Tuberculosis). Just in 6 cases, the sanitary evaluation was performed because a lesion had been observed in the routine population monitoring program (through both photo-trapping and radio-tracking). In the first case, some sporadic minor lesions were observed (ocular lesions due to trauma and skin injuries due to fights), not compromising the fitness of the patient. When necessary, cures were applied to injuries, and long-endurance antibiotherapy was provided to patients. In individuals showing lesions thought to compromise the individual fitness and thus the body condition, a close monitoring was performed in order to evaluate the survival capability in the wild. Given the wild condition of the Iberian lynx, every actuation was carefully considered in order to evaluate if the benefits of checking-up an individual worth the stress due to handling. In those individuals captured because lesions jeopardized its survival in the wild, an exhaustive clinical evaluation was performed, including complementary diagnostic techniques, such as radiology, ecography, retinography, etc. Individuals showing starvation (one case), serious injuries (four cases) or paralysis of the pelvic limb (one case) were extracted from the nature and transported to a wildlife recovery centre or to quarantine enclosure of a captive breeding centre. Capture methods employed with the Iberian lynx in the Iberlince LIFE project are box-trap and anesthetic darts shot with blowpipe. Out of the six individuals extracted from the field, three could be recovered and brought back to the nature (those showing injuries due to intra-specific fights), while two other individuals were included in the captive breeding program (the starved individual and one with an amputated leg). The last individual (the one showing paralysis) died because the effects of *Clostridium sordellii*.

[310] CONSERVATION MEDICINE PROGRAM IN THE CRITICALLY ENDANGERED IBERIAN LYNX

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The critically endangered Iberian lynx *(Lynx pardinus)* is considered the most endangered cat in the world, and diseases have recently pointed out as a possible threat for its population. A comprehensive Iberian lynx conservation LIFE project was implemented in 2002, and populations have grown since that from 94 to 290 individuals. This LIFE project includes a conservation medicine program (CMP) which main goal is to reduce disease-related threats. The CMP is based on specifically-developed clinical evaluations, and complete necropsies. Clinical evaluations include physical examination, haematology, biochemistry, proteinogram, urinalysis, PCRs for the detection of infectious agents, serology, parasite coprology, microbiology and preservation of genetic material through skin biopsies. Moreover, blood, hair, faeces and urine samples are preserved in a biological resource bank for future needing. Necropsies include veterinary pathology, toxicology, microbiology and detection of infectious agents (PCRs and serology). Between 2002 and 2012, 352 clinical evaluations and 98 necropsies have been performed. Diseases have shown to be of concern, mainly Feline Leukaemia Virus, Tuberculosis, Pasteurellosis, Canine Distemper Virus, Feline Parvovirosis and *Leptospira interrogans*. The importance of infectious diseases is probably related to the low genetic diversity. Under this scenario, the CMP is considered essential in future Iberian lynx conservation projects.

[311] DOLPHIN MORBILLIVIRUS INFECTION IN A CAPTIVE HARBOR SEAL (*PHOCA VITULINA*)

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Dolphin Morbillivirus (DMV) represents a major biological threat for free-ranging cetaceans. In June 2011, one bottlenose dolphin (*Tursiops truncatus*) and one striped dolphin stranded along the Tyrrhenian coastline. Both animals were found molecularly positive to DMV. Personnel from a marine park helped the stranding network with the bottlenose dolphin, standard quarantine protocols were applied to avoid any contact with cetaceans of the park. On July 25th, 2011, an 8-years-old male harbor seal (*Phoca vitulina*) hosted inside the aforementioned park was submitted for post-mortem examination. Histopathological and molecular analyses were carried out to look for the presence of Canine Distemper Virus (CDV), Phocine Distemper Virus (PDV) and Cetacean Morbillivirus (CeMV). Pathological findings suggested a morbilliviral infection, which was confirmed by RT-PCR, with CeMV-specific genome sequences being subsequently amplified from the brain of this seal. CDV and PDV molecular analyses were negative. Phylogenetic analysis confirmed clustering of the harbor seal CeMV sequence with previously reported DMV sequences from cetacean species. A possible route of virus (DMV) entry into the facility could have been represented by either the personnel and/or the instruments used for the rehabilitation of the DMV-infected bottlenose. This report emphasizes that, apart from cetaceans, also pinniped species such as harbor seals are susceptible to DMV infection and related mortality.

[312] UROLITHIASIS IN FREE-RANGING HARBOR SEALS

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Uroliths have been described in pinnipeds, however reports of urolithiasis in harbor seals (*Phoca vitulina*) are scarce. Simultaneous ammonium urate and uric acid nephroliths have been reported in a captive adult harbor seal and magnesium triple phosphate crystals in three wild pups. A review of necropsy records from 1,469 stranded harbor seals for evidence of uroliths revealed five cases (prevalence 0.3%). Two cases were pre-weaned male pups with fine granular material in the bladder as well as mineralized debris and dilatation of multiple renal calices. In one case the stones were microscopically identified as magnesium triple phosphate and bacterial culture of urine yielded light growth of alpha *Streptococcus* spp., suggesting a possible infectious role in urolith development. The other cases were adult male seals. Stones were identified in the renal calices and/or common collecting ducts with attendant dilatation. In one case the nephroliths were identified as ammonium urate. There was no statistically significant sex predilection. This review confirms that uroliths in wild harbor seals are rare.

[313] A NOVEL DERMATOPATHY IN ALASKAN GRAY WOLVES (*CANIS LUPUS*)

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We describe a novel dermatopathy in 16 Alaskan gray wolves, grossly characterized by symmetrical alopecia of guard hairs over the trunk and caudal thighs sparing the head, front limbs, dorsal back, and tail. The remaining undercoat was often curly. Histologically, mild to moderate follicular dysplasia and atrophy were present often with varying degrees of orthokeratotic basket-weave hyperkeratosis, epidermal atrophy and less commonly follicular keratosis. Inflammation rarely accompanied these microscopic changes and external ectoparasites were excluded. The etiology of this condition was not determined but the lesions are suggestive of a hormonal or seasonal-based etiology. In domestic canines, various endocrine dermatopathies, cyclic (seasonal) follicular dysplasia and follicular dysplasia and atrophy that occur in a condition termed alopecia X are associated with similar lesions. Alopecia X is an incompletely understood disorder that may be associated with abnormal levels of sex hormones originating from the adrenal glands. Additionally, dysfunctional hormonal receptors on hair follicles may play a role. Alopecia X is seen typically in young adult dogs of plush-coated arctic breeds such as Chows, Keeshonds, Samoyeds, Siberian Huskies, and Alaskan Malamutes. Since the northern breed domestic canines with alopecia X are closely related to wolves, it cannot be ruled out the wolf dermatopathy represents the same condition with a similar pathogenesis. Evaluation of blood for a possible endocrine etiology may prove useful in future studies. Additionally, based on the prevalence and geographic distribution of effected wolves, investigations of a genetic component may shed light on the cause of this unusual dermatopathy.

[314] CAUSES OF DEATH AND HEALTH MONITORING IN FREE-LIVING WOLVES FROM GERMANY

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The grey wolf (*Canis lupus*) was considered extinct in Germany, until a small population was founded by wolves which came from Poland in 2000. The first pack settled on a military exercise area in the Lausitz, Saxony. Since then the population continuously grew and consist nowadays of ~60 individuals living in 12 packs, as pairs or individuals. Due to the successful re-establishment, the wolf population is continuously expanding in size and range and a health monitoring of this large carnivore became necessary. From 1999 to 2011 we examined 26 wolves which died through collision with cars or have been illegally shot. Identifying the cause of death was important in cases of illegal persecution due to the involvement of the public prosecution department which also required careful documentation. In addition to diagnosing the cause of mortality, a standardized health screening was performed for rabies, canine distemper, canine parvovirus and hepatitis contagiosa canis. Parasitological investigations focused on endoparasites. The overall prevalence for endoparasites in the wolves was 81%. Infections with helminths were found in 17 and with protozoans in 15 wolves. The only protozoan parasite identified so far was *Sarcocystis* sp. Among the helminths we identified the cestode *Taenia* sp. in 13 cases and the nematodes *Toxascaris leonina* and *Trichinella* sp. in 2 cases each, *Capillaria plica* was found in 1 wolf. Furthermore, the trematode species *Alaria alata* was identified in one wolf. Further investigations will concentrate on the molecular characterization of the collected specimen, since morphological identification is hampered due to the decomposed condition of the material. Therefore, modern PCR techniques will be applied to identify the organisms on species and even strain level.

[315] PRESENCE OF *ECHINOCOCCUS MULTILOCULARIS, TOXOCARA CATI* AND *TOXOPLASMA GONDII* IN VEGETABLE GARDENS, NORTH-EASTERN FRANCE

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Echinoccocus multilocularis (Em), *Toxocara cati* (*Tc*) and *Toxoplasma gondii* (*Tg*) are intestinal parasites of foxes, dogs and/or cats that are responsible of human zoonosis through the accidental ingestion of parasite eggs spread on the soil and the vegetation with carnivore feces. *Em* is notably responsible for alveolar echinococcosis (AE), a zoonosis of primer human health concern in northern Europe. We conducted a 6 months preliminary study to have a first evaluation of the presence of *Em*, *Tc* and *Tg* in vegetable gardens located in an endemic area. The study took place in a rural area located in the Ardennes Region, north-eastern France, where 50 % of the fox are infested with *Em*. Carnivore's feces were monthly collected in 35 vegetable gardens. A real-time PCR was used for their specific identification and for the detection of parasite eggs they could carry. Fossorial water voles (*Arvicola terrestris scherman,* intermediate host) were also trapped in vegetable gardens and their sera were tested for antibodies to *Tg* using the modified agglutination test. One hundred carnivore's feces were collected: 45 % were from cats, 36 % from foxes and 4 % from dogs. *Em* and *Tc* were frequently found in fox and cat feces respectively and 11/58 (19 %) of the voles trapped in garden had *Tg* antibodies. These preliminary results would alert us about the risk of human contamination due to the consumption of raw vegetables cultivated in gardens opened to carnivore species.

[316] A CASE REPORT OF INFESTATION OF C*ALODIUM HEPATICUM* (*SYN. CAPILLARIA HEPATICA*) IN EUROPEAN BROWN HARE (*LEPUS EUROPAEUS*) IN THE CENTRAL ITALIAN ALPS

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In Octoberber 2009, during the biometric checks on health and hunted game in the province of Sondrio, was hunted , in the town of Civo (N 46°9’, E 9°34’), 720 m.s.l. altitude, an adult female hare with obvious epatodistrofia and splenomegaly. The biometric measurements and the absence of identification systems suggest that it belonged to local population and not by artificial translocation. The liver was grossly and histologically examined for the presence of C. hepatica adults, eggs and typical C. hepatica-induced lesions. This is the first reported case of about 400 inspections carried out since 2004 in this area. Calodium hepaticum (Syn. Capillaria hepatica) is a zoonotic liver nematode of mammals distributed worldwide. Several mammalian species, including insectivores, rodents, lagomorphs, carnivores, artiodactyls, non-human primates and man, are also susceptible to the infection. The high prevalence and low pathogenicity of the infection in brown rats have suggested the role of rats as primary hosts for this parasite. The occurrence of such infestation in the brown hare raises interesting questions about the epidemiological role of the species is on the potential zoonotic risk to consumers of games.

[317] REPORT ON DERMATITIS OBSERVED IN WILD RUMINANTS OF STELVIO NATIONAL PARK, CENTRAL ALPS, ITALY

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Since 2008 there have been reports of lethal infection by PPV in red deer. Scagliarini et al described the genomic characterization of Parapoxvirus (PPV) isolated from 6 different wild ruminants of Stelvio National Prak. In the last two years the disease goes on affecting 13 animals with proliferative skin lesions. The aim of this work is to describe and compare macro and microscopic lesions of a eruptive dermatological disease in a selected samples (3 red deer calf, 2 chamois, 1 alpine ibex). In deer scabs about 6 mm diameter were distributed exclusively on the muzzle, nose, lips and oral mucosa. Pustules were present on the gums and on dental pad. In Chamois and Ibex, severe proliferative lesion affects lips and coronary band. From skin and mucosa samples PPV particles were detected by electron microscopy. Histological exam revealed severe epithelial hyperplasia with balloonig degeneration of keratinocytes, dyskeratosis and eosinophilic cytoplasmic inclusion bodies. Parapox, is an epitheliotropic virus, induces weight loss and death in calves that are reluctant to eat due to oral pain. The control of the disease in wildlife is very important to limit the diffusion, to avoid epidemics and losses of young animals. In spring, the diffusion of the disease is easier because new born of sympatric species, sensible to infection, share the same pastures with adults and domestic ruminants.

[318] FATAL BABESIOSIS IN FREE-RANGING CHAMOIS (*RUPICAPRA RUPICAPRA*) IN ITALIAN ALPS

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Babesiosis, caused by intraerythrocytic protozoan parasites of the genus *Babesia,* is a globally important tick-transmitted disease. It has been reported in several domestic and free-ranging mammal species and is gaining importance as an emerging zoonosis in humans (Taylor et al., 2001; Herwaldt et al., 2003). Disease ranges from silent infections to haemolytic anaemia, cardiovascular shock, and multiple organ failure, depending on factors such as age, immunocompetence, and coinfections of hosts with other pathogens (Homer et al., 2000). In spring of 2011 , fatal haemolytic anaemia due to acute babesiosis was diagnosed in a 4th ears old female of chamois as found dead in Fusine (46°9’22647’’N - 9°42’50283’’E), province of Sondrio (Italy). Next to the female was also found, near the village, the kid alive, who died within one week. Complete necropsy of the carcasses was carried out. Serum and EDTA blood were collected from the heart cavities for microscopic examinations and PCR analysis. Spleen samples were also collected for biomolecular analysis. Tissue specimens of the inner organs (lung, liver, spleen, kidney, lymph nodes, adrenals, urinary bladder) of animal were collected and fixed in 10% formalin buffered. Many *Ixodes ricinus* ticks were found on the adult and the kid chamois. Predominant macroscopic findings in adult chamois consisted of pale mucous membranes, waterlike bloods, swollen spleen with soft pulpy consistency and haemoglobinuria. In the adult chamois, blood smear examination revealed slight anisocytosis, hypochromasia and small, round to pyriform, basophilic inclusions. No inclusions could be detected in the blood smear of the kid. No significant microscopical lesions were observed in the kid. The molecular genetic analyses of blood, spleen and *I. ricinus* samples revealed 99–100% identity with *Babesia divergens*. Serum sample result positive also for *B. bovis* and *Anaplasma phagocytophilum.* Fatal babesiosis in chamois has been reported in five animals from Switzerland but in Italian central Alps has not been observed during the past ten years in free-ranging chamois. The potential impact of this apparently emerging disease on the affected chamois population remains unclear. In case of a newly introduced *Babesia sp.,* many fatal cases might be expected.

[319] *CERVUS NIPPON* AS AN ALIEN SPECIES IN ITALY: REPORT ABOUT PATHOLOGICAL FINDINGS ON THE FIRST CASE OF SIKA DEER CULLED ACCORDING TO A NATIONAL LAW ON ALIEN SPECIES

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In March 2012 a sika deer (C*ervus nippon),* first report of this species in Italy, was shooted by the Provincial Police Modena after been traced among a group of red deer *Cervus elaphus* abundant in the area, in the municipality of Polinago and Prignano s/S, province of Modena, in the region Emilia-Romagna (Italy). The killing was the final act of an eradication operation promptly decided by the Province of Modena on the basis of alloctonous status of this species (which can hybridize with the red deer *Cervus elaphus*) after the approval of ISPRA (Istituto Superiore Protezione e Ricerca Ambientale) and then planned also with the Veterinary Service of to start mandatory inspections before the commercialization of the meat. Immediately after killing have taken steps to start evisceration and the cooling to <7℃, in fur, in the game storage facility of a nearby Hunting Farm. The viscera were sent to the local section of Modena of the Zooprophylactic Institute of Lombardia and Emilia-Romagna for anatomo-hystopathologycal analysis, bacteriological and parasitological tests. The main pathological findings were abscesses in the lungs, lymphodes and muscles where was isolated a strain of *Pasteurella multocida*. Other bacteriological, ecto-endoparasitological and hystopathologycal findings are discussed along with the possible consequences of the presence of species not know as farmed in the Emilia Romagna region and the nearby Toscana, Liguria, Marche and Lombardy.

[320] HANDLING OF WILD RUMINANTS IN LEVEL-3 BIOSAFETY FACILITIES

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Experimental infection is the most reliable way to study the relationship between infectious agents and their hosts, including pathogenesis of diseases, pathogen excretion and transmission, comparative analysis of strains virulence, immune response and genetic resistance to infections, and vaccine testing, among others. All this information is needed when trying to accurately determine the epidemiological importance of a given host species for a given disease. Nevertheless, experimental infection studies in wild ungulates are scarce, due to the challenge of handling these species in constrained spaces, as well as the stress-associated risks for the animals, which may also affect the results of the study. The aim of this communication is to present the methodology of four experimental infections carried out in three different species of wild ungulate species; two experimental infections with bluetongue virus (BTV) in red deer (*Cervus elaphus*), one BTV experimental infection in Spanish ibex (*Capra pyrenaica*), and one experimental infection with chamois Border Disease Virus (ch-BDV) in Southern chamois (*Rupicapra pyrenaica*). Facilities modification, handling procedures, drugs and dosages administered (including sedatives and long-acting neuroleptics), restraint techniques, and sample collection procedures used allowed a safe handling (for both the animals and the operators) and the fulfilment of the objectives of all four experimental infections.

[321] THE METABOLIC EFFECTS OF PHYSICAL VS CHEMICAL CAPTURE OF FREE-RANGING BIGHORN SHEEP (*OVIS CANADENSIS*)

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Rocky Mountain bighorn sheep (*n*=26) were captured in Alberta, Canada, via chemical immobilization (CI) with medetomidine (0.16 +/- 0.04 mg/kg) and ketamine (4.2+/- 1.6 mg/kg). Animals were darted from a vehicle or on foot. Arterial blood was collected for analysis. Desert bighorn sheep (*n*=52) were captured via helicopter net gun (NG) in Nevada, USA. Sheep were transported to a processing area where jugular venous blood was collected for analysis. Samples, corrected for body temperature, were analyzed immediately with an i-STAT^{R}1 analyzer. All values, except blood glucose, were significantly different between capture techniques.

	pH	PCO_2 (mmHg)	HCO_3 (mmol/L)	BE (mmol/L)	Lac (mmol/L)	Temp (°Celsius)
NG	7.0 +/- 0.12	24.1 +/- 5.0	5.9+/-2.3	-24.9 +/- 4.0	> 20 (49 of 52)	40.3+/- 0.7
CI	7.4 +/- 0.03	55.0 +/- 5.7	33.1+/- 3.8	7.9 +/- 3.4	1.0 +/- 0.6	39.3+/- 0.6

	Na^+(mmol/L)	Cl^- (mmol/L)	K^+ (mmol/L)	BUN (mmol/L)	Gluc (mmol/L)	Hct (%)
NG	145.9 +/- 3.9	111.0 +/- 2.7	3.8 +/- 0.4	6.0 +/- 2.3	7.9 +/- 1.6	46 +/ -5
CI	142.5 +/ - 3.8	102.7 +/- 2.7	4.2 +/- 0.6	4.3 +/- 1.3	8.6 +/- 2.6	32 +/- 5

The major effect of NG is extreme lactic acidosis with compensatory hyperventilation. NG animals also had biochemical findings of hemoconcentration compared to CI. These findings are attributable to physical exertion. CI resulted in normal pH with a respiratory acidosis and concurrent metabolic alkalosis. Similar blood gas changes as in the CI animals have been previously documented in ungulates immobilized with medetomidine-ketamine.

[322] VALIDATION OF THE INFRAORBITAL NERVE BLOCK TO PROVIDE LOCAL ANESTHESIA FOR UPPER CANINE EXTRACTION IN AMERICAN ELK (*CERVUS CANADENSIS*)

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Nine mature female elk weighing 283+/-17kg were restrained in a hydraulic squeeze chute and sedated with 0.35+/-0.4 mg/kg of xylazine, administered intranasally. The anode and cathode of a peripheral nerve stimulator was applied to the rostral and caudal aspect of the upper ipsilateral canine tooth. The stimulator was ramped up (level 0-10) until an active avoidance response was displayed (baseline). Lidocaine HCl 2% with epinephrine (5-10 ml) was than administered into the infraorbital canal via a 20 gauge 3.5 cm needle. The tooth was stimulated every 30 seconds, until an active avoidance response was noted. The block was considered to be effective when no response was observed at the maximum level (10) for 2 consecutive time points. The first of the points was taken as onset. Stimulation was terminated 5 minutes following block administration and a "failed block" was noted. After determination of onset, tolazoline was administered at a dose of 2.1+/-0.6 mg/kg IM and the animal was released. An effective block was produced in 7 of 9 animals, with an onset time of 3.6+/-2.4 minutes. The two animals with failed blocks received an initial lidocaine dose of 5 ml total. All animals that received 10 ml of lidocaine (0.036+/-0.002 ml/kg), as an initial dose, developed an effective block. Lidocaine HCl 2%, administered into the infraorbital canal, at a dose of 0.036 ml/kg, will provide an effective block of the ipsilateral upper canine tooth in mature American elk.

[323] DISEASE CONDITIONS OF SOME WILD ANIMALS IN IRAQ

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Many species of wild animals in Iraq have been vanished at the earliest time of 20th century from their natural habitat due to uncontrolled hunting, other decrease in their numbers to the point of extinct. Some of these animals such as Gazella (*Gazella subgutturosa*) and wild goat (*Capra aegagrus*) reared in enclosures as an aid of their survival. Diseases prevalence were carried out during the 1985 – 2011, diseases were diagnosed either on clinical symptoms or confirmed by laboratory examination. This includes FMD, EHD, HS (Pasteurellosis), Colibacillosis, Enterotoxemia, Caseeous lumphadinitis, Hydatid Syst, mange mite, Helminths, Coccidia, capture Myopathy and Abscesses, predator such as wolf and eagle. Most of these diseases were seen in domestic livestock nearby or grassing surrounding these enclosures.

[324] MORTALITY OF CAPTIVE MOUNTAIN GAZELLES (*GAZELLA GAZELLA*) AT KING KHALID WILDLIFE CENTER, SAUDI ARABIA (1988-2011)

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Mountain gazelle (*Gazella gazella*) mortality was investigated based on the pathology records of 1219 captive animals at King Khalid Wildlife Research Center, Saudi Arabia, from 1988 through 2011. The largest number of deaths were due to trauma (n=379; 31%). Trauma was subdivided in three categories: self-inflicted (n=140; 11%); predator (n=83; 7%) and intraspecific (within pen) aggression (n=156; 13%). Self-inflicted trauma declined during this 24-year period, being near zero in 2011. Respiratory infection (n=186; 15%) was another major cause of mortality cause, being more prevalent during the winter (November - March). Other causes of death included gastro-intestinal diseases (n=108; 9%), such as clostridiosis and salmonellosis, nutritional disorders (n=113; 9%), primarily maternal neglect (n=101; 8%), and stress-related pathology (n=35; 3%), particularly capture myopathy. We discussed here, the significance of these findings for the improved captive management of this Vulnerable Arabian species.

[325] ALPHAHERPES AND PESTIVIRUS IN ALASKAN CARIBOU

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Research suggests Cervid herpesvirus 2 (CvHV2) is endemic in Alaskan reindeer and wild caribou, and serological tests point to a pestivirus being also present in the reindeer. Changes in migration of the Western Arctic caribou herd have resulted in co-mingling of reindeer and caribou for the past 15 years, and it is unknown whether these viruses were introduced to wild caribou from reindeer or if they were already endemic. We did a retrospective study examining 30 years of sera from two populations of Alaskan caribou: the Western Arctic Caribou herd (contact with reindeer since 1998) and the isolated Denali herd. A total of 358 caribou serum samples were tested using commercial blocking ELISA kits validated for reindeer. A subset of 42 samples was selected for virus neutralization (VNT) against different alphaherpesviruses. Results showed high seroprevalences in both herds across all 3 decades for alphaherpesviruses. VNT results showed highest titres against CvHV2 in both herds. These results suggest that CvHV2 has been present in caribou for over 30 years and recent contact with reindeer is not responsible for its introduction. Pestivirus seroprevalence in the Western Arctic herd has a similar profile to that found in reindeer with almost no traces of the infection before 1996 and being present ever since. The Denali herd remained seronegative over the 30 years period. This asymmetry and establishment of the virus after 1996 raises questions on its origin and spread.

[326] POXVIRUS IN PUDU (*PUDU PUDU*)

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A poxvirus was first described in pudu at the Bronx Zoo in 1994, and later in other zoos in NA, in both imported and locally born animals. The signs and clinical course include severe ulcerative and crusting skin with and without oral lesions. Some cases have been self-resolving; others have had significant morbidity and death despite aggressive treatment. And in some survivors, disease has recurred months or years after resolution of initial lesions. Frozen tissue from an animal that died of the disease and skin biopsies from three clinically ill animals were analyzed by PCR using degenerate primer sets that recognize DNA polymerase of chordopoxvirinae. Three were positive by PCR for a 543 base pair product of poxvirus DNA polymerase. Approximately 489bp of DNA sequence was obtained from each positive sample. DNA sequence analysis revealed that the pudu poxvirus is 99.4% identical to Deerpox virus W 1170-84 by BLASTn. Sequence alignment demonstrated 100% identity among the 3 cases. PCR analysis of the topoisomerase gene of poxvirus from the animal that died also showed 99.7% identity with Deerpox W1170-84. Deerpox is a cervid poxvirus and falls within it's own genus of chordopoxvirinae and is most similar to capripoxvirus, suipoxvirus, and leporipoxvirus genera. There has been no known contact between captive pudu and free-ranging cervids in North America; poxvirus may be of clinical significance to wild South American cervids and may have a carrier state in pudu.

[327] A WINTER OUTBREAK OF CONTAGIOUS ECTHYMA (ORF) IN MUSK OX (*OVIBOS MOSCHATUS*) AT A POPULATION PEAK

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Contagious ecthyma (orf) is a skin disease caused by a Parapoxvirus and is characterized by pustular, wart-like lesions around the mouth and nostrils. The disease is common among sheep, goats and various wild ruminant species. In a free-ranging musk ox (*Ovibos moschatus*) population in Dovrefjell, Norway, an abandoned and debilitated calf was euthanized mid-January 2012 with severe orf-like lesions. During February, further five affected calves were found. Grossly, characteristic orf lesions of variable severity affecting the skin around the mouth and on the muzzle were seen. Histology revealed a pustular dermatitis consistent with orf (epidermal proliferation with ulcerations, vesicle formation and intracytoplasmatic eosinophilic inclusion bodies in keratocytes). During the annual population census performed in March, additional five of the 42 observed calves had signs of orf. The calf-cow-ratio was 0.44, compared to 0.63 in an average year. The outbreak has thus affected at least 11 calves and, based on the low number of calves counted, further mortality due to orf is suspected to have occurred. This is the second orf epizootic in this musk ox population affecting the entire pasture area. The first occurred July-October 2004, in which 19 young individuals were diseased. A peak in the population size and density, and harsh winter conditions with icebound pastures are factors considered significant in triggering an orf outbreak in the Dovrefjell musk ox population.

[328] WEST NILE VIRUS SEROSURVEILLANCE IN FERAL HORSES IN GUADALQUIVIR MARSHES, DOÑANA, SPAIN

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In Spain, wetland areas with large populations of birds and mosquitoes provide an optimal habitat for the emergence of West Nile virus (WNV). Our previous studies in Doñana National Park (Guadalquivir marshes, Southern Spain) found seroconversions in common coots in 2004 and a seroprevalence of 8% in free ranging horses in 2005. This horse population is considered to be feral, since it lives free outdoors in the marshes all year round, and far away from anthropogenic habitats, so they can be useful for surveillance of WNV in this area. For that reason, in 2005 we initiated a follow-up study to monitor the evolution of the seroprevalence, based on analysis by virus-neutralization tests. First results showed a sharp decline in neutralizing antibodies from 2005 to 2008, concluding that the horse population could have had contact with WNV in 2005 or shortly before, and that contact declined in the following years. Continuing with this serosurvey, here we present the results obtained in 2009-2010. In this period, the prevalence rised up again. Moreover, seroconversion was observed in some individuals, indicating WNV circulation again in 2009-2010. Consequently, we can establish two periods of more intense WNV circulation, the first in 2005 or before, and the second in 2009-2010. During the study this population has remained asymptomatic but, remarkably, in 2010, WNV disease affected domestic horses and humans in Cadiz (Southern Spain) less than 100 km away from our sampling sites.

[329] SEVERE DERMATITIS IN SWEDISH MOOSE (*ALCES ALCES*)

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Since the fall of 2011 there was a dramatic increase in reports (about 30) of moose from southern Sweden with severe and extensive skin lesions, with alopecia, crusts and ulcers mostly on the dorsal neck and the dorsal back. This has only occasionally been reported before in moose. Two carcasses submitted for necropsy and skin parts from eight adult bull moose were examined. Five moose had lost one or both antlers. Two animals were in bad nutritional condition and two were emaciated. Macroscopically, all animals had extensive areas of alopecia, ulcerations, crusts and exudative inflammation. All animals were infested with deer ked, *Lipoptena cervi*. Four moose skins were also infested with ear mites, *Chorioptes* sp. Histologically, in the non-ulcerated alopecic skin there was chronic hyperplastic perivascular dermatitis and severe crusting with debris and bacteria on the surface. In ulcerated parts there was chronic deep granulation tissue with destruction of adnexal structures, leading to alopecia. Bacteriologically, from six cases *Staphylococcus aureus* was cultured and from one case *Staphylococcus* spp. Pyotraumatic dermatitis with secondary bacterial infection, caused by scratching and rubbing an itch is fairly common in domestic animals. Male moose can scratch the dorsal area with their antlers, and therefore, deer ked and mange may have a role in the pathogenesis of the dermatitis.

[330] IS EURASIAN WILD BOAR CULLING A SUSTAINABLE ALTERNATIVE FOR DISEASE CONTROL?

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Worldwide there is a need to develop strategies to control relevant infectious disease agents, such as *Mycobacterium bovis* and Aujeszky's disease virus (ADV) in wildlife reservoirs. We describe observational data on the effects of Eurasian wild boar (*Sus scrofa*) abundance reduction of approximately 50%, through increased culling on the maintenance of two chronic infectious diseases: tuberculosis (TB) and Aujeszky's disease (AD) in a high prevalence region. Wild boar TB prevalence remained stable in the 10 control sites, whereas it decreased by 21-48% in the 3 treatment sites. In one treatment site, the annual wild boar abundance positively correlated with the annual percentage of skin test reactor cattle, suggesting a bovine TB reduction effect. In another treatment site, red deer (*Cervus elaphus*) *M. bovis* infection prevalence decreased after wild boar culling. ADV seroprevalence in wild boar remained unaffected in the treatment sites. The reduction in wild boar TB was achieved despite no alternative *M. bovis* host being included in the culling strategy. Although culling alone is not a sustainable option for TB control in our area, wild boar culling could become a part of integrated control strategies including habitat and game management changes and vaccination, particularly if an initial short term but substantial reduction of host abundance and disease prevalence could contribute to increase the success likelihood of other control tools, or to reduce the total expenses.

[331] STUDIES ON MODIFICATIONS OF THE SAMPLING INTERVAL AND -SIZE IN THE FRAMEWORK OF CSF SURVEILLANCE OF WILD BOAR

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Classical swine fever is a serious viral animal disease affecting pigs and wild boar. In wildlife population surveys data are often reported as small-area counts in time and space. Necessary resources are tremendous to detect low virological prevalences for CSF in wild boar. A methodology has been applied to show, that modifications of the sampling interval and / or the sample size will lead to saving resources. As study area 65 hunting grounds (about 230 sqkm) located in the district Euskirchen of the Eifel region in the Federal State North Rhine-Westphalia belonging to the municipalities Euskirchen and Bad Münstereifel were used. A time period of 72 month starting from 1st January 2004 until 13th December 2009 has been examined. During this time period 4.652 virological investigations with 72 virological positive results were recorded. Within a simulation study, the monthly virological investigations were merged into different sampling periods of 2, 3, 4 or 6 months duration. Out of these pooled investigations 45 or 90 samples were selected randomly and tested for the detection probability of at least one positive sample to be found. Repeating this sampling scheme 100 simulation runs, results in the probability estimation for the detection of a CSF infection. Despite the fact, that large numbers of investigations are accumulated if time periods are merged, the highest probability to detect CSF still is reached in the monthly testing scheme.

[332] A NEW APPROACH FOR MODELLING MONITORING AND SURVEILLANCE DATA FROM WILDLIFE DISEASES: CLASSICAL SWINE FEVER IN WILD BOAR

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The analysis of epidemiological field data from monitoring and surveillance systems (MOSSs) in wild animals is of great importance to evaluate existing systems and to specify guidelines for future measurements. Our main goal is to implement an evaluation model for these systems. Our new approach is based on the non-homogeneous Poisson process which describes the number of individuals with specific epidemiological properties. For an epidemic scenario, an underlying SIR model has been assumed which drives the intensities of the observed process. A sampling rate has been defined which describes the specifics of data collection for MOSSs and also takes diagnostic procedures into account. The implementation and evaluation of the combined model by simulation studies demonstrates its ability to correctly estimate epidemic parameters. Thus, it can help to evaluate existing disease control systems. The model has been tested on real data from a classical swine fever outbreak in wild boars. The results for the adjustments of simulated and real data will be presented in the contribution. However, due to an insufficient amount of data for the time period during which vaccination procedures were practised, interpretation of the model is difficult. Therefore, the use of the DIVA marker vaccines would allow a much better understanding of the vaccination processes due to the sophistication of the epidemiological model.

[333] DOES INNATE IMMUNITY CORRELATE WITH THE PROBABILITY OF WILD BOAR TO GET INFECTED BY CLASSICAL SWINE FEVER?

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Constitutive innate humoral immunity (CIH) is thought an important determinant of host-parasite dynamics since it provides a first-line of protection against invading pathogens. However, clear demonstrations that CIH correlate with individual resistance to infection are rare. In the current study, we assessed variation in the probability to get infected and to survive among free ranging wild boar exposed to classical swine fever (CSF) using a capture-mark-recapture design. We further examined whether individuals' survival and probability to get infected were related to variations in levels of natural antibodies or complement activity. As expected, linear mixed regression and capture-mark-recapture models put in evidence a negative correlation between individuals' immunocompetence and their probability to get infected and to survive. Both results suggested a possible protective effect of CIH against CSF infection and highlight the potential of hemolysis–hemagglutination assays to assess individual susceptibility to this emerging epizootic in wild populations.

[334] CSF OUTBREAK IN WILD BOAR AND DOMESTIC PIGS IN ISRAEL: WHAT WAS THE SOURCE?

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Israel experienced a CSF outbreak in domestic pigs (*Sus scrofa domesticus*) in February 2009. The outbreak took place in a farm located in northern Israel, after an absence of 62 years from this region. Consequently, the Israel Nature and Parks Authority initiated a field survey among wild boar (*Sus scrofa*) including sampling from live and dead animals. The RT-PCR tests were positive for CSFV in 17 of 151 individuals, where positive results ranged from the farm's boundary up to 30 km away. The first mortality of wild boar due to CSF was a few hundred meters from the farm's boundary. A study was conducted to find the source of the virus and its trajectory, due to the fact that transmission of the disease between domestic pigs and wild boar is well known, and because we could not readily determine which was the source. To test the hypothesis that wild boar were the source of the epizootic, an ELISA test was conducted on 49 sera samples collected prior to the epizootic from wild boar (2007-2008). Identification of a previous exposure could support the hypothesis that the virus persisted in the population, and circulated among susceptible and immune animals. This hypothesis was rejected as our results showed no past seroconversion. Analysis of the virus sequence that had 99% homology to strains that were isolated from pigs in China supports this conclusion. While the pathway of the virus into the farm remains unknown, we are convinced that diseased carcasses of pigs and swill from the farm contaminated the wild boar population.

[335] ISOLATION OF *AEROMONAS HYDROPHILA* FROM LUNGS AND NASAL FOSSAE IN WILD BOARS (*SUS SCROFA*)

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Aeromonas hydrophila is a facultative anaerobic gram-negative bacillus that has been associated with different diseases in warm-blooded animals and humans. However to the authors' knowledge there are no published data on the presence of *A. hydrophila* associated with diseases or isolated from wild boars. This study describes the isolation of *A. hydrophila* from a dead wild boar piglet with clear signs of respiratory disease and the subsequent research to verify its presence in the respiratory tract of wild boars. 12 nasal fosse swabs from live animals and 34 lung tissues samples were collected at a hunting held in the same estate where the dead wild boar piglet was found. All samples were cultured on Aeromonas isolation agar and lung tissue samples were also taken for histopathological examination. *A. hydrophila* was isolated in pure culture from three lungs samples and two nasal fosses swabs (10.87% of animals tested). All isolates showed -haemolysin phenotype and were positive for a specific PCR to determine the presence of the haemolysin-encoding gene as virulent factor. Despite that fact, it was not possible to establish significant statistical differences between hystopathological evidences found in lungs where *A. hydrophila* was isolated and lungs where this microorganism was not found. So their role as primary pathogen in wild boars remains unclear. Nevertheless the presence of *A. hydrophila* in wild boars has zoonotic implications for people who hunt these animals for sport and consumption since this microorganism has been associated with a while range of illnesses in humans. These people may be at risk for getting infection when they fail to use proper hygienic precautions during butchering and food preparation. In addition the microbiological conditions of meats from hunted animals can be compromised by poor placement of shots, the usual evisceration and sometimes further dressing of carcass in the field, and ageing of carcasses at ambient temperatures.

[336] BOVINE TUBERCULOSIS IN NON-MANAGED WILD BOARS FROM A PROTECTED AREA IN CATALONIA (N.E. SPAIN): EPIDEMIOLOGY AND MANAGEMENT

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In 2004, bovine tuberculosis (bTB) was detected in wild boars (*Sus scrofa*) from a protected area in Catalonia. To date, 740 wild boars and 250 Iberian ibexes (*Capra pyrenaica*) have been examined for compatible lesions (TBCL), and by bacteriological and molecular analyses. Epidemiological data available from sympatric cattle have been contrasted. In 2008, stamping out of a bTB infected cattle herd was officially decreed. Simultaneously, harvesting of wild boar was increased by hunting and box-trapping. By means of a tree modelling approach we evaluated the role of environmental- (e.g., climatic conditions), human- (e.g., livestock removal and hunting intensification) and population-related factors (e.g., wild boar abundance) on bTB prevalence trend. So far it is the first description of tuberculosis in wildlife from Catalonia and its prevalence is amongst the highest described in a free-ranging, non-intensively managed ungulates population in Spain. Epidemiological link between cattle and wild boar was confirmed by shared spoligotypes. Ten different spoligotypes have been identified in wild boars. Although eradication of bTB has not been achieved in wild boar, prevalence has been reduced from 75% to 23% (TBCL) and cattle remain TB-free after two years since its reintroduction. To date, bTB has not observed in ibex. Jump-back from wild boar to cattle can occur and a more sophisticated approach would be needed to eradicate bTB.

[337] *MACRACANTHORHYNCHUS HIRUDINACEUS*: A CASE REPORT OF INTENSE PARASITIC INFESTATION IN HUNTED WILD BOARS IN PORTUGAL

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In the last decades, wildlife has assumed an important role in disease transmission not only because of its potential impact on human and domestic animal health, but also in what concerns wildlife conservation. *M. hirudinaceus* is an Acanthocephala parasite that has domestic pig and wild boar as the main definitive hosts. Nevertheless, this parasite has already been reported in other animal species (dogs, foxes, jackals) as well as in human beings, and for this reason it is considered a zoonotic agent. The presence of the adult parasite in the intestine may cause an inflammation process with the presence of granulomas and, in severe infestation, the animals may present significantly decrease in growth rate and/or some health compromise. This study was carried out on 105 wild boars shoted during the hunting season 2011/2012 in northern Portugal. From those, only three captured in the same drive hunting procedure, revealed the presence of numerous worms fixed in the mucosa that were collected and placed into a container with 70% alcool, for further analysis. Morphological characterization of the worm suggested that it might be a case of *M. hirudinaceus* infestation. To our knowledge, this is the first case described in wild boars in Portugal. Despite the regional occurrence observed, these results underline the importance of game examination after the hunting procedure in order to detect pathological processes and to minimize disease transmission.

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[338] DETECTION OF A NOVEL GAMMAHERPESVIRUS IN CHAMOIS IN NORTHERN ITALY

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Respiratory infectious diseases are frequently reported in chamois population. In the Italian Central Alps (Lombardy region, Bergamo province) during a yearly sanitary control program, outbreaks of pneumonia infection have been monitored since 2000. The most frequently recovered pathogens have been *Pasteurella spp.*, *Chlamydophila pecorum* and Respiratory Syncitial Virus (RSV), that caused a severe mortality in the past years. More recently a novel gammaherpesvirus (GHV), never reported before in Italian chamois population, was detected in four animals. In particular the virus was found in the lungs of two adult hunted chamois, collected by the hunters that recognized suspicious lesions in the parenchyma, and in the lungs of two young animals discovered death. All the four samples were submitted for bacteriological and virological assays and they tested negative for all the exams aside from the PCR for Herpes virus. A specific set of primers was designed to amplify a fragment of 943 bp of the gene coding the glycoprotein B (gB). The amplicons were sequenced and blasted in NCBI GenBank. All the samples show a 100% homology with the sequence of GHV detected in Alpine chamois. Based on the sequence analyses, a phylogenetic phylogentic tree was built showing three different clusters: one encompassing all four samples showing high similarity with rupGHV1, a second one typical for the ovine/caprine herpes viruses, and a third one for bovine herpes viruses.

[339] EVALUATION OF TWO ELISAS FOR PARATUBERCULOSIS IN FOLLOW DEER USING TISSUE AS COMPARISON

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Methods to improve the ELISA test to detect *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in cattle have been explored over several years. However, ELISA evaluation for MAP in wildlife mammals is generally poor. The present study describes the evaluation of two ELISAs to detect specific antibodies to MAP in sera from free fallow deer compared to analysis using the “gold standars” thechnique histopathological lesions. Blood and tissues samples were collected during necropsy of 95 hunter-harvested fallow deer. Histological lesions were classified as: (1) Focal and multifocal lesions. (2) Diffuse lesions (3) No lesions. Two traditional ELISAs were evaluated using a commercial MAP protoplasmatic antigen (CPPA) and a homemade PPA antigen (HPPA) obtained from a fallow deer MAP strain (SU-98). In addition, two different peroxidase conjugates were assayed to evaluate the binding ability to fallow deer antibodies, a protein A/G-conjugate and a homemade specific fallow deer antiglobulin (AFD). The average optical density (OD) of antibody responses to HPPA antigens using the AFD conjugate were higher than the ones obtained with the CPPA antigen and the A/G-conjugate. In addition, the OD values for the ELISA using HPPA antigen and AFD conjugate showed greater sensitivity and specificity in all histopatological lesions groups than the one using CPPA antigen and A/G conjugate. In receiver operating characteristic (ROC) curve analysis, the result of HPPA Ag-AFD conjugate ELISA yielded an area under the curve value of 0.864, which indicated that this Ag using AFD conjugate is more accurate indicator of MAP than commercial Ag using the A/G conjugate.

[340] CULLING RED DEER TO ERADICATE *MYCOBACTERIUM BOVIS* INFECTION IN WILDLIFE IN THE BROTONNE FOREST (NORMANDY, FRANCE)

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Bovine tuberculosis (TB) was discovered for the first time in France in wild ungulates in 2001. Tuberculosis-like lesions were detected accidentally in a red deer (*Cervus elaphus*) hunted in the Brotonne Forest (Normandy), and *Mycobacterium bovis* (same genotype as nearby cattle strains) was isolated. The first survey (2001–2002) showed an TB prevalence of 28% (n=84) in wild boars (*Sus scrofa*) and 14% (n=77) in red deer. Control measures, *i.e.* increasing hunting pressure, ban on artificial feeding and viscera destruction, were implemented to stop TB spread. Nevertheless, the situation worsened in 2005-2006 (prev.: 23 % [n=145] in red deer; 37% [n=155] in wild boars), with gross lesions preferentially in mesenteric (red deer) or retropharyngeal (wild boars) lymph-nodes. In 2006, it was decided to wipe out red deer (*ca*. 500 heads, considered as the primary TB reservoir) and to strengthen the reduction of wild boar's density (considered as a spillover host). In 2010-2011, less than 10 deer remained in the area and those inspected were healthy. Since 2007, the prevalence in wild boars has regularly decreased (2008: 19%; 2009: 11%; 2010-2011: <1%). Since 2001, only one case has been found in roe deer (n=425), foxes (n=162) and badgers (n=160), indicating a marginal role of these species. Moreover, no TB cases have been reported in nearby cattle since 2006. In conclusion, red deer culling and viscera elimination appeared as the appropriate strategy to eliminate TB in wildlife in the area.

[341] EXTRAINTESTINAL LESIONS ASSOCIATED WITH *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* IN WILD CERVIDS

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Paratuberculosis is a chronic enteritis of ruminants caused by *Mycobacterium avium* subsp*. paratuberculosis* (*Map*). Major lesions are mainly observed in the lower part of the small intestine and associated mesenteric lymph nodes. Disseminated infections are rarely reported in wild cervids. In this study, we report two cases with extraintestinal lesions associated with *Map* in adult red deer (*Cervus elaphus*). The first one was found dead in 2011 and the second was culled for sanitary reasons in 2012. After necropsy, samples of feces, ileocaecal junction, spleen, lung and lymph nodes (mesenteric, mediastinal and bronchial) were processed for bacterioscopy, histopathology, *Map* PCR and culture. Gross lesions were observed in intestinal tract, mesenteric lymph nodes, lung (focal consolidation, case #1) and bronchial/mediastinal lymph nodes (enlargement, case #2). Microscopic changes were characterized by granulomatous enteritis, pneumonia and associated lymphadenitis, with abundant acid-fast bacilli in macrophages and giant cells (multibacillary form). The presence of *Map* was shown by direct PCR performed on bronchial, mediastinal, mesenteric lymph nodes and spleen. *Map* cultures are currently in progress. Taken together, these observations suggest that systemic dissemination of *Map* may occur in paratuberculosis.

[342] ENDOPARASITIC INFESTATION LEADING TO MORTALITY IN WILD SPOTTED DEER (*AXIS AXIS*) IN WEST BENGAL, INDIA

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A survey study was conducted to study the incidence of significant parasitic diseases in spotted deer in different national parks, sanctuaries and deer parks of West Bengal, India. Wild life survey was carried out in different National Parks, sanctuaries and deer parks of West Bengal, India in which information on the causes of mortality in wild spotted deer was obtained based on clinical and post-mortem examinations along with bacterial isolation studies from three National parks (Buxa, Gorumara and Sudarban), six wild life sanctuaries (Mahananda, Jaldapara, Bethuadahari, Bibhutibhushan, Ballavpur and Ramnabagan) and five deer parks (Kumari Kangshabati, Adina, Gorchumuk, Jaipur and Jhargram) respectively. Mortality rates in spotted deer due to fascioliasis, amphistomiasis and oesophagostomiasis were 4.69%, 3.12% and 1.56% respectively in Buxa National park; 3.79%, 12.65% and 1.26% respectively in Gorumara National park; 0.78%, 0.64% and 0.12% respectively in Sundarban National park; 6.17%, 3.70%, and 1.23% respectively in Mahananda Wild life sanctuary; 6.36%, 7.27% and 0% respectively in Jaldapara Wild life sanctuary; 4.07%, 7.25% and 1.44% respectively in Bethuadahari Wild life sanctuary; 4.07%, 5.88% and 0% respectively in Bibhutibhusan Wild life sanctuary; 6.66%, 5.06% and 1.26% respectively in Ballavpur Wild life sanctuary; 4.44%, 6.66% and 0% respectively in Ramnabagan Wild life sanctuary; 4.25%, 6.38% and 0% respectively in Kumari Kangshabati Deer park; 0%, 7.31% and 0% respectively in Adina Deer park; 9.52%, 0% and 0% respectively in Jaipur Deer park and 3.63%, 5.45% and 0% respectively in Jhargram Deer park. The overall study revealed that the incidence and mortality caused due to endoparasitic infestations were more in National parks and Wildlife sanctuaries than in Deer parks.

[343] A MINIMALLY INVASIVE APPROACH TO DIAGNOSE A POORLY DIFFERENTIATED SINO-NASAL CARCINOMA IN A ROE DEER (*CAPREOLUS CAPREOLUS*): COMPLEMENTING PATHOLOGY AND COMPUTER TOMOGRAPHY

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A female roe deer (*Capreolus capreolus*) that appeared disoriented, numb and blind in the right eye was found in the Swiss Alps in December 2009. Because of its low chances of survival, it was shot by the game warden. The head was sent for investigation. It presented an ovoid, compact mass (15 x 20cm) delimited by a thin capsule. Computer tomography revealed that the mass was expanding from the right frontal sinus, with displacement and compression of the right eye, destruction of the right orbit and invasion of the brain cavity. On cut surface, multiple bony trabeculae were expanding centripetally from the inner aspect of the external margin of the mass. Histologically the mass was composed of poorly defined islands of polygonal to spindloid cells separated by fibrous septa, bony spicules and myxoid-like stromal areas with a patchy distribution. The neoplastic cells were arranged in anastomosing and branching cords, occasionally forming acinar-like elements. The bony spicules scattered within the sections were composed of woven bone and frequently embedded within fibrous sprouts expanding from the external periostium. The features of the mass were consistent with a poorly differentiated epithelial tumor. Similar tumors have been previously documented in deer species but never investigated by computer tomography. This method allows a minimally invasive approach without precluding a conclusive diagnosis and with full preservation of the skull specimen.

[344] UTILITY OF BLOTTING PAPER FOR SEROLOGICAL TESTS TO PERFORM MONITORING PROGRAMS FOR EUROPEAN BROWN HARE SYNDROME (EBHS)

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Since mid '80 the European brown hare (*Lepus europeaus*) populations have been progressively declining due to several causes, including the occurrence of EBHS. After the first outbreaks in North Italy in the '90, the periodical EBHS cases imposed the adoption of an articulate monitoring plan of the different hare populations, including those from protected areas and the hunting territory. In addition to the examination of dead animals for viral detection, such monitoring activity takes advantage from serological survey i.e. by checking the presence of antibodies to EBHSV. Since different types of blood sampling may be adopted according to each situation, from 2005 to 2012, we planned to compare the serological titres obtained by testing with cELISA: a) the "classical" serum b) samples of blood dried onto blotting paper and c) bloody fluid from the heart cavities. The major aim was to establish the utility of each sampling method for verifying hares' health status and the possibility to get data from low density areas, as hunting ones. We analysed the following samples: a) + b = 305 animals; b) + c) = 182 animals; a) + c) = 95 animals. Even if blotting paper and cardiac blood slightly underestimate the EBHSV antibody titres, both these "alternative" sampling methods may be useful for field studies. Moreover, the slightly underestimates of antibody titres do not prevent to correctly interpret the sero-epidemiological results with regard to the understanding of spatial/time exposure of the population to EBHS and the ability of single hares to resist the EBHSV infection.

[345] LOW PATHOGENIC AVIAN INFLUENZA VIRUS IN A COMMON SYNANTHROPIC MAMMAL, THE COTTONTAIL RABBIT

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Cottontail rabbits (*Sylvilagus* spp.) are common mammals throughout much of the U.S. These lagomorphs are often found in peridomestic settings, potentially interacting with humans, livestock, and poultry operations. Thus, this species potentially poses a risk for avian influenza virus (AIV) trafficking if they are susceptible to AIV infections and shed the virus in sufficient quantities. To assess the potential for AIV shedding in cottontail rabbits, we nasally inoculated fourteen cottontails with an AIV H4N6, a subtype frequently found in waterfowl in the U.S. All inoculated animals shed large quantities of viral RNA nasally and relatively high viral RNA orally. However, oral shedding tended to decline more quickly than did nasal shedding. No animals showed any obvious signs of disease throughout the study. All infected animals demonstrated evidence of serological activity at 22 days post infection. Cottontail rabbits are often found in highly peridomestic situations, such as farms, parks, and suburban neighborhoods, often becoming habituated to human activities. Thus, if infected these mammals could potentially play a role in the epidemiology of AIV in local situations.

[346] MYXOMATOSIS IN THE WILD RABBIT OVERPOPULATION OF LEMNOS ISLAND, GREECE

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Myxomatosis is one of the most frequent viral diseases of wild and farmed rabbits in Europe. In Greece, a Myxomatosis outbreak in farmed rabbits was observed in 2007. Since then no further cases were reported. As part of the eradication strategy for the disease vaccination is not permitted. In the present study, the diagnosis and isolation of a wild, highly pathogenic Myxoma virus is reported, causing an outbreak in the wild rabbit overpopulation of the Greek island Lemnos. Affected and dead wild rabbits were reported from hunters in Lemnos in autumn 2011. Local official veterinarians recognised the typical signs and lesions. 18 wild rabbits were processed for Myxoma virus detection. Total DNA was extracted from affected tissues and a 492bp PCR product was amplified targeting the major envelope protein gene of myxoma virus. After sequencing and alignment with available Myxoma strains in Genbank, the myxoma virus of wild rabbits had 100% homology with Lausanne strain. From positive samples viral isolation was performed according to OIE standard protocol using the established RK-13 cell line. Typical Myxoma virus cytopathogenic effect was always present after the second passage. Samples from eyelids and dermis presented histologically myxoid material mixed with collagenous tissue. There is no information on how the causative agent entered the island and affected the wild rabbit population. Also, no Myxomatosis cases are reported in farmed rabbits or other lagomorphs in the zone.

[347] SEEKING ADDITIONAL BIOLOGICAL CONTROL AGENTS TO AUGMENT RABBIT HAEMORRHAGIC DISEASE (RHD) AND MYXOMATOSIS: MANAGING AUSTRALIA'S RECOVERING PEST RABBIT POPULATION

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The release of myxoma virus in 1950 and rabbit haemorrhagic diseases virus (RHDV) in 1995 reduced Australia's pest rabbit (*Oryctolagus cuniculus*) population providing billions of dollars in economic returns, and major environmental benefits. Both viruses initially reduced rabbit numbers in many areas by up to 95% however, as expected with pathogen/host relationships, the viruses changed and genetic resistance developed in the rabbits allowing their numbers to recover, albeit to levels lower than those before the virus releases. With seedling recruitment in native vegetation being prevented at rabbit densities as low as 0.5/ha, and increasing agricultural and pastoral impacts, further rabbit control is still necessary. This could be achieved economically in arable lands using poisons and rabbit burrow destruction, but in the remote, vast (>1 million km^2) and sparsely populated semi-arid and arid parts of Australia where management of rabbits is problematic, biocontrol agents are the only realistic solution. Agents additional to myxoma and RHDV have been suggested though not as yet assessed. In conjunction with expert review of these agents, we are developing wildlife and commercial rabbit industry disease networks to detect other control agents that might assist Australia in its continuing battle with the rabbit.

[348] OUTBREAK OF TULAREMIA IN BROWN HARES (*LEPUS EUROPAEUS*) IN FRANCE, JANUARY TO MARCH 2011

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Tularaemia is a cosmopolitan bacterial zoonosis caused by *Francisella tularensis*. Although this bacterium is found in many animal species, the European brown hare (*Lepus europaeus*) play an important role in the disease ecology in France and is a significant source for human infection. Hare tularaemia has been reported in most French departments, with endemic areas in northern France. Although tularaemia is often fatal to hares, hare tularaemia in France is usually sporadic and does not significantly affect hare populations. However, a tularaemia outbreak occurred in brown hares in Pas-de-Calais (northern France) from January to March 2011 and was characterised by a high mortality rate in the local hare population. In total, the SAGIR network (an outbreak surveillance network that aims at determining the etiology of wildlife mortalities) reported 51 tularaemia cases. Investigations were performed to confirm that tularaemia was responsible for theses hare mortalities. 16 % of carcasses were analysed by both bacterial culture and real-time PCR (polymerase chain reaction). Epidemiological field investigations were unable to identify a source of infection. However, several epidemiological factors, which likely favored the emergence of the disease in the area, were identified. The epizootic form of this outbreak raises many epidemiological questions and leads to reconsider the impact of tularaemia on French hare populations and the risk factors for zoonotic transmission.

[349] AN UNUSUAL PRESENTATION OF TULAREMIA IN A EUROPEAN BROWN HARE (*LEPUS EUROPAEUS*) IN SWEDEN: PATHOLOGY AND IMMUNOHISTOCHEMISTRY

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Tularemia is endemic in northern Sweden affecting mountain hares (*Lepus timidus*), but has in the last decade emerged in European brown hares (*Lepus europaeus*) in south-central parts of the country. The typical pathological presentation in both species is acute disseminated disease with no inflammation. The European brown hare described here showed prominent tularemia-meningitis and necroses associated with inflammation in multiple organs. The hare was an adult, emaciated, female, which showed circling movements before she died. The spleen was severely enlarged with grossly visible confluent necroses. Histopathology showed necrotic foci in the spleen, liver, bone marrow, lung, brain leptomeninges, uterus and ovaries. The inflammatory reaction was prominent in the meninges and lungs. It was dominated by macrophages while T cells (CD3+) constituted approximately one third of the inflammatory cell population and B cells (CD79+) were scarce. Immunohistochemistry to detect *F. tularensis* demonstrated a combination of extracellular location in the necroses, and intracellular presence mostly in macrophages. No other bacterial infection could be demonstrated by culture. This case shows a clear inflammatory response to *F. tularensis* and an unusual pathological presentation of tularemia, not observed before in Sweden. Inflammatory lesions caused by *F. tularensis* are however, reported in E. brown hares in other countries, like Germany (Sterba and Krul, 1985) and Hungary (Gyuranecz, 2010).

[350] A CASE OF MENINGITIS AND ORCHITIS IN A HARE (*LEPUS EUROPAEUS*) DUE TO *FRANCISELLA TULARENSIS*

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The last occurrence of tularaemia in Belgium was in 2003 with two human cases diagnosed by serology; the two patients showed clinical signs of the disease and reported a close contact with a dying hare. In this later, isolation of the bacteria was positive. In September 2012, in southern Belgium (province of Liège), a young male brown hare was found moribund by a hunter and killed by cervical dislocation with bare hands. The animal was transmitted to the Surveillance Network of Wildlife Diseases for necropsy. At post-mortem examination, numerous whitish foci, less than 1 mm of diameter, were recorded bilaterally on the surface of the testis, the epididymis and the deferent duct. The spleen was doubled in volume, with a firm consistence. The animal showed a poor body condition. Various organs were examined by histopathology. Lung and kidney showed congestion. A light subacute multifocal necrotising inflammation was observed in the liver. Major findings were reported in the brain with generalized subacute necrotising meningitis with local congestion, and in testis with several foci of subacute necrotising orchitis at the periphery of the gland in the tunica albuginea. Infection with *F. tularensis* subsp. *holarctica* was confirmed by both bacterial culture and real-time PCR. This is the first report of meningitis due to *F. tularensis* in a brown hare. Moreover this case shows that tularaemia still occurs in Belgium and can be a potential threat for people in contact with wildlife.

[351] IMMUNOPHENOTYPIC ANALYSIS IN HEALTH STUDIES OF TWO VULNERABLE ENDEMIC AUSTRALIAN SPECIES: THE KOALA (*PHASCOLARCTOS CINERIUS*) AND THE AUSTRALIAN SEA LION (*NEOPHOCA CINEREA*)

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The adaptive immune response plays a pivotal role in mediating outcomes of the host- pathogen- environment interaction and provides a potentially useful indicator of the health of individuals and populations. An ability to measure this response is important to studies of pathogenesis and epidemiology of hookworm disease of Australian sea lion pups, and koala retrovirus (KoRV) and chlamydial infection of koalas. Unfortunately, the immune system is under significant evolutionary pressure, and the resulting diversity among wildlife species limits application of many of the reagents developed commercially for humans or domestic animals, and also limits validity of extrapolation among species when interpreting findings. Here we report the use of commercial cross-reactive anti- T and B lymphocyte and MHCII antibodies to describe species-specific immunophenotypic patterns in the koala and Australian sea lion, and changes to these in response to age, sex, season, health status, and *in-vitro* mitogen stimulation.

[352] DOES HIBERNATION IMPAIR IMMUNOCOMPETENCE OF *MYOTIS MYOTIS*? LEUKOCYTE COUNTS BEFORE AND AFTER AROUSALS FROM HIBERNATION

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Mammalian hibernation consists of prolonged periods of reduced basic metabolic rate and body temperature, which are interrupted by intermissions of arousal when most of the physiological functions are temporarily restored. Hibernation affects as well the immune function, and it has been hypothesized that arousals may activate the dormant immune system to combat accumulated pathogens. Despite a plethora of studies on rodents and insectivores, we know virtually nothing about the immunocompetence of hibernating bats. However, this field is of highest priority due to emergence of the White-Nose Syndrome in North America. We collected blood samples from greater mouse-eared bats (*Myotis myotis*) in order to compare the total and differential white blood cells of the pre-hibernating, hibernating and aroused individuals. In accordance with previous studies on non-Chiropterans, hibernating bats showed leucopenia, both lymphocytes and monocytes being affected. After arousal, the numbers of these cells were restored, however slower compared with other hibernators, while the number of neutrophils was not affected as their values remain similar for all three physiological conditions. Our study is one of the first to demonstrate an effect of hibernation on immune system in Chiroptera. Contrary to all animal species studied so far, hibernation of greater mouse-eared bats has no influence on circulating neutrophils indicating a continuous preparedness of the immune system against pathogens.

[353] DISEASE INVESTIGATIONS IN BATS FROM MONGOLIA

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Chiroptera are one of the most successful animal orders with up to 1240 different species inhabiting a broad range of ecosystems. Despite major achievements in bat biology and ecology research, knowledge on bat diseases is still limited. A field expedition in summer 2011 to the Gobi desert, Mongolia, included a research project on bat biology. Thirty-four bats were mist netted and captured comprising the following species: *Eptesicus gobiensis* (3 males, 3 females), *Hypsugo alaschanicus* (11 females), *Myotis aurascens* (5 males, 9 females), *Plecotus* sp. (3 females). From each bat a set of samples was taken: Saliva, blood and feces as well as tissue samples of the inner organs. Histo-pathologic examination of randomly selected wild caught animals is most often limited to descriptions of healthy organ tissues as chances to detect a diseased animal are rather low. Surprisingly, despite the low number of individuals histology results of the investigated bats revealed two thirds of animals with pathologic changes in one or more organs. Moreover, there was a clear difference between animal species and organ lesions, e.g. the larger *H. alaschanicus* had numerous granulomas within their livers caused by parasitic nematodes while the bat species *E. gobiensis* and *M. aurascens* were lacking such changes. However, a number of *E. gobiensis* and *M. aurascens* caught in one location had distinct pulmonary changes indicating suppurative pneumonia of varying degrees. One bat had additional acute necrosis of a liver lobe, another bats mild localized myocarditis. Beside the histo-pathologic analysis results of virologic investigations on selected viral infectious agents as well as examination of blood smears for blood parasites will be presented.

[354] NATURAL CIRCULATION OF MARBURG VIRUS IN *ROUSETTUS AEGYPTIACUS* POPULATIONS IN SOUTHWESTERN UGANDA

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Marburg virus (family *Filoviridae*) causes sporadic outbreaks of severe hemorrhagic fever (HF) in Africa. The Egyptian fruit bat (Rousettus aegyptiacus) has been implicated as a likely natural reservoir, based most recently on the isolation of infectious virus directly from bats caught at the Kitaka mine, Uganda where miners fell ill with Marburg HF in 2007. The ecological drivers that lead to virus spillover to humans are poorly understood. Within a year, two separate cases of Marburg HF occurred in American and Dutch tourists respectively after visiting Python cave, Uganda, a tourist attraction 25 miles from Kitaka mine. Python cave also contained large numbers of *R. aegyptiacus*. These two cases prompted a long-term ecological investigation of *R.aegyptiacus* in Python cave to determine if, 1) the *R. aegyptiacus* in the cave carried infectious Marburg virus similar to that found in the tourists, and 2) what ecological factors might influence virus transmission and spillover to humans. In this report, we present results from our investigation in which we find that, 1) approximately 2.5% of the ~40,000 bats is actively infected with Marburg virus, 2) the sequences from *R. aegyptiacus* virus isolates closely matched those from the tourists, and 3) older juveniles ~ 6 months of age are 5-6 times more likely to be actively infected than adults tested at the same time. These data present the first long-term study of filovirus circulation in its natural reservoir.

[355] INITIAL SURVEILLANCE OF *GEOMYCES DESTRUCTANS* IN SWEDISH BATS AND BAT HIBERNACULA

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White nose syndrome WNS caused by the fungus *Geomyces destructans* has led to massive mortalities in bat colonies in North America and has been found in bats in several European countries, but without apparent mortality events. The aims of this study were to initiate the first surveillance for possible presence of *G. destructans* in Swedish bats and bat hibernacula, and to evaluate what methods could be used for a future surveillance of WNS in bats. Hibernacula in four counties were visited in late winter during annual bat inventories. Environmental samples for fungal culture were taken adjacent to bats to avoid manual handling and minimize disturbance. Hibernating bats were only visually inspected, without handling them. In the summer, 90 bats caught in mist nets for surveillance of bat Lyssa virus were sampled from facial fur for culture. From hibernacula, 135 samples from the environment were analyzed and 277 hibernating bats were visually inspected. All culture results in this initial study where negative for *G. destructans* and changes indicating WNS were not seen in any bat. Several other fungal organisms were found in hibernacula and on bat skin. In conclusion, *G. destructans* has yet not been found in Sweden. Annual bat inventories of hibernacula should help to detect increased mortality and morbidity, and including PCR-techniques would probably improve future screening sensitivity. The study was funded by the Swedish Environmental Protection Agency and WildTech (EC 7FP).

[356] FIRST GENOTYPIC CHARACTERIZATION OF *TOXOPLASMA GONDII* FROM FREE-LIVING BATS

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Bats belong to the Order Chiroptera, Suborders Megachiroptera and Microchiroptera. Only microchiropteran bats occur in Brazil, where they are widely distributed. Increased numbers of bat population in the urban areas worldwide are related to the destruction of the environment by man. Little is known about the role of these mammals in toxoplasmosis. Toxoplasma gondii is a globally distributed protozoan parasite infecting almost all warm-blooded animals, including man. Toxoplasma gondii isolates are genetically highly diverse in domestic animals from Brazil, however little is known about the genetics of this parasite from wild animals in this country. Here we reported for the first time the genotypic characterization of two recently isolated T. gondii strains from free-living bats captured in the metropolitan area of São Paulo city, São Paulo state. Eleven PCR-RFLP markers were used to genotype the isolates. The genotype revealed for the isolate TgBatBr1 (from the insectivorous Molossus molossus) has already been identified in one isolate from a capybara from São Paulo state. The genotype revealed for the isolate TgBatBr2 (from the vampire bat Desmodus rotundus) has already been described in 16 isolates from different hosts (cats, capybaras, chickens, sheep, a rabbit and a mouse) in the southeast, south and middle west of Brazil. This study demonstrates for the first time that bats can share T. gondii genotypes that circulate in terrestrial domestic mammals and birds.

[357] POOR HEALTH OR BAD LEADERSHIP? ASSESSING THE ROLE OF DISEASE IN A PILOT WHALE MASS STRANDING EVENT, SCOTLAND, JULY 2011

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The cause of cetacean mass stranding events (MSEs) have long been a mystery and many factors, both natural and anthropogenic, are suspected to play a contributory role. In July 2011 a large mass stranding of pilot whales occurred in north-west Scotland. Here we describe the logistical, diagnostic and investigative pathways used to attempt to establish whether the stranding mechanism was an intrinsic "error of navigation" or a result of one or more external factors. A pod of approximately 70 long-finned pilot whales *(Globicephala melas)* entered the tidal estuary of Kyle of Durness and about 40 stranded. Approximately half of these were successfully refloated, however 19 animals were known to die during the MSE. Sixteen animals were recovered and necropsied to investigate potential trigger factors for the MSE. The investigation included detailed pathological examination and diagnostic testing to attempt to assess health and the burden of disease. These included microbiology, histopathology, morbillivirus (RT-PCR) and quantitative analyses for multiple contaminants including algal toxins, organochlorine pesticides, PCB congeners and total tissue metal concentrations. External triggers, such as unusual climatic conditions and sources of underwater noise were also investigated. Results suggest a combination of bacterial disease (in one case) and external acoustic events were influential. Issues with evaluating multifactorial causes of cetacean strandings will also be discussed.

[358] CETACEAN MORBILLIVIRUS AND POXVIRUS EPIDEMIC IN BOTTLENOSE DOPHINS ASSOCIATED WITH A SEASONAL WATER QUALITY DECLINE

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From June to October 2009 six bottlenose dolphins (*Tursiops aduncus*) from a resident population of 20 to 25 animals were found dead in the Swan-Canning Estuary, Perth, Western Australia. Opportunistic bacterial and mycotic infections, severe ulcerative dermatitis, and lymphoid depletion suggested reduced immune competence. Ulcerative dermatitis was associated with intracytoplasmic eosinophilic inclusions typical of cetacean poxvirus. Morbillivirus antigen was detected by immunohistochemistry in lymphocytes, endothelium and biliary epithelium from two dolphins. We hypothesize that morbillivirus-induced immunosuppression exacerbated poxvirus dermatitis causing unusually severe ulcerative dermatitis and predisposed to bacterial and *Aspergillus spp*. infections. Blubber toxicology also revealed high levels of pollutants such as dieldrin (mean: 17.13 µg/g lipid). Temporal analysis of this event and historical records found an association between mortalities and seasonal flushing of the estuary by heavy spring rains and marked salinity declines. A similar event occurred among resident bottlenose dolphins in the Gippsland Lakes, Victoria, in October and November 2007. It appears that the Swan-Canning Estuary dolphin population, and likely other costal resident bottlenose dolphin stocks, are regulated by a balance between pathogens, environmental stressors and possibly, anthropogenic pollutants.

[359] IS THIS NORMAL?

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It is a well known fact that female birds can lay their eggs in nests of other birds. This behavior can be observed in birds within the same species and is then called intraspecific nest-parasitism. This is observed in ducks such as common goldeneye (*Bucephala clangula*) and ruddy duck (*Oxyura jamaicensis*). Interspecific nest-parasitism is when birds place their eggs in nests of other species, like the cuckoo (*Cuculus canorus*). Nest-parasitism is by many ecologists regarded as a normal behavior and explained in different ways. However, we have during studies of thiamine (vitamin B_1) deficiency since 2005 at several localities in the Baltic sea, in gulls (*Laridae* sp.) and common eider ducks (*Somateria mollissima*) among other sea birds, observed an increasing number of eggs from one species placed in nests of another species. During this study, on one affected island in Blekinge archipelago (southern Sweden) there were eggs from other species observed in nests of common eider, barnacle goose (*Branta leucopsis*), herring gull (*Larus argentatus*), mallard (*Anas platyrhynchos*), Canada goose (*B. canadensis*) and mute swan (*Cygnus olor*) in a percentage between 2 and 15 per cent of the nests. This high misplacing percentage of eggs among these species has not been observed back in time at localities along the Swedish coastline in the Baltic Sea. We have also observed foreign items such as stones and bits of plastic waste in nests of gulls, geese, swans and ducks, that the birds apparently have collected to include into the clutch. Although some ecologists may regard this behavior as normal, we consider the observed placing of eggs in nests of other species in this seabird colony as unnatural, since the offspring most certainly will die from starvation and/or other erroneous attendance of the adult parent(s). Thiamine deficiency is well-known to cause damaged brain functions, including altered behavior. Since we have found severe thiamine deficiency in both adult birds and chicks of ducks, geese and gulls from islands where this behavior is observed, our main working hypothesis is that this egg-laying disorder may have its origin in the deficiency of thiamine. The relationship between misplacing of eggs and thiamine deficiency will be further studied.

[360] CAUSES OF DISEASES AND DEATHS OF FRENCH PROTECTED WILD BIRDS BY USING THREE PASSIVE SURVEILLANCE SYSTEMS

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Causes of diseases and deaths of game birds in France are diagnosed by the SAGIR network and are well known. Regarding French protected wild birds, those causes remained to be studied. This was the main goal of this study. To do so, we used data from three different passive surveillance systems of wildlife diseases: the SAGIR network[2], the CVFSE[1] and the RFPVFS[3]. The other goals were to assess those systems and to determine ways to improve the knowledge on wild birds' health status in France.

Causes of diseases and deaths of three groups of birds' species sharing the same risks (birds of prey, water birds, garden birds) were determined in a descriptive epidemiological approach. Traumatic injuries were the main casualties (37%). Intoxications were frequently diagnosed on birds of prey (80%) while avian botulism and oil pollution affected mostly water birds. Infectious diseases were sparse (8%) and involved mainly water birds and garden birds. Our results were consistent with previous studies from Europe or North-America. However in our study casualties were indefinite in one third of the cases. The three passive surveillance systems seem to be complementary because they cover different area and involve different species of birds. However they have different and variable sensitivities. An improvement of the diagnostic abilities of those systems is necessary to increase their sensitivities and to reduce the high proportion of indefinite causes of diseases and deaths of French protected wild birds.

[361] SEROLOGICAL SURVEILLANCE OF BAGAZA VIRUS IN GAME BIRDS AFTER THE 2010 OUTBREAK IN SOUTHERN SPAIN

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In September 2010 an outbreak of Bagaza Virus (BAGV) occurred in Southwestern Cádiz (Andalusia, Spain) affecting Red-legged partridges (*Alectoris rufa*) and Common pheasants (*Phasianus colchicus*). Apparently at least, no other bird species were affected. BAGV had only been reported in Subsaharan Africa and India and thus the 2010 outbreak meant the first introduction of a new Flavivirus into Europe. After the outbreak, a surveillance program was established in order to determine the epidemiological situation in the affected and surrounding areas. During the next hunting season (October-December 2011), samples from hunted-harvested partridges (n=132) and pheasants (n=13) were collected in 10 hunting properties in the affected province (Cádiz). No relevant mortalities in the monitored populations were observed in this period. Serum samples were tested for antibodies against BAGV by micro virus-neutralization test (VNT). Mean seroprevalence was 19% ± 6.7% in partridges and 61.54 ± 26.4% in pheasants. Specific antibodies were found in 7 out of 10 sampled locations. Although antibodies were detected all over the sampling period, seroprevalence was higher in those sites sampled in December. This fact together with seropositivity observed in juvenile partridges suggests that BAGV circulated in the area during the monitoring season.

[362] BUILDING A MODEL OF SPREAD OF HPAIV H5N1 IN AQUATIC WILD BIRD POPULATIONS AS A TOOL FOR AN EARLY WARNING SYSTEM

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The spread of HPAIV H5N1 was investigated using an agent-based, stochastic epidemiological model assuming a three-species scenario with intra- and interspecies transmissions in Mallards, Mute Swans and Common Pochards at fixed geographic localities and during migration. Starting with a single-location scenario, each modelling step simulates randomized contacts between individuals of different model compartments (SEIR). Different characteristics of the considered species, environmental factors mediating or facilitating virus spread and all year-round LPAI prevalence with focus on homo- and hetero-subtypic immunity were taken into account. The onset of migration involved three locations and the basic assumptions regarding migratory behavior were as follows: Mute Swans were present at all three sites as purely resident/sedentary populations; Mallards undertook a simplified "stepping-stone" migration with a prolonged stay at a staging site, whereas Pochards reached the wintering grounds in a single movement. Burn-in runs had highlighted parameters to which the model reacted sensitively. Changes in the overall length of the epidemic course, as well as amplitude and time point of maximally infected birds were used as readouts. If scenarios were related to the onset of migration, the chance of migration-capable birds propagating virus and spreading it over larger distances was examined. The results were evaluated to come up with proposals for optimized surveillance strategies.

[363] REVIEW OF SURVEILLANCE FOR AVIAN INFLUENZA IN WILD BIRDS IN GREAT BRITAIN

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The aim of this study was to compare the performance of the surveillance system for detecting Avian Influenza virus (AIV) infections in wild birds in Great Britain (GB) before and after a change in surveillance design. The AI wild bird surveillance (AIWBS) system in GB comprised two sampling components: (i) wild birds that were live trapped or shot (active surveillance) and (ii) birds found dead (passive surveillance). In October 2008, the AIWBS system in GB was changed to focus on the detection of wild birds found dead by warden patrols and decrease the number of birds tested through public reporting of dead birds. Active surveillance was also decreased, with the total number of live-trapped birds reduced and cessation of shot bird sampling. We investigated the impact of these changes by comparing the 12-month period prior to October 2008 with the subsequent 12-month period. For each surveillance system component we considered: (i) the number of wild birds tested, (ii) whether birds tested were considered Higher Risk Species, (iii) the location of birds tested with respect to priority areas for surveillance due to an increased risk of H5N1 HPAI incursion into commercial poultry and (iv) probability scores that each component would yield detections of H5N1 HPAI, H5 or H7 LPAI or all AIV compared with detection rates in the European Union from 2006-2008. Our results show differences in surveillance performance pre- and post- AIWBS design changes, particularly for H5N1 HPAI.

[364] ACTIVE AVIAN INFLUENZA (H_5N_1) SURVEILLANCE IN SEASHORE BIRDS IN COSTA RICA

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The increasing consumption of poultry products in Costa Rica and other Central American countries has created a very well established poultry industry in Costa Rica. Costa Rica is part of the migratory routes to birds from North, Central and South America. Several features such a geography, extensively kept domestic birds and wildlife bird populations generate the risk that avian influenza virus carried by wild birds could spread into local poultry populations and humans. This makes it necessary to monitor the presence of avian influenza virus and especially highly pathogenic strains in our country. The aim of this project was to determine the presence of high or low pathogenic avian influenza virus strains in a reservoir population of sea bird. During a three years (2009-2011) time period, a wetland located in the central pacific coast (N 10 01.644, W 084 54.117) was used as sampling area once a month. This area is well known to be used by seashore birds as feeding station and resting place by migratory as well as small groups of resident birds. Following international biosecurity recommendations, birds were captured using mist nets. All birds were cloacal and traqueal sampled using sterile swabs, which were placed inside sterile transport medium and stored in liquid nitrogen for 24-36 hours before being sent to the laboratory. A total of 380 birds belonging two families of seashore birds (*Scolopacidae and Charadriidae*) plus 30 specimens belonging to otherbird families were also assessed. All the swaps were submitted to RNA extraction (QIAamp®MinElute® Virus Spin Kit) and cDNA transformation (Kit RevertAid by Fermentas). Pools of five samples belonging to the same species were prepared. We followed the *Fouchier et al* 2000 PCR protocol using a Gene H and N Matrix as a sample to screen for the presence of any Avian Influenza A. In the total 410 samples tested we could not find evidence of AI. However, we cannot simply assume that the virus is not present in this population just because we did not find any positive case. Nevertheless, according to the study design the actual prevalence among the seashore birds must be less than 5%. It is necessary to maintain this surveillance forlonger to be certain that the virus is not present or if it were, which strain could be circulating among these bird populations.

[365] EMERGENCE OF A NOVEL AVIAN POX DISEASE IN BRITISH TIT SPECIES

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Avian pox is a viral disease with a wide host range and is considered endemic in a range of non-Paridae hosts in Britain. Avian pox in Paridae species was first diagnosed in a great tit (*Parus major*) from south-east England in 2006 with large skin lesions. We utilised a database of opportunistic reports of garden bird morbidity and mortality (2006-2011) to analyse spatial and temporal patterns of avian pox throughout Britain. A number of Paridae species are susceptible to Paridae pox, however, the majority of incidents involved great tits with multiple individuals affected. Results showed an annual seasonal peak of reports in August/September, likely driven by environmental and demographic factors. Spatial spread of Paridae pox from an origin in south-east England was also observed. Sequencing of the avian poxvirus 4b core protein produced a single sequence from each of 30 great tits tested from Britain. This sequence was identical to that of avian pox from great tits in central Europe and Scandinavia. In contrast, sequence variation was evident amongst virus tested from multiple non-Paridae hosts. Our findings show Paridae pox to be an emerging infectious disease in wild birds in Britain, possibly originating from viral incursion from central Europe or Scandinavia. Ring recovery data do not support great tit migration as a likely route of viral spread; instead vector movement, perhaps via wind-borne spread, offers a more likely explanation for the method of incursion.

[366] IDENTIFYING FACTORS ASSOCIATED WITH THE SPATIAL DISTRIBUTION OF WEST NILE VIRUS IN WILD BIRDS IN NORTHERN GREECE

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West Nile Virus (WNV) has been isolated from avian species worldwide. Birds play an important role in the epidemiology of WNV as the main amplifying host of the virus in nature. From the other hand bird migration provides a mechanism for the establishment of new endemic foci of disease at great distances from where an infection was acquired. Many different types of habitats (agro ecosystems, wetlands, rivers, forest etc.) are located in Northern Greece counties such as Thessaloniki and Serres, which are used by birds for resting, feeding etc., during their migration from Europe and Greece to Africa and the opposite. This study was conducted in order to identify environmental factors from areas where different species of birds found to be exposed to WNV associated with WNV distribution using the ENFA (Ecological Niche Factor Analysis) method and GIS. Out of the 295 bird samples belonging to different species, namely mallards (*Anas platyrynchos*), European turtle doves (*Streptopelia turtur*), European magpies (*Pica pica*) and hooded crows (*Corvus cornix*), 53 were found seropositive to WNV. ENFA method showed that irrigated cultivations, distance from permanent water and altitude are important attributes for the prediction of spatial distribution of WNV. Spatial analysis with GIS is a useful tool in mapping areas with high risk of exposure to WNV and can provide the authorities information in order to control the spread of the disease in human and animal populations.

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[367] MOVEMENTS OTHER THAN MIGRATION MAY ALSO MATTER: SPATIAL DIMENSIONS OF THE EVOLUTIONARY ECOLOGY OF SEABIRD-PARASITE INTERACTIONS

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It is clear that migration is likely to play an important role in the dynamics of host-parasite interactions, but other rather neglected types of movements may also be important. For instance, the dispersal of individuals among spatially subdivided breeding populations, but also the mere visits of individuals prospecting potential breeding patches, may contribute to the dynamics of interactions between host and parasite phenotypes. Focusing on a study system involving seabirds (notably the Black-legged kittiwake *Rissa tridactyla*), their ectoparasitic tick *Ixodes uriae* and Lyme disease agent *Borrelia burdgoferi sensu lato*, the implications of these other types of movements and of the spatial dimension of host-parasite interactions will be illustrated by results obtained using a combination of ecological approaches, from field observation, population genetics, serology to specific experiments. It is argued that studies host-parasite systems with contrasting migratory behaviours can lead to a better understanding of key spatial processes which are of broad eco-epidemiological relevance.

[368] CONJUNCTIVITIS, RHINITIS, AND SINUSITIS IN CLIFF SWALLOWS ASSOCIATED WITH *MYCOPLASMA STURNI* INFECTION AND CRYPTOSPORIDIOSIS

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Fledgling cliff swallows developed clinical signs of ocular disease characterized by conjunctivitis, epiphora, and hyperemia of palpebrae and nictitans. One cliff swallow died, and three were euthanized due to poor therapeutic response. Mycoplasma cultures and molecular diagnostics identified *Mycoplasma sturni*. Histopathologic examination revealed lymphoplasmacytic conjunctivitis, rhinitis and infraorbital sinusitis with follicular lymphoid hyperplasia, epithelial hyperplasia, and protozoal stages compatible with *Cryptosporidium* sp. Identification of concurrent *M. sturni* and *Cryptosporidium* sp. infections in these cliff swallows demonstrates an alternative infectious condition that can produce gross and microscopic lesions comparable to those commonly observed in *M. gallisepticum* infections of house finches and other passerine species. Conjunctivitis associated with *M. sturni* and *Cryptosporidium* sp. may represent an emerging disease of considerable risk to a naïve, high density and colonial species such as colony nesting cliff swallows.

[369] *MYCOPLASMA COROGYPSI* ASSOCIATED POLYARTHRITIS AND TENOSYNOVITIS IN BLACK VULTURES

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Three American black vultures were presented to rehabilitation centres for inability to fly. Clinical examinations revealed swelling of multiple joints including elbows, stifles, hocks and wrist joints, and of the gastrocnemius tendons. Radiographic exam in 2 vultures demonstrated periarticular soft tissue swelling and irregular articular surfaces with subchondral bone erosion. Antibiotherapy did not improve clinical signs. Necropsy and histology demonstrated a chronic lymphoplasmacytic arthritis involving multiple joints and gastrocnemius tenosynovitis. Articular lesions varied in severity and ranged from moderate synovitis and cartilage erosion and fibrillation to severe synovitis, diffuse cartilage ulceration, subchondral bone loss and/or sclerosis, pannus, synovial cysts, and epiphyseal osteomyelitis. Mycoplasma PCR was positive in at least one affected joint from each bird. Sequencing 16S rRNA gene amplicons identified *Mycoplasma corogypsi. M. corogypsi* is a likely cause of arthritis and tenosynovitis in black vultures, and similar cases in New World vultures should be investigated for *Mycoplasma* spp., especially *M. corogypsi*.

[370] DEVELOPMENT OF MOLECULAR TOOLS FOR THE IDENTIFICATION OF *EIMERIA* SPP. IN KIWI (*APTERYX* SPP.)

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Infection with coccidial organisms may cause significant morbidity and mortality in kiwi (*Apteryx* species). These parasites have been shown to affect multiple organs in kiwi, including both enteric and extra-enteric tissues. Examination of sporulated oocysts confirms at least two species of coccidia infecting kiwi are *Eimeria* spp., and histological studies of the endogenous stages suggest kiwi may be infected with at least three intestinal and one extra-intestinal species of coccidia. In this study, we describe the development and application of molecular diagnostic tools for the detection of *Eimeria* species that infect kiwi. Species-specific primers were designed from both the first and second internal transcribed spacers (ITS-1 and ITS-2) of ribosomal DNA sequences obtained from cloned isolates. Using polymerase chain reaction (PCR), various faecal and tissue samples from infected kiwi were examined to identify and discriminate various species of *Eimeria* for the first time.

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[371] TRICHOMONOSIS IN AN EURASIAN SPARROWHAWK IN FRANCE

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An adult female Eurasian Sparrowhawk (*Accipiter nisus*), was found shut in a pigeon house in February 2011 near Nantes (North-West France). The physical examination revealed a wound on her head and buccal capillariosis (ova observed from a scrape preparation). The wound and buccal capillariosis healed rapidly with treatment but the animal suddenly died, just before the planned release, after a three-week period of hospitalization. At necropsy, we observed multiple, oval, oesophageal ulcers, from 2 to 7 mm in diameter, one of them perforating the wall, leading to a subcutaneous focal extensive inflammatory lesion. Histologic examination revealed a multifocal, severe, acute, necrotic, focally transmural, oesophagitis, with severe superficial bacterial proliferation and the presence of flagellates consistent with *Trichomona*s sp. A PCR targetting the ITS1/5.8S/ITS2 ribosomal region of *Trichomonas gallinae* was performed on DNA extracted from oesophageal lesions. The result was positive, with 100 % sequence identity to published *T. gallinae* isolates from columbids in a range of countries and also the UK strain associated with epidemic mortality in British and French finches in recent years. Additional research is required to investigate the impact of finch trichomonosis on European predatory or scavenging wild bird populations.

[372] FIRST OUTBREAKS OF TRICHOMONOSIS IN YELLOWHAMMERS (*EMBERIZA CITRINELLA*) IN FINLAND

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The protozoan avian parasite, *Trichomonas gallinae*, emerged in passerine birds in Finland in 2008. Trichomonosis has since caused considerable mortality among greenfinches (*Carduelis chloris*). So far, the disease has apparently had only minor influence on other species. Cold winters limit the spread of the disease as *T. gallinae* cannot survive freezing. Yellowhammer (*Emberiza citrinella*) is a granivorous bird like the greenfinch but unlike greenfinch, forms large flocks that visit feeding places only in winter. In January 2012, increased mortality among yellowhammers was reported at a feeding place in Central Finland. Necropsy and parasitological examination of two birds confirmed ingluvitis caused by *T. gallinae* as the cause of death, for the first time in Finnish yellowhammers. After this, similar outbreaks were reported in 8 other locations (12 birds examined) in different parts of the country during January – March 2012. Chronic lesions suggested a prolonged course of disease. Exceptional weather conditions most likely facilitated the spread of disease: December 2011 was the warmest since the emergence of trichomonosis with mean temperature above or at 0ºC in South and Central Finland. For the first time, winter flocks of yellowhammers could be exposed to *T. gallinae* at feeding places shared with greenfinches. Changing climate and the behaviour of birds are major factors in the spread of trichomonosis. New populations may be affected by *T. gallinae* in the future.

[373] AFLATOXIN INHIBITS PHAGOCYTIC ACTIVITY OF MACROPHAGES IN BOBWHITE QUAIL

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An increasingly common practice in the United States is baiting roads with grains to facilitate quail harvest. However, baiting roads might have a negative impact on quail survival due to aflatoxin. Aflatoxins are potent disease agents, which can occur in wildlife feed beyond recommended levels. Also, it is estimated that 30-50% of quail shot during the hunting season are wounded and not retrieved, the prognosis of which is unknown. We investigated the likelihood of survival of wounded northern bobwhites (*Colinus virginianus*) exposed to aflatoxin. Twenty wild-caught bobwhites were randomly divided into those that received an oral dose equivalent to 100ppb aflatoxin once a week for 4 weeks and a control group. We surgically implanted each bird with one pellet of number 6 shot into the peritoneal cavity. The peritoneal cavity of each bird was washed 5 days later, macrophages counted, infected with *Candida* cells, and fixed with 4% formaldehyde. The above experiment was repeated sans the *Candida* to determine mortality of birds. Bobwhites within the control group produced more macrophages, had a greater percentage of phagocytic macrophages, and internalized more yeast cells per macrophage than those in the treatment group. Nine of 10 birds within the aflatoxin group died due to infection within 12 days of pellet implantation, while 3 of 10 birds died within the control group. Aflatoxin in combination with wounding during the hunting season decreases the chances of bird survival.

[374] ACUTE EFFECTS OF AFLATOXIN ON NORTHERN CARDINALS

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Urban and suburban landowners use backyard feeders to attract birds for viewing and photography. However, supplemental feeding of birds, although well intended, may present an insidious problem in the form of aflatoxin at backyard bird feeders. Aflatoxin is a widely occurring and harmful mycotoxin produced by strains of *Aspergillus* spp. growing on grain. Our study investigated the concentration of aflatoxin needed to impair normal physiological responses and induce acute morbidity and mortality in northern cardinals (*Cardinalis cardinalis*). Eighty wild-caught adult cardinals (40 males and 40 females) from southern Texas were randomly assigned to each treatment group (N = 10; 0, 25, 50, 75, 100, 500, 1,000, and 2,000 ppb aflatoxin). One-hundred microliters of aflatoxin, derived from *A. flavus*, were orally administered once per week for 4 weeks. Bird weight, daily feed consumption, liver histology, and blood chemistries were determined. An *in vitro* white blood cell proliferation test was conducted using spleen tissue to determine the effect of aflatoxin on immune response. Mortality was not observed in the control group, whereas mortalities were <10% in cardinals that received <100 ppb aflatoxin, but >77% in cardinals that received >100 ppb aflatoxin. LD_{50} for cardinals appears to be a single dose of 500 ppb aflatoxin. Aflatoxin reduced cardinal feed consumption, weight, and affected blood chemistry levels that indicated cellular damage to the liver. Immune response of cardinals given >25ppb aflatoxin were <70% of those birds in the control group. Short-term, acute doses of aflatoxin are harmful to northern cardinals.

[375] MONITORING THE EFFECTS OF PLANT PROTECTION PRODUCTS ON BIRDS AND MAMMALS: A FRENCH NATIONAL ACTIVE PROGRAM

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Bird and mammal risk assessments for plant protection products are carried out under EU regulation using the guidance document issued by EFSA (Sanco/10997/2009) which outlines the procedure for a range of scenarios and proposes different options for refinement. Field studies designed to detect non-intentional effects of plant protection products in fields are one of the options. Moreover, post-authorization data may be additionally useful to assist in the regulatory process. This is the case of PPP in solid formulations like granules or seed treatments where the first tier assessment usually does not match the regulatory trigger by a great extent. A specific bird and mammal monitoring national program has been performed for 3 years by the French SAGIR wildlife network during the drilling period of methiocarb-treated rape seeds and use of molluscicides from 2009 to 2011. Any dead or moribund bird or mammal in or in the vicinity of oilseed rape fields was collected and systematically analyzed for methiocarb and metaldehyde, and eventually for other chemicals according to the carcass finding context. From this monitoring, 1 case was attributed to methiocarb and 3 to metaldehyde poisonings on 207 cases detected. Although the absence of recorded incidents does not necessarily indicate a low risk, for our case, the high theoretical predicted risk is not confirmed. Even if results cannot be quantitatively analysed, SAGIR surveys are able to highlight unusual mortality phenomenon.

[376] ACTIVE WIDLIFE MONITORING PROGRAMS FOR ASSESSMENT OF ACUTE AND SHORT-TERM RISK TO BIRDS AND MAMMALS FROM GRANULES AND SEED TREATMENTS

Emmanuelle Bonneris[1,4], Ralf Barfknecht[2], Pascal Buffet[3], Xavier Crébassa[3]
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Regulatory authorities require comprehensive environmental risk assessments to be conducted before a pesticide can be registered for use on specific crops. As mentioned in the guidance document issued by EFSA (EFSA, 2009[1]), an holistic weight-of-evidence approach based on the results of laboratory studies, modelling exercises, field studies, and available incidents reports can be presented as final step of the risk assessment to birds and mammals. However, in some case, in order to confirm under practical conditions of use the efficacy of the mitigation measures and get additional information, post-approval monitoring programs may be requested. This is the case for some pesticides in solid formulations like granules (i.e. metaldehyde) or seed treatments (i.e. imidacloprid) known for their acute toxicity to birds. In such context, two specific active wildlife monitoring programs were initiated in autumn 2011 during the drilling period of cereal seeds, one for treated seeds and the second for the use as molluscicide using similar protocols. 167 farmers participated to these programs in different locations in France covering 3023 ha of treated cereal fields. Farming practices were recorded. Bird observations and carcass searches were done involving farmers. No abnormal behaviour was observed on survey fields. Four bird carcasses were discovered. According to all information available, none of the birds found is considered to have died as a result of organophosphates, carbamates, metaldehyde and imidacloprid uses.

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[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (Sanco/10997/2009). EFSA Journal 2009 7(12) : 1438 [358 pp.]; Available online: www.efsa.europa.eu

[377] EVALUATION OF 12 INORGANIC ELEMENTS IN COMMON GUILLEMOTS (*URIA AALGE*) FROM THE PRESTIGE OIL TANKER ACCIDENT

Percy Rodríguez[1], Julián Alonso[1], María Ángeles García[1], Marcos Pérez-López[2], and María Julia Melgar[1,3]

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Seabirds are very sensitive to the contamination of oil; in many cases these have elevated levels of inorganic toxic elements. The purpose of this study is the evaluation of 12 inorganic elements: arsenic, cadmium, cobalt, copper, chromium, iron, manganese, mercury, lead, selenium, vanadium and zinc in common guillemots dead during the Prestige oil tanker accident (Galicia, Spain) in November, 2002, collected from the Recovery Center of Oleiros (A Coruña - NW Spain). There were used samples of liver and kidney of seabirds affected by the petroleum (46) and other group of clean animals (8) as a control. After microwave acid digestion of the samples, the levels of the 12 inorganic elements were measured by inductively coupled plasma - mass spectrometry (ICP-MS). The results were transformed to obtain a normal distribution with the Box-Cox method, then the levels of the 12 inorganic elements of the two groups (seabirds affected by petroleum and clean animals) were compared, and a significant statistical difference ($p < 0.05$) was observed for selenium in liver, and additionally arsenic and manganese in kidney. These levels were more elevated in seabirds affected by petroleum when compared to clean animals. These results suggested that petroleum produces an acute increase of some toxic and essential elements on seabirds.

Acknowledgements: This work was financially supported by Xunta de Galicia (INCITE08PXB261087PR).

[378] FATAL IMPACT OF TWO GOLDEN EAGLES (*AQUILA CHRYSAETOS CHRYSAETOS*) IN THE AUSTRIAN ALPS

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Due to protection laws the population of Golden eagles (*Aquila chrysaetos chrysaetos*) has increased continuously in the central Alps since the middle of the last century. Today the density of Golden eagles has reached the level of saturation in some Alpine areas. The main regulation mechanism for the population density is the reduced reproduction success of breeding pairs due to increased territorial activities against frequently intruding non-territorial single eagles. These territorial activities often results in aggressive intraspecific conflicts with sometimes lethal consequences. We report a fatal impact of two Golden eagles in the province of Vorarlberg in Austria in September 2008. The two eagles descended at what was probably very high speed, going straight through the roof of a house situated in the peripheral area of a small town. Both eagles were female, one adult and the other one subadult. The gross section of the two birds, both in a good condition, yields polytraumatic fatal lesions. The post mortem examination gave no definitive evidence of the reason for the incident. We conclude that the two eagles were fighting in the air by digging their talons in each other, interlocking in such a way that neither of them was able to get free, resulting in a loss of flight control and the impact.

[379] PHYLOGENETIC RELATIONSHIP OF *TRYPANOSOMA* SP. IN SPECTACLED CAIMAN (*CAIMAN CROCODILUS*) FROM MATO GROSSO, BRAZIL

Arlei Marcili, Andrea P. Costa, Herbert S. Soares, Igor L.C. Acosta, Julia T.R. Lima, Antonio H.H. Minervino and Solange M. Gennari

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Trypanosoma genus comprises flagellated protozoa able to infect fishes, amphibians, reptiles, birds and mammals. In Crocodilydae family three trypanosomes specie were described, *T. grayi* in African crocodilian and *T. cecili* and *Trypanosoma* sp. in caimans species from Amazon and Pantanal region in Brazil. *T. grayi* was transmited by tse-tse flies and the vector of Brazilian caimans trypanosomes is unknown. We characterized first Brazilian trypanosome isolated from Spectacled Caiman (*Caiman crocodilus*) in Confresa County from Mato Grosso State in Brazil. Morphological findings in epimastigotes forms from axenic culture showed high similarity with *Trypanosoma* sp. from *Caiman yacare* described in Brazilian Pantanal. Phylogenetic studies performed with SSU rDNA and *g*GAPDH (glyceraldehydes-3-phosphato dehydrogenase glycosomal) supports clustering of *C. crocodilus* trypanosome in *T. grayi* Clade and together to genotype Cay 01 from unnamed specie from *C. yacare*. The phylogenetic position of this new isolate suggests sympatric species of vertebrate hosts of same vector for both species.

[380] A NON-INVASIVE METHOD FOR HEAVY METALS DETERMINATION IN LOGGHERHEAD TURTLE (*CARETTA CARETTA*) CARAPACE BY SQUARE WAVE ANODIC STRIPPING VOLTAMMETRY (SWASV)

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The aim of this study was to determine the concentration of copper (Cu), cadmium (Cd), lead (Pb) and mercury (Hg) in plasma and carapace samples of loggerhead turtles (*Caretta caretta*) catched around Linosa Island. A simple and portable instrument was chosen to estimate potential hazard level of heavy metal and to evaluate carapace as a suitable target organ for non-invasive monitoring of heavy metals in sea turtles. Concentrations of Cu, Cd, Pb and Hg were measured by using an electroanalytical method based on square wave anodic stripping voltammetry (SWASV) coupled to an acid digestion protocol. Eleven loggerhead turtles (seven juvenile and four subadult specimens) were accidentally caught in commercial fishing nets and then brought to the Linosa's Turtle Rescue Center for a first physical examination. After biometric measurement, blood and carapace samples were collected for heavy metal analysis. Mean concentrations of Cu, Cd, Pb and Hg were 9.75±0.06, 0.44±0.49, 2.97±1.63, 0.36±0.05 μg/g in carapace and 479.8±132.3, 268.7±, 12.29 44.3±99.45, 5.46±0.25 ng/g in plasma samples, respectively. The results are in agreement with previous studies conducted on Mediterranean turtles Cu, Cd and Hg levels in the carapace do not correlate with plasma concentrations. By contrast, carapace Pb levels were correlated with plasma concentrations ($p<0.05$) suggesting that the carapace could be a useful non-invasive indicator for monitoring lead levels in the live sea turtles.

[381] ROAD-KILLED COMMON TOADS (*BUFO BUFO*) IN FLANDERS (BELGIUM) REVEAL LOW PREVALENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* AND RANAVIRUSES

An Martel[1,3], Mojdeh Sharifian Fard[1], Pascale Van Rooij[1], Robert Jooris[2], Francine Boone[3], Freddy Haesebrouck[1], David Van Rooij[4], and Frank Pasmans[1]

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Healthy amphibians can be asymptomatic carriers of diseases responsible for devastating amphibian population declines. Most importantly, ranavirus and *Batrachochytrium dendrobatidis* are primary pathogens of amphibians, and disease outbreaks are believed to be driven by environmental factors. A thorough risk analysis to predict future scenarios in indigenous amphibian species should include the identification of principle pathogen reservoirs. In the spring of 2011, 1740 road-killed common toads were collected from 104 migration sites in Flanders. A qPCR for detection of DNA of *B. dendrobatidis* was performed on a skin sample of each toad. For detection of ranavirus, a liver sample was examined by PCR. To assess whether the presence of one or both pathogens in a toad population coincides with obvious population declines, numbers of migratory toads of 2011 and previous years were extracted using the database obtained from the working group Natuurpunt Hyla. None of the samples were PCR-positive for ranaviruses. At nine sites, 11 *B. dendrobatidis*-positive toads were found (0.63% prevalence). Levels of *B. dendrobatidis* in the positive samples were relatively low with an average of 0.25 genomic equivalents ±0.11 (SD). For all positive sites, no significant decrease in the number of migrating toads was observed during the years monitored.

[382] PATHOGEN DETECTION IN INVASIVE BULLFROGS (*LITHOBATES CATESBEIANUS*) IN BELGIUM AND THE NETHERLANDS

An Martel[1,3], Connie Adriaensen[1], Mojdeh Sharifian-Fard[1], Annemarieke Spitzen-Van Der Sluijs[2], Gerald Louette[3], Sander Devisscher[3], Tim Adriaens[3], Kristof Baert[3], Ben Crombaghs[4], Freddy Haesebrouck[1], and Frank Pasmans[1]

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Exotic invasive bullfrogs (*Lithobates catesbeianus*) are considered to exert a considerable negative impact on native amphibian communities. This can be due to competition and predation, but they are also a notorious source of the infectious diseases chytridiomycosis and ranavirosis, affecting amphibian populations globally. Little is known regarding their carriage of other microbial agents that might be transferred to humans or other animals. In this study we determined the occurrence of the amphibian pathogens Ranavirus and *Batrachochytrium dendrobatidis* and of the zoonotic agents *Coxiella burnetii*, *Neospora caninum*, *Leptospira* sp., *Toxoplasma gondii*, *Mycoplasma* sp., *Campylobacter*, *Salmonella* and ESBL producing *E. coli* in 164 bullfrogs from Belgium and The Netherlands. *B. dendrobatidis* was present in 104/164 samples. All tested samples were negative for the other infectious agents examined, suggesting a limited role of bullfrogs as carrier of these pathogens.

[383] RANAVIRUS EMERGING IN THE NETHERLANDS?

Jolianne Rijks[1,3,4], Annemarieke Spitzen-Van Der Sluijs[2], Alphons Van Asten[3], Marisca Stege[3], Andrea Gröne[1,3], and Marja Kik[1,3]

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Ranavirus infection was diagnosed for the first time in free-living amphibians in the Netherlands in 2010, when it caused a mass die-off in a pond in National Park Dwingelderveld (NP-Dwingelderveld). It was unclear if the disease was emerging or not. A study was set up with two aims. First, to obtain more insight into ranavirus species present, susceptible host species and geographical spread, the general public was requested to report marked mortality events, and to submit amphibians for post-mortem examination and screening for ranavirus (PCR and sequencing). Second, to obtain more insight into the impact on amphibian populations and factors triggering die-offs, amphibian populations and environmental factors were monitored regularly between May and October in the pond where the 2010 die-off occurred and four other ponds in NP-Dwingelderveld. In total 117 amphibians and 6 fish were submitted for post-mortem examination, corresponding to mortality events at 20 locations outside NP-Dwingelderveld, and 15 monitoring moments in NP-Dwingelderveld. Among other causes of amphibian mortality in 2011, we found evidence for ranavirus-associated mortality in several ponds in NP-Dwingelderveld and four locations outside but in vicinity of NP-Dwingelderveld. All sequenced products showed high homology with that of the ranavirus detected in 2010. Additional amphibian species were found to be susceptible. There was no straightforward relation between environmental factors and observed mortality at monitored sites. The results suggest that the disease may be emerging in the Netherlands, and activities have been proposed to delay the spread of the virus.

[384] ISOLATION AND CHARACTERIZATION OF TWO DIFFERENT RANAVIRUSES FROM EDIBLE FROGS (*PELOPHYLAX KL. ESCULENTUS*) IN SWITZERLAND

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Edible frogs (*Pelophylax kl. esculentus*) were collected from ponds from several European countries for behavioural studies and transported to a research facility in Switzerland. Several days after their arrival in Switzerland frogs began dying with reddening of the skin (legs, abdomen) and haemorrhages in the gastrointestinal tract. A ranavirus was detected from several of the dead frogs using PCR and virus was isolated in cell culture. New animals were added to the surviving group yearly. In the following three years, another two outbreaks with low to high mortality in between asymptomatic periods took place. During the first two years, the same ranavirus was detected repeatedly. Consequently; a new ranavirus was isolated in association with the second mass-mortality event. The two different ranaviruses were characterised based on nucleotide sequences from four genomic regions: major capsid protein (MCP), DNA polymerase (DNApol), ribonucleoside diphosphate reductase alpha (RNR-α) and beta (RNR-β) subunit genes. The obtained sequences showed slight variations to each other and in comparison to frog virus 3 (FV3), the type species of the genus *Ranavirus*. Furthermore, a quiescent infection was demonstrated in two individuals. In retrospect, it was possible to detect a ranavirus in wild amphibians in Germany for the first time.

[385] COMPARATIVE PATHOLOGY OF SELECTED INFECTIONS IN FREE LIVING ANURAN AMPHIBIANS IN THE BRAZILIAN ATLANTIC FOREST

Catia Dejuste De Paula[1], Allan Pessier[2], Julieta Catarina Burke[3], Selene Dall'Acqua Coutinho[3], Kathleen Fernandes Grego[4], Lika Osugui[3], Maria Christina Christovão Ramos[5], Miguel Trefaut Urbano Rodrigues[6] and José Luiz Catão-Dias[7]

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In recent years, amphibian populations have suffered severe declines. Infectious diseases are among the various causes of these processes. This study investigated the occurrence of amphibian diseases in the Brazilian Atlantic Forest, the most threatened biome in Brazil, in order to contribute to better understanding the potential conservation impacts. One-hundred-and-twenty anuran amphibians of 33 different species from the Biological Station of Boracéia, Biritiba- Mirim, state of São Paulo, were collected and submitted to post-mortem examination, followed by histopathology, parasitology, microbiology and conventional PCR to detect *Batrachochytrium dendrobatidis*. At necropsy, endoparasites and ectoparasites were found in 45.8% (55/120) and 0.8% (1/120) of specimens, respectively. *B. dendrobatidis* was detected in 19.1% (22/115) animals. Microbiological results were non-contributory or negative. Significant macro and microscopic lesions were not observed in the vast majority of animals studied. When present, lesions affected mainly the digestive, integumentary, lymphoid and urinary systems, but no correlation with infectious agents was reported. Given the fragile condition of the Atlantic Forest and the susceptibility of anurans to habitat and climatic changes, we believe that long-term studies should be conducted to properly understand how infectious organisms might interfere with the dynamics of amphibian populations in this very unique biome.

[386] A SEVERE PARASITIC NEMATODE INFECTION IN THE SOUTH AMERICAN RED-FOOTED TORTOISE ("MORROCOY") GEOCHELONE CARBONARIA" (CHELONIA; TESTUDINAE) FROM UPATA (BOLÍVAR, VENEZUELA)

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Geochelone carbonaria is one of the two species of tortoises present in Venezuela. In this work we report a severe parasitic infection with strongylid and pinworm nematodes in 05 out of 15 red footed tortoises *G. carbonaria*, after they were captured in a cattle farm in Upata (Bolívar state, Venezuela). This farm is open to tourist and has a wild population of these tortoises which usually "visit"the farm to be feeded. The owner decided to capture them and allocate in a restricted area where they were confined with a fence to show them to the public. A few months later, several animals become sick, showing different behavior, anorexia and lethargy. Faeces were diarroeic, mucoid and haemorragic. Five animals died. A veterinarian took faecal samples and performed the necropsies. Intestines were enlarged, and hundreds of nematode parasites were found inside; mucosae were haemorragic and liver showed fibrin and haemorragic tracts. Faecal samples and part of the intestinal content was sent to Helminthology Laboratory, (FCV-UCV)). Faecal examination showed a severe strongylid infection and intestinal content was plenty of pinworm nematodes identified as *Atractis* and *Thelandros* genus (Order Oxyurida), first time reported in Venezuela. Animals were treated orally with Albendazole (10 mg/kg), once a day for 03 days. A lot of nematodes were expulsed and condition of the animals improved. Stress is a common cause of outbreak of parasitism in recently captured reptiles.

Workshops

Monday July 23rd – 09:30-12:00 & 13:30-16:00

Main organizer	Title
Sonia M. Hernandez	American College of Zoological Medicine Ultrashort course
Ted Leighton	Capacity Development for Wildlife Health Management in Low and Middle Income Countries
Céline Richomme	Biodiversité et santé animale (Workshop in French)
Estelle Rousselet	Red-eared slider turtle and domestic pigeon necropsy: they didn't teach me that in veterinary school! (student workshop)
Marie-Pierre Ryser	Infectious keratoconjunctivitis in wildlife: a comparative approach for new perspectives
Stephanie Shaw	Amphibian chytridiomycosis
Michelle Verant	WNS in cave hibernating bats: pathology, epidemiology, host-pathogen ecology, diagnostic techniques, and management
Lisa Yon	WildTech Technology Transfer Workshop: New Technologies for Screening and Diagnosing Pathogens in Wildlife
Sophie Rossi	Vaccinating wildlife: sharing experience about tools and efficacy

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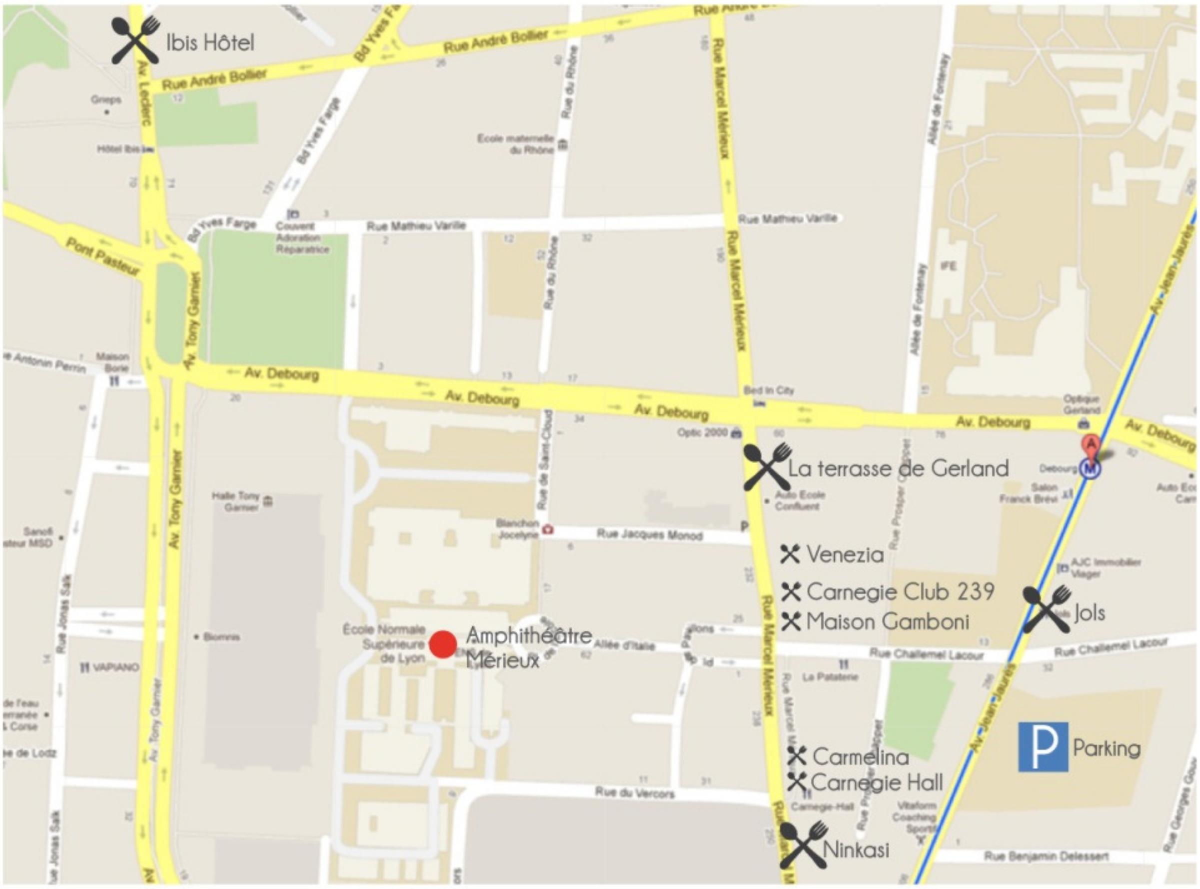

Conference Overview

Sunday July 22nd	08:00-12:30	Business meetings (JWD editorial board) at the Novotel, Gerland
	12:30-13:30	Lunch
	13:30-18:00	Business meetings (WDA council, other) at the Novotel, Gerland
Monday July 23rd	07:45	Bus departure for workshops
	09:00-12:00	Workshops
	12:00-13:30	Lunch
	13:30-16:00	Workshops
	16:00-18:00	Visit of the VetAgro Sup, Veterinary Campus
	19:00-20:30	Ice breaker, Rhône Department House / Hotel du Département du Rhône
Tuesday July 24th	08:30-09:00	Opening Address
	09:00-12:00	One health Session 1
	09:00-09:45	Keynote lecture 1: Aaron Bernstein
	11:00-12:00	Morning Break / Poster session 1
	12:00-13:00	One health Session 2
	13:00-14:00	Lunch
	14:00-15:00	EWDA Business meeting + Latin American WDA Business meeting
	15:00-16:45	One health Session 3
	16:45-17:30	Afternoon Break
	17:30-19:00	Population health assessment Session 1
	19:30	Student / Mentor Mixer
Wednesday July 25th	08:00-09:45	Population health assessment Session 2
	08:00-08:45	Keynote lecture 2: Marc Cattet
	09:45-10:30	Morning Break
	10:30-11:15	Population health assessment Session 3
	11:15-11:30	Migration Session 1
	11:45	Conference trip: visit of the “Parc des Oiseaux”, lunch on site
Thursday July 26th	08:00-09:00	Migration Session 2
	08:00-08:45	Keynote lecture 3: Sonia Altizer
	09:00-10:30	Terry Amundson Award Student Session 1
	09:00-09:30	WDA Student Research Recognition Award: Karoun Bagamian
	10:30-11:30	Morning Break / Poster session 2
	11:30-13:00	Terry Amundson Award Student Session 2
	13:00-14:00	Lunch
	14:00-15:00	WDA Business Meeting
	15:00-16:00	Terry Amundson Award Student Session 3
	16:00-17:00	Afternoon Break
	17:00-18:30	Terry Amundson Award Student Session 4
	18:30	Auction at the Amphitheatre Merieux, ENS
Friday July 27th	08:00-10:15	Disease control Session
	08:00-08:45	Keynote lecture 4: Richard Delahay
	10:15-11:15	Morning Break / Poster session 3
	11:15-13:00	Pathogenesis Session
	13:00-14:30	Lunch
	14:30-15:15	Pathogen discovery and disease emergence Session
	15:15-16:00	Afternoon Break
	16:00-16:45	Translocation and reintroduction Session
	16:45-17:30	Closing lecture: Philippe Berny
	17:30-18:00	Closing address
	19:00	Banquet and Awards Ceremony, Lyon City Hall / Hotel de Ville de Lyon