

## Dynamic Slide Show Abstracts

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### **CANINE DISTEMPER VIRUS AND ITS IMPLICATIONS IN THE CARNIVORE CONSERVATION IN CENTRAL CHILE: THE RISK OF DOG-WILDLIFE COEXISTENCE**

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**AIM OF THE STUDY:** To assess the effect of host population size on the risk of transmission of canine distemper virus from domestic dogs to free-ranging foxes in urban and rural selected sites in a semi desert area in north-central Chile.

**MATERIAL AND METHODS:** In 2003, a CDV outbreak involving two South-American fox species was documented in Chile. In this area there are two main centres of urbanisation connected to a National Park through varying land use. Two transects were created connecting these urban areas to the park: 80 km N-S from Coquimbo (human pop~300,000) and 40 km E-W from Ovalle (human pop~70,000). Seven sites (6.5 km radius) were selected in rural areas. Within each site, household questionnaires were conducted. Blood samples were collected from dogs during the questionnaire survey and from foxes during captures to test antibodies against CDV. Sample sizes were calculated assuming a CDV seroprevalence of 50% in domestic dogs (confidence 90%). Also, abundance of foxes was estimated through scent stations along transects.

**RESULTS:** The seroprevalence of CDV in rural dogs is inversely related to distance from urban sites. CDV seroprevalence of rural domestic dogs is highest in older dogs born before 2003. Scent station and questionnaire data indicate that wild carnivores occur frequently in peridomestic rural environments. CDV incidence in foxes varied widely between years with a peak incidence recorded in 2003, and seasonal peaks in summer months.

**DISCUSSION:** Virulent viruses, such as CDV, require large populations to persist and we suggest that, in Chile, these are likely to occur only in large urban centres. In our study area, the size of the rural dog population is probably below the critical community size required for maintenance but dogs may act as a source of infection to fox populations. The inverse relationship between rural dog seroprevalence and distance to urban centres is consistent with this interpretation. As might be expected in a non-maintenance population, the incidence of CDV in foxes varied widely, with household questionnaire data indicating a peak of cases in 2003 (consistent with the previously recorded epidemic in wildlife) and showing significant seasonal fluctuations. Further analysis will be carried out to investigate factors affecting seasonal prevalence, including climatic factors and inter-species interactions.

**CONCLUSION:** Our results suggest that free-roaming dogs and foxes come into close contact throughout the rural study sites, providing frequent opportunities for disease transmission. A high seroprevalence in domestic dogs born before 2003 further supports a link between CDV patterns in rural dog and fox populations.

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### **ARTIFICIAL FEEDING REDUCES FAECAL PARASITE EXCRETION IN IBERIAN RED DEER: A PSEUDO-EXPERIMENTAL APPROACH**

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**AIM OF THE STUDY:** To establish and correctly interpret infection rates as health monitoring indicators requires the study of the underlying factors. Resource

availability is critical to determine host fitness and capacity to defence against parasites. Therefore, we hypothesize that parasite outputs may reflect individual fitness. Their use would be very valuable to characterize population performance in managed wild ungulates. Our objective was to test any nutrition-mediated effect on the faecal excretion of parasites in wild naturally infected Iberian red deer (*Cervus elaphus hispanicus*).

**MATERIAL AND METHODS:** Two experimental groups of 19 wild red deer hinds were allocated in two contiguous areas (about 14 ha each) which consisted of natural Mediterranean woodlands and open pastures. They both were similar in age, body condition and parasite outputs at the beginning of the trial. One group was provided with a high protein feed, whereas the other group remained as a control and did not receive artificial supplementation. During a two year period, we monthly collected faecal samples which were subjected to coprological analyses to quantify nematode larvae and eggs. We used GLMz to test the effect of food supplementation on faecal parasitic propagules while controlling for the effect of season.

**RESULTS:** Faecal larvae 1 of the meningeal worm *Elaphostrongylus cervi* were highly prevalent in the study animals (overall over 90 %), whereas *Dyctiocaulus* spp was less represented (40 % of the samples). Thrichostrongylidae were the most common gastrointestinal nematode eggs found in the faeces. After controlling for seasonal variation in parasite excretion, both groups statistically differed in *Elaphostrongylus cervi* and Thrichostrongylidae abundances, the supplemented group presenting lower parasite loads than the control group. Although *Dyctiocaulus* spp only presented low abundance figures and there were no differences between groups, it statistically and positively correlated with *Elaphostrongylus cervi* outputs.

**DISCUSSION:** Our experimental design allowed us relating the differences in parasite excretion to food provisioning, since the only differential factor between both groups was the presence of artificial food. We can only speculate about the role of resource availability in reducing, somehow, the parasite excretion levels for several taxa. More evidence regarding the potential involved factors could be distilled from studies of individual traits, which revealed that body condition negatively correlated with parasite loads, and positively affected the size of an immune defence organ (the spleen), which in turn negatively correlated with parasites (Vicente et al. 2007). Therefore, the partitioning of limited individual resources between body functions, which include parasite defence, could explain our results.

**CONCLUSION:** Parasite excretion loads could be useful for monitoring population health status in deer in the context of different management schemes (density, habitat richness, food provision).

## PREVALENCE OF HEPATITIS E VIRUS IN EUROPEAN WILD BOARS

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**AIM OF THE STUDY:** The aim of the present study was to investigate hepatitis E virus infection in European wild boar population in Spain. Also, we aimed to compare HEV strains from Spanish wild boars with other available strains of the virus.

**MATERIAL AND METHODS:** A total of 150 wild boars from different ages (juveniles, sub-adults and adults) and management (open, fenced and intensive) from 8 South-Central Spanish regions [Albacete (n=11), Cuenca (n=11), Guadalajara (n=5), Guadiana (n=31), Montes Toledo (n=28), Ruidera (n=12), Sierra Morena (n=39) and Toledo(n=13)], were collected by the Instituto de Investigación en Recursos Cinegéticos (IREC, Ciudad Real, Spain). Serum samples were tested for specific anti-HEV IgG, IgA and IgM antibodies by in-house ELISA tests and by RT-PCR for

virus detection. Positive RT-PCR samples were sequenced and phylogenetically analysed.

**RESULTS:** Overall, 64 animals (42.7%) were positive for at least one of the immunoglobulins tested and 19.6% (27/138) were viraemic. Virus and antibodies were detected in all studied regions. Viraemia was related with IgM presence but not with IgG neither with IgA. Wild boars intensively reared had higher seroprevalence to IgG and IgM and the analyses by age and management showed that a higher proportion of juvenile animals were viraemic compared to other age groups ( $p < 0.05$ ). On the other hand, juvenile animals in intensive management had higher seroprevalence to IgG and IgA.

Wild boar HEV analyzed sequences showed high percentage of homology between them (77-100%). Moreover, 76.1-99.4% and 78.5-95.8% identities were found between wild boar HEV strains and domestic swine and human VH2 strains, respectively. On that score, wild boar strains were 76.7-91% identical to other domestic swine strains from genotype 3.

**DISCUSSION:** Studies on HEV in wild boars were previously limited to Japan and Australia. The present study represents the first description of HEV infection in the European wild boar. We detected that more than 40% of studied wild boars had anti-HEV IgG, IgA or IgM antibodies and around 20% of them were viraemic. Anti-HEV antibodies and virus were detected in all studied Spanish regions, suggesting the ubiquitous presence of HEV in wild boars, similarly to what has been reported in domestic swine in Spain and other countries like USA, Japan and Taiwan. Moreover, the prevalence of HEV reported in the present study is high when compared with the one reported in wild boars in abovementioned countries. In Australia, a prevalence of 25% to IgG in wild-caught pigs was recorded, while this was of 9% in Japanese captured wild boars. The studied wild boar HEV sequences showed high similarities. Sequences clustered within the genotype 3 together with other Spanish HEV sequences, although a group of them formed an isolated cluster. This may be indicative of the existence of heterogeneity among HEV strains in wild boars, as it has been suggested for domestic swine.

**CONCLUSION:** Results of this study showed that HEV is apparently widespread in Spanish wild boar population since anti-HEV antibodies were detected in all studied Spanish regions. This is relevant considering that wild boar may act as a reservoir for the infection, with implication not only on domestic swine but also public health.

## **RANAVIRUSES: POTENTIAL AGENTS OF EXTINCTION OF AMPHIBIAN COMMUNITIES**

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**AIMS OF THE STUDY:** 1. To examine the potential for the ranavirus to be an agent of extinction in amphibian communities in the United Kingdom. 2. To determine the phylogeography of the Ranavirus in *Rana temporaria* in the United Kingdom. 3. To examine the host diversity and potential sources of introduction of the Ranavirus in the United Kingdom. 4. To establish a cryoarchive of ranavirus isolates from the UK for future work.

**MATERIAL AND METHODS:** Firstly, animals will be screened using PCR primers for the major capsid protein (MCP) of frog virus 3 (FV3). Animals which show the presence of MCP will undergo further analysis, including a screen for two other FV3-specific loci and sequencing of the relevant viral genes. The sequence data will be used to analyze the relationship between the ranavirus(es) found in different areas of the UK. Virus will be isolated from infected individual animals via established methods using the fathead minnow (*Pimephales promelas*) cell line.

**ANTICIPATED RESULTS:** The elucidation of the relationship between ranaviruses found in different populations of *Rana temporaria* and other species which are affected by the ranavirus in the UK. Also, the determination of other species which

have the potential to act as reservoir species and the vectors that had the potential to bring the ranavirus into the UK.

**DISCUSSION AND CONCLUSION:** Emerging infectious diseases have the potential to cause both local extirpation and extinction of species. Ranaviruses exhibit several characteristics which have the potential to result in disease induced extinction. This makes the ranaviruses a concern for conservation efforts in many amphibian species, especially common frogs, as they seem to be the most adversely affected in the UK. Therefore, it is imperative that research into the phylogeography of the ranaviruses, the alternate hosts, and the evolution of the ranavirus present in the UK is performed.

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## **EFFECTS OF LIVESTOCK OWNERSHIP AND INSECTICIDE TREATMENT ON THE RISK OF HUMAN MALARIA: A CASE-CONTROL STUDY IN ETHIOPIA**

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**AIM OF THE STUDY:** In regions where the mosquito vectors of human malaria feed on both animals and humans, the presence of animals close to the household may affect the probability of vector-human contact, and hence the risk of malaria transmission to humans. It has recently been suggested that in these regions, the treatment of livestock with insecticides could be a complementary tool for controlling malaria vectors, and hence reducing the incidence of human malaria, while contributing also for the integrated control of livestock diseases caused by ticks, tse-tse flies and other vectors. As part of my PhD research these questions are being investigated, using three approaches: (1) mathematical modelling, to predict the impact of insecticide treatment of livestock on malaria transmission under different intervention scenarios; (2) linking mathematical modelling with GIS, to identify the African regions where the intervention could be most beneficial; and (3) an observational case-control study in Ethiopia, to assess the effects of livestock ownership and insecticide treatment on malaria risk, which is the focus of the presented poster.

**MATERIAL AND METHODS:** The study was conducted in Southern Ethiopia, where the main malaria vector feeds considerably on livestock. All the laboratory-confirmed malaria cases diagnosed during the peak malaria transmission season of 2004 (June to December), were identified from the local health facilities in Konso district. The household of each malaria case from 30 selected villages was traced and a comprehensive questionnaire was conducted there and in the household of a neighbourhood age-matched control. An array of potential risk factors for malaria was investigated, including: livestock ownership and husbandry practices, human demography, house construction, and environmental surroundings. The statistical analysis was conducted using conditional logistic regression.

**RESULTS:** The study included 107 pairs of malaria cases and matched controls. The results indicate that people who kept livestock, especially cows, within their household compound, were at an increased risk of malaria. Surprisingly, insecticide treated cows seemed to be associated with an additional increase in malaria risk, suggesting a repellent effect that might have diverted the mosquito vector from treated cows to nearby humans.

**DISCUSSION:** Although the effect of insecticide treatment contrasts with findings from experimental studies near the study area and from a community trial in Pakistan, the possible repellent effect in our study might be due to the lower treatment coverage and/or dose of insecticide on the animals.

**CONCLUSION:** Notwithstanding the limitations of the study, the data suggest that the impact of livestock on Afro-tropical malaria deserves further investigation. In particular, a specifically designed large-scale field trial to assess the effect of insecticide treated livestock on malaria transmission in a specific ecological setting in Africa would be useful. It is hoped that the final results from the other two components of this study - the mathematical modelling and the linking of modelling with GIS - may lead to the implementation of such a trial, and contribute to the integrated control of human malaria and animal diseases.

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## **TOXOPLASMA GONDII INFECTION IN WILD CARNIVORES IN THE BASQUE COUNTRY (NORTHERN SPAIN)**

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**AIM OF THE STUDY:** To determine the importance of wild carnivores as reservoir of *Toxoplasma gondii* in the Basque Country by direct detection of the parasite using PCR.

**MATERIAL AND METHODS:** Carcasses of 192 wild carnivores belonging to 9 different species were obtained between 2001 and 2006. These animals were found dead principally with traumatism (mainly run over) or hunted. A complete necropsy was performed on each animal, and the tissues conserved. Brain and heart samples were analyzed individually in all animals by PCR. In wild cats intestines were also analyzed. If any of the samples were positive, all the tissues available from these animals were also analyzed by PCR.

**RESULTS:** *T.gondii* infection was detected in the 9% of the studied animals in at least one of the tissues examined. Eight of the nine species studied were infected but the percentage of animals giving a positive reaction varied significantly among species (1,6%-50%). The parasite was detected in heart, brain, intestine and lung. None of liver or spleen samples from infected animals were PCR positive.

**DISCUSSION:** The prevalence observed and the fact that 8 of 9 species studied were infected, suggest that toxoplasmosis is widespread among wild carnivores in the Basque Country and that they may play an important role in the epidemiology of the sylvatic cycle of the parasite. Concerning previous reports of *T.gondii* infection in wild carnivores several authors have described presence of antibodies in serum samples. Only results published recently in the Czech Republic describe the presence of DNA of *T.gondii* in brain tissues from naturally infected stone martens (4,9%) and red foxes (1,32%).

**CONCLUSION:** We can conclude that this technique serves as a good indicator of *T.gondii* infection among wild carnivore species and this study involves the first report of prevalences *T.gondii* in wild carnivores in the Iberian peninsula.

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## **HEMANGIOSARCOMA IN A WILD RED DEER (*CERVUS ELAPHUS*)**

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**AIM OF THE STUDY:** Since 2001, a Health Surveillance Program for Wildlife surveys the causes of mortality amongst wild animals populations in Wallonia (Southern Belgium). The red deer (*Cervus elaphus*) is one the major wild ungulates present in this region; from September 2001 to December 2006, 128 deer found dead were necropsied. The 3 major mortality causes are paratuberculosis (37/128), polyparasitism (27/128) and traumatic lesions (25/128). Besides these common pathologies, more rare ones like tumors (3/128) have been diagnosed in our study. This abstract reports one of these tumors, a hemangiosarcoma. This kind of tumor has been described only once in deer (case involving an captive aged Père David's deer).

**MATERIALS AND METHODS:** The case concerns a 14-yr-old female red deer found dead in March 2003 in the region of Bièvre (Southern Belgium). The animal was necropsied at the Faculty of Veterinary Medicine, Liege, Belgium. Various samples were collected for histopathology.

**RESULTS:** At necropsy, numerous nodular, well-demarcated, 0,5-3 cm in diameter, red-black, soft to firm masses were scattered throughout the lungs. On section, the masses were dark red and oozed blood. On the left thoracic flank of the animal, a

soft invasive sub-cutaneous mass (approximately 15 cm in diameter) was adhering to the 7<sup>th</sup> rib, with bone lysis. Section of the mass revealed large areas of necrosis and hemorrhage. At histopathology, the paracostal mass and the pulmonary masses displayed a similar morphologic pattern. The latter showed conspicuous accumulation of blood, either filling small clefts or giant cavernous channels, or freely dissecting the tissues (haemorrhages). The clefts or channels were clearly delineated by endothelial cells, some of them were visibly ruptured or thrombosed. The stroma interspersed between channels and clefts was obviously constituted by neoplastic cells, varying in size and shape, but being usually elongated. The nuclei of these cells were round or ovoid, very hyper chromatic and commonly displayed mitotic figures. All the masses comprised numerous macrophages filled with large amounts of hemosiderin. The paracostal mass also displayed very large areas of necrosis, with foci of neutrophilic accumulation.

**DISCUSSION:** These histological features are compatible with a diagnosis of cavernous hemangiosarcoma, a malignant tumor of vascular endothelial cells. The primary tumor probably developed within the 7<sup>th</sup> left rib, then extended to the pleurae and the thoracic muscles and finally, showered the lungs with metastases.

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## **THE PHYLOGENETIC ANALYSIS OF AVIAN POXVIRUS ISOLATES FROM HUNGARIAN, SPANISH AND DUTCH WILD AND EXOTIC BIRDS**

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**AIM OF THE STUDY:** As the potential impact of avian pox on endangered wild bird species can be substantial, it is essential to clarify the question of diversification, host specificity and pathogenicity of avian poxviruses.

**MATERIALS AND METHODS – RESULTS:** We have developed a new PCR system for the detection of avian poxviruses, based on polymerase gene sequences available from GenBank. With this method we have determined nucleotide sequences of fifteen poxvirus isolates originating from wild and exotic bird species, mainly raptors (Booted eagle, Imperial eagle Goshawk, Common buzzard, Red kite, Red-legged partridge, Peacock). A PCR method used for the amplification of a fragment of the 4b core protein gene was adapted from Adams et al. (2005). Eighteen sequences were obtained with this method from our samples. Alignments were created using the Clustal V and the Megalign software. Partial polymerase and 4b core protein sequences were subjected to phylogenetic analysis by the Distance Matrix followed by Fitch method (Phylip programme package) together with corresponding avipox virus sequences available from GenBank.

**DISCUSSION – CONCLUSION:** The analysis of the 4b core protein sequences confirmed the results of Adams et al. (2005) defining 5 distinct clades of avian poxviruses. However, the diversity demonstrated within clade 4. supports the suggestion of Lüschoew et al. (2004) dividing this group further into three distinct subgroups. One of these has been reaffirmed as a distinct falconpox group. Another group could be define as a raptorpox group. These distinctions were supported by the results of the analysis of polymerase gene sequences.

We conclude as well, that the polymerase gene based approach gives good results both in phylogenetical analysis and the detection of avian poxviruses by PCR.

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## THE TRANSMISSION CYCLE OF *BORRELIA BURGDORFERI* AND THE ASSOCIATION WITH LYME BORRELIOSIS RISK IN SCOTLAND

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**AIM OF THE STUDY:** To quantify the risk of acquiring Lyme borreliosis (LB) in Scotland by examining the transmission cycle of *Borrelia burgdorferi* sensu lato and relating this to human cases. This will be accomplished on two levels: 1. Countrywide: examination of the distribution of cases of LB cases and the clinical manifestations of these patients in an attempt to associate symptoms with *Borrelia* genospecies. 2. Site specific: individual sites with high reported cases of LB will be investigated in order to elucidate why this may be so. The transmission cycle of *B. burgdorferi* in *Ixodes ricinus* ticks and their hosts will be examined (diagram of life cycle of *I. ricinus*).

**MATERIAL AND METHODS:** Countrywide: Sufferers of Lyme borreliosis are contacted and questioned regarding where they received the tick bite that gave them LB [sample of questionnaire and map of cases]. Sufferers are also questioned on the clinical manifestations of their disease in order to associate this with the genospecies of *Borrelia* (e.g. dermatological, rheumatological, neurological symptoms etc).

Site specific: Sites with known infected tick bites are identified through patient questionnaires [map of study sites]. 16 sites will be sampled (8 major and 8 minor) twice a year (one spring and one summer/autumn). Major and minor sites are paired and are close geographically, although may vary in habitat type, land management (e.g. deer fencing/unfenced) or environmental/abiotic characteristics. Major sites will be blanket dragged to collect questing ticks and small mammals trapped to collect feeding ticks (using Longworth traps and live trapping). In the second year of the PhD, ear punch biopsies will be taken from small mammals in order to test tissue samples for *B. burgdorferi* s.l. Minor sites will be blanket dragged for questing ticks. Other species: Additional ticks will be collected from deer (via gamekeepers), birds (via the British Trust for Ornithology and the Scottish Agricultural College) and from squirrels (via the Institute of Zoology). Molecular analysis: Ticks will be tested for *B. burgdorferi* s.l. by nested PCR. Real Time PCR will then be used on positive samples to determine the *Borrelia* genospecies through melting point analysis. GIS: A predictive map will be created in association with TickMAP (developed by the Macaulay Institute) to create a risk map for Lyme borreliosis by elucidating the associations between ticks, their hosts, *B. burgdorferi* and human cases.

**CONCLUSION:** It is anticipated that the results of this work will provide a detailed understanding of the transmission cycle of *B. burgdorferi* in Scotland (which is a little-studied area of the Europe in terms of *Borrelia* research). It is possible that the information gathered can be used to implement prevention and protection measures.

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## WEST NILE VIRUS IN WILD PERIDOMESTIC BIRDS, SOUTHERN FRANCE, 2004

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**AIM OF THE STUDY:** During late summer and autumn 2004, an equine outbreak of West Nile virus (WNV) occurred in the Camargue region, a wetland area in the south of France where the virus was first reported in humans and horses in 1962 and reemerged in 2000. We conducted epidemiological investigations on wild

peridomestic birds (house sparrows *Passer domesticus*, tree sparrows *Passer montanus* and common magpies *Pica pica*) in order to determine whether these species were likely to be associated with WNV emergence in horses.

**MATERIAL AND METHODS:** Birds were captured using mist nets or traps and bled from the brachial vein. Plasma samples were screened for WNV immunoglobulin G using an indirect ELISA test and positive samples were confirmed by a microneutralization assay. WNV specific RT/Nested-PCR assay and virus isolation on C6/36 cells were performed on brain samples from two dead birds (a house sparrow and a magpie). The complete genome of both isolates was sequenced.

**RESULTS:** Four birds out of 228 tested positive in serology, indicating the circulation of WNV in the peridomestic bird population. Pair wise alignment of both isolates showed that they were identical and multiple alignment with other sequences available on GenBank database revealed that they were related to WNV lineage 1 strains belonging to the European/Mediterranean/Kenyan cluster.

**DISCUSSION:** To our knowledge, our isolates are the first WNV avian isolates from the western Mediterranean basin to be entirely sequenced. Although no WNV strain could be isolated from horses in 2004, it is quite likely that our WNV avian strain was the one involved in the 2004 equine outbreak. Indeed, WNV is believed to be transmitted to horses by mosquitoes able to feed on both birds and mammals. Mosquito species suspected to act as epidemic vectors in the Camargue are likely to get infected while feeding on birds and subsequently to transmit the virus to horses. As sparrows and magpies are closely associated with human settlements and farming activities, they appear to be ideal avian hosts for WNV amplification and transmission to horses.

**CONCLUSION:** Our data indicate that WNV circulated among wild resident birds in the Camargue region before and during the 2004 equine outbreak and support the hypothesis that these bird species might be involved in WNV emergence in horses. Further studies should include experimental infection using the isolated WNV strain and investigations on the host feeding pattern of mosquito vectors.

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## **DEER KEDS (*LIPOPTENA CERVI*) ON MOOSE IN SOUTH-EASTERN NORWAY**

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**INTRODUCTION:** Deer ked (*Lipoptena cervi*) is a species of biting fly from the family of louse flies (*Hippoboscidae*). This ectoparasite is commonly encountered in temperate areas of Europe, Asia and Northern America, but was only recently observed in Norway (1983).

The main host of the deer ked is moose and other cervids. Adult deer keds are 6 mm in length and dark brown. The adult fly sucks blood and lays prepupae that immediately pupate in the fur of the host. The pupae fall to the ground and hatch into flies during August, September and October. Deer keds can only fly a short distance and once the insect reaches its host, it sheds its wings. They are believed to only reproduce on cervids. They can however, occasionally, bite humans.

**MATERIALS AND METHODS:** In October 2006, hunters found several hairless animal cadavers and a number of emaciated moose, with extensive hair-loss, were observed in the south-eastern part of Norway. During January to February 2007, seven affected animals were euthanized, due to animal welfare concerns, and submitted to the National Veterinary Institute for examination. The body condition, parasite burden and pathological lesions were noted at necropsy. Biopsies from affected skin were collected for histopathology, bacteriology, parasitology and mycology. All visible deer keds and pupae in the fur were collected and counted from three of the animals.

**RESULTS:** All the animals examined were in poor body condition, and the only significant finding at necropsy was various degrees of alopecia and dermatitis. A consistent finding was the presence of large numbers of deer ked in the fur.

**DISCUSSION:** Good insulation is a key factor for the survival of a mammal during the cold part of the year. A moose that lacks a large proportion of its hair coat will loose



heat and has to increase its metabolic rate in order to maintain its body temperature. Moose with severe alopecia may therefore freeze and starve to death during winter. CONCLUSION: Our preliminary conclusions are that the deer ked is probably the main factor causing alopecia in moose in south-eastern Norway. Extensive alopecia in moose can be a severe animal welfare issue. However, more research is needed to evaluate the long term effects of deer keds on the Norwegian moose population.

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## PROPOSED NORTH AMERICAN WILDLIFE DISEASE SURVEILLANCE NETWORK

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**AIM OF THE STUDY:** Across North America, there are thousands of organizations and individuals involved in the care of injured and orphaned wildlife. Generally known as wildlife rehabilitation, this field of endeavour seeks to care for these wild animals and return them to the wild. Where wildlife rehabilitation centers exist, they are essentially the “first responders” to situations involving wildlife health. Animals presented to rehabilitation facilities and wildlife veterinary hospitals are likely to be the first indicators of a new or newly-emerging wildlife disease. The proposed North American Wildlife Disease Surveillance Network (NAWDSN) is a network designed to connect these first responders and to provide “real time” surveillance by detecting aberrant trends. This surveillance method could be used as an early warning system for emerging infectious diseases in wildlife populations or serve as a means of continued detection.

**MATERIAL AND METHODS:** The proposed network would provide an accurate, comprehensive and sensitive “real time” surveillance system, including an analytical GIS-linked database of case records and disease reports. The primary data sources would be wildlife care providers, including wildlife hospitals and veterinary schools. Data would also be collected from state and federal agencies, laboratories, and law enforcement personnel across North America. Baseline data collected through NAWDSN will include descriptive epidemiologic information, including numbers of animals seen, common clinical presentations, and variations in morbidity and mortality classified by species, age, and gender that can be classified temporally, seasonally and geographically. Syndromic surveillance techniques will be used to condense the clinical presentation into a standardized language for further analysis.

**RESULTS:** The network will link 20 to 25 of North America’s largest and most progressive wildlife hospitals, via the Internet, to a comprehensive epidemiological database. Through the “real time” reporting and analysis of disease data, the surveillance network will assist in identifying diseases in wildlife that may adversely affect ecosystem, human, or agricultural health.

**DISCUSSION:** In a 2004 joint report issued by the WHO/FAO/OIE on emerging zoonotic diseases, one recommendation to improve prevention and control of these diseases in the Americas was to develop the capacity for surveillance of wildlife disease. Wildlife cases presented to wildlife hospitals and rehabilitation centers across North America provide a unique data source that can greatly enhance the early detection of emerging diseases. By identifying patterns of endemic disease associated with critical disease agents, a baseline against which future disease incidence can be compared will be established. Once baseline information is established, it can be used to monitor incoming cases for aberrations and statistical anomalies. Deviations from normal can act as “trip-wires” for early detection of newly emerging disease or the presence of an environmental toxin. Early detection will enable targeted investigations, prompt notification of appropriate agencies and institutions, and rapid response for disease control.

**CONCLUSION:** Wildlife hospitals and care centers have a tremendous potential to deepen and broaden the information available to conservation organizations and government agencies, as well as public health agencies. The data which can be provided through NAWDSN is simply not available elsewhere.

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## CLONING, SEQUENCING AND EXPRESSION OF WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*) INTERFERON-GAMMA (IFN- $\gamma$ ) AND THE PRODUCTION OF RHINOCEROS IFN- $\gamma$ SPECIFIC ANTIBODIES

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**AIM OF THE STUDY:** Bovine tuberculosis (BTB) is endemic in African buffalo (*Syncerus caffer*) in the Kruger National Park (KNP). In addition to buffalo, *Mycobacterium bovis* has been found in at least 14 other mammalian species in South Africa, including kudu (*Tragelaphus strepsiceros*), Chacma baboon (*Papio ursinus*) and lion (*Panthera leo*). This has raised concern about the spill over into other potentially susceptible species like rhinoceros, thus jeopardising breeding and relocation projects aiming at the conservation of biodiversity. Hence procedures to screen for and diagnose BTB in black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) need to be in place. The Interferon-gamma (IFN- $\gamma$ ) assay is used as a routine diagnostic tool to determine infection of cattle and recently African buffalo, with *M. bovis* and other mycobacteria. The aim of the present work was to develop reagents to set up a rhinoceros IFN- $\gamma$  (RhIFN- $\gamma$ ) assay.

**MATERIALS AND METHODS:** The white rhinoceros IFN- $\gamma$  gene was cloned, sequenced and expressed as a mature protein. Amino acid (aa) sequence analysis revealed that RhIFN- $\gamma$  shares a homology of 90% with equine IFN- $\gamma$ . Monoclonal antibodies, as well as polyclonal chicken antibodies (Yolk Immunoglobulin-IgY) with specificity for recombinant RhIFN- $\gamma$  were produced.

**RESULTS:** Using the monoclonals as capture antibodies and the polyclonal IgY for detection, it was shown that recombinant as well as native white rhinoceros IFN- $\gamma$  was recognised. This preliminary IFN- $\gamma$  Enzyme-linked Immunosorbent assay (ELISA), has the potential to be developed into a diagnostic assay for *M. bovis* infection in rhinoceros.

## AVIAN IMMUNITY TO WEST NILE VIRUS

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**AIM OF THE STUDY:** Study aims included characterizing the duration of protective immunity to West Nile virus (WNV) in adult birds, and of passively acquired WNV immunity in chicken chicks.

**MATERIALS AND METHODS:** Two studies addressed the duration of experimentally or naturally-induced WNV antibodies in adult birds. House sparrows (*Passer domesticus*) were experimentally inoculated with WNV (NY99 strain) to induce immunity. At 6-month intervals (3 years anticipated total), antibody levels are measured in all sparrows, and a subset is challenged to determine immune protection against morbidity, mortality, and viremia. Also, WNV antibodies of naturally-infected captive raptors are monitored monthly (4 years anticipated total). In addition, duration of passively acquired WNV immunity was assessed in chickens, with eggs and chicks from WNV seropositive chickens (*Gallus domesticus*) tested for maternally-derived WNV antibodies. We documented the decay of maternal antibody

in chicks over time, as well as protection against morbidity, mortality, and viremia by challenging maternal antibody positive chicks at various times post-hatch. In addition, seronegative chicks of various ages (from 1-70 days post-hatch) were infected with WNV to examine the age-associated response to infection. Viremia profiles were determined by Vero cell plaque assay and antibody levels by plaque reduction neutralization test.

**RESULTS:** Thus far, WNV antibodies persist and are protective for  $\geq 1$  year post-inoculation in sparrows and  $\geq 3$  years in raptors. All eggs and chicks derived from seropositive chicken hens were WNV-maternal antibody positive. Maternal antibodies were no longer detectable in chicks by 4-5 weeks post-hatch, but remained protective against viremia until 6 weeks post-hatch. Seronegative chicks succumbed to WNV infection at 1 day post-hatch while experiencing relatively high viremia levels (up to  $10^{7.9}$  plaque forming units/ml serum), while their seropositive counterparts showed no clinical signs and did not develop viremia. Thereafter, susceptibility to morbidity and peak viremia levels declined relatively rapidly in chicks of increasing age.

**DISCUSSION:** Results from this study have implications on the health of free-ranging avian populations in North America, where New World strains of WNV have been circulating and spreading since 1999. Both the persistence of protective WNV antibodies and of maternal antibodies in birds affects WNV transmission dynamics and survivability of birds. First, birds that survive WNV infection appear to be protected against morbidity and viremia for at least several years after initial infection. Second, chicks born of seropositive mothers are protected when most vulnerable to WNV infection. These neonatal chicks are less likely to experience WNV-associated morbidity and mortality or to have a role in WNV transmission. These results should be considered when interpreting WNV serosurveys of free-ranging birds because previously exposed birds remain seropositive in subsequent years and therefore do not imply recent transmission, and seropositive chicks may not signify WNV exposure and transmission, but rather WNV maternal antibody transfer from seropositive mothers.

**CONCLUSION:** Both experimentally and naturally-induced WNV immunity in adult birds is protective over the long-term, and chicks derived from seropositive mothers are also protected for a limited period post-hatch by maternally-acquired antibody.

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## **VIRAL INFECTIONS IN ARCTIC WILDLIFE: PERSISTENT ALPHA-HERPESVIRUS INFECTIONS IN SEMI-DOMESTICATED REINDEER IN FINNMARK COUNTY, NORWAY**

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**AIM OF THE STUDY:** Finnmark County in northern Norway is the biggest reindeer herding area in Norway with a total area of 56 682 km<sup>2</sup> and a total of 168 599 animals in 2004/2005. Reindeer mortality rates in Finnmark are estimated to be as high as 9% in adults and 15% in marked calves (2004/2005) or 36% in the total number of born calves (marked or not). Predators account for approximately 85% of the losses. Persistent viral infections, such as Alpha herpesvirus (CeHV-2) are believed to exist in Finnmark<sup>1,2</sup>, and might partially explain up to 10% of the mortality. These infections could have an impact on reproduction (abortion) and calf survival causing relevant economical losses. The present work was aimed at identifying the seroprevalence of Alpha herpesvirus in reindeer in Finnmark as well to attempt to isolate a Norwegian strain of the Alpha herpesvirus for sequencing and phylogenetic studies.

**MATERIAL AND METHODS:** A total of 3300 animals were sampled from 2003 to 2006 at slaughterhouses in Karasjok, Kautokeino, Varangerbotn and Suoššjävri as well as from live animals in district 16C and 16A. Sera, obtained from these 3300 animals, were tested using a Bovine gB-blocking ELISA kit known to be functional in reindeer<sup>3</sup>. A total of 450 samples from the trigeminal ganglion of the fifth cranial nerve were collected and DNA was extracted. Specific primers (CR13 and CR14) for the detection of Reindeer Alpha herpesvirus (RanHV-2) were used for amplification by PCR<sup>4</sup>. The PCR amplicons from some of the individuals were sequenced.