Tuberculosis Survey in Cape Buffalo (Syncerus caffer), Cattle and Humans at the Wildlife / Domestic Interface in Uganda

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Tuberculosis (TB) caused by Mycobacterium bovis and M. tuberculosis is an important debilitating disease of domestic and wild animals, and humans with implications for public health and conservation. A survey was done of TB in buffalo in the interior of Queen Elizabeth National Park (QENP), Uganda, in 1997 using the gamma interferon test. TB was found in 21.4% of a random sample of 42 live captured free-living cape buffalo (Syncerus caffer) in good body condition. Fresh post-mortem tissue samples cultured from granulomatous lesions of one buffalo and one warthog revealed M. bovis.

The study concluded that the TB prevalence and species infected were similar to a previous survey carried out in the 1960's by Woodford on post-mortem tissue samples of 116 animals. Uganda went through a period of poor governance and civil wars from 1971 to 1985 where buffalo numbers dropped by 76.7% (18,000 to 4,200). In spite of this drop in buffalo numbers over 24 years, the TB prevalence appears to have remained similar. This could reflect the chronic nature of TB in buffalo, or the decreased densities of buffalo may not have allowed an increase in the rate of TB infection. As buffalo numbers and densities increase, TB could become a more significant cause of morbidity and mortality, and of TB exposure for cattle and humans.

The prevalence of TB in cattle and humans around QENP and Bwindi Impenetrable National Park (BINP), Uganda, is considered high. A follow up study is assessing the risk of TB disease transmission at the human/wildlife/domestic animal interface in QENP and BINP. This is being done by determining the prevalence of TB in buffalo, cattle and humans dependant on these cattle at the periphery of QENP; and in cattle and humans that overlap with mountain gorillas in BINP. Results from these studies will be used to determine the type and source of infection and risks of transmission between species, to formulate recommendations to manage TB in Uganda, and to promote public health and conservation.
Serologic Survey of Selected Viral, Bacterial, and Protozoal Agents in Captive and Free-Ranging Ungulates from Central Kenya

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Over 200 serum samples from captive and free-ranging ungulates in Central Kenya were collected between 1993 and 1999. Animals were sampled during any procedure that required chemical immobilization (e.g., translocation) or within minutes after death. None of the wild animals exhibited clinical signs or lesions suggestive of prior exposure to the agents examined in this study. Burchell's zebra (Equus burchelli) were seropositive for African horse-sickness virus (AHSV), equine viral arteritis virus (EAV), equine herpesvirus type-1 (EHV-1), equine herpesvirus type-4 (EHV-4), equine rhinovirus type-1 (ERV-1), equine rhinovirus type-2 (ERV-2), and equine influenza virus (EIV). Unvaccinated captive camels (Camelus dromedarius) were seropositive for bluetongue virus (BTV), EHV-1, ERV-1, Brucella abortus (BRUCELLA) and seronegative for infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV), anaplasmosis (ANAPLAS), foot and mouth disease virus (FMDV), malignant catarhral fever virus (MCFV), rinderpest virus (RPV), and trypanosomosis (TRYP). Buffalo (Syncerus caffer) were seropositive for ANAPLAS, BTV, bovine parainfluenza -3 virus (PI3), bovine respiratory syncytial virus (BRSV), and IBRV, and seronegative for bovine leukemia virus (BLV), BVDV, BRUCELLA, FMDV, MCFV, RPV, and TRYP. Grant's gazelle (Gazella granti) were seropositive for BTV, PI3, BRSV, and IBRV but seronegative for ANAPLAS, BLV, BVDV, BRUCELLA, FMDV, MCFV, RPV, and TRYP. Impala (Aepyceros melampus) were seropositive for BTV, PI3, BRSV, and IBRV and seronegative for ANAPLAS, BLV, BVDV, BRUCELLA, FMDV, MCFV, RPV, and TRYP. Giraffes (Giraffa camelopardalis) were seropositive for PI3 and BRSV, but seronegative for ANAPLAS, BLV, BVDV, BRUCELLA, and IBRV. Domestic cattle (Bos indicus) had been vaccinated for FMDV and RPV, and were seropositive without vaccination for BTV, PI3, BRSV, BVDV, BRUCELLA, and IBRV, and seronegative for ANAPLAS and BLV. Free-ranging zebras in nature preserve had a higher prevalence of ERV-1 than zebras inside the fence. For all other species and all other disease agents, seroprevalence was not significantly different inside or outside the fence. This study is the first report of seropositivity to ERV-1 and -2, EIV, and EAV in zebra, PI3 in Grant's gazelle and giraffe, BRSV in buffalo, gazelle, impala, and giraffe, and IBRV in Grant's gazelle. Results of serologic testing for domestic animal diseases in these samples could have been altered by the cross-species application of the tests, and by irradiation of the samples upon entry into the United States. This study highlights the possibility of transmission of these agents from domestic species into wildlife or vice-versa at our study site.
Oral Rabies Vaccination of African Wild Dogs (*Lycaon pictus*): Vaccine Testing and Bait Development

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The African wild dog *Lycaon pictus* is a highly endangered species. Rabies is an important cause of mortality amongst wild dogs, particularly in small and/or declining populations. Previous attempts at protecting wild dogs against the disease have focused on parenteral vaccination methods. Such attempts have been largely unsuccessful, and are logistically difficult and expensive. Attention has turned to the development of effective oral vaccination techniques, which would enable large packs comprised of all age groups to be vaccinated without excessive disturbance. The aims of the current study are to determine the efficacy and the duration of the immune response elicited by the SAG-2 oral rabies vaccine in African wild dogs, and to develop an effective bait and baiting system for the delivery of the vaccine to a pack of free-ranging wild dogs.

Cafeteria-style bait preference trials testing seven candidate baits were conducted in captive wild dogs. Results showed a significant preference for chicken head baits (June trials: \( p = 0.023 \), September trials: \( p = 0.021 \)). Trials using a topical biomarker (Rhodamine B) showed that chicken head baits are sufficiently chewed 71.4% (n=7) of the time to rupture the vaccine container and spill vaccine on the oral mucosa. Significant dominance of bait intake by a single individual was seen in four of six study packs, and in the three packs in which an alpha pair could be distinguished it was noted that the dominant individual was an alpha animal. Pattern of bait distribution and degree of satiation had no effect on pack coverage (proportion of pack ingesting at least one bait). Pack coverage was found to be significantly related to trial number \( (r^2 = 0.51, p < 0.001) \), with pack coverage increasing with increased exposure of the pack to the baits. Free-ranging wild dogs and young puppies (10 weeks) successfully ingested chicken head baits. During 45.9 hours of diurnal observations only two baits were lost to non-target species.

Vaccine efficacy trials in captive wild dogs are underway. These trials aim to determine the persistence of protective antibody titres following administration of single and booster doses of SAG-2, and to determine the efficacy of the oral vaccine when given by chicken head baits.

It is recommended at this stage that oral rabies vaccination techniques form part of an integrated rabies control programme in African wild dogs, with previous exposure of wild dogs to chicken head baits (‘priming’) an important part of the technique.
Dynamics of Disease Invasion in a Metapopulation

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Motivated by the growing bovine tuberculosis epidemic in African buffalo (*Syncerus caffer*) of Kruger National Park, South Africa, we develop a metapopulation model for the spread of an infectious disease through a spatially-structured susceptible population. Subpopulations (herds) are distributed randomly on a bounded lattice, and individuals (susceptible, exposed or infected) can migrate between herds within a pre-set distance. The disease is modeled as an SEI epidemic, with no recovery from the infected state, and both transmission and migration are treated stochastically. The model is kept general, so conclusions are relevant to other systems involving migration between spatially-distributed populations.

Dynamic aspects of the epidemic are emphasized to learn which factors most influence the rate of disease spread. Rules for within-herd transmission and between-herd movement are varied, and their impact is measured via the average time it takes for the disease to cross the entire space or for a set proportion of herds to become infected. Implications for control strategies are considered, including vaccination of buffer areas and the effect of physical barriers.
A “Cervus” Genotyping Kit Based on Automated Fluorescent Multiplex PCR for Rapid Characterisation of Genetic Diversity in Several Deer Populations: A Tool for Wildlife Management

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Recent techniques in molecular biology and advances in statistics are very useful to characterise the genetic structure of populations and to deduce adapted rules for wildlife management and farming. Consequently, it becomes possible i) to infer demographic changes, to assess the level of gene flow between populations and the impact of human activities (hunt, habitat fragmentation) on wild populations; ii) to estimate the impact of farming system on genetic diversity of captive populations, to help the farmers on parentage control in their herds and to set up efficient breeding schemes. In the tropical countries, deer production, including farming and hunting, is economically very important but most often empirical. We have no information either on the populations genetic diversity or on the potential negative impacts of the human practices (on the genetic resources). In this context, we set up a genotyping tool for several tropical deer species using microsatellites markers. Thirty eight microsatellites markers (30 loci from bovine and 8 from ovine origins) were screened in 4 tropical deer species (Rusa, Eld’s, Swamp and Vietnamese Sika deer). They were tested for their ability to give polymorphic PCR products. Then 12 polymorphic microsatellites were chosen to set up multiplex PCRs. The 12 primer sets were labelled with three different fluorochromes. For all species, 3 groups of 4 markers were realised depending on their allelic size range. The automatic analysis was performed using an ABI 377 sequencer and PE Genotyper software. The multiplex PCR and automatic analysis have numerous advantages : security using fluorescent molecules, reproducibility, accuracy, use of small quantity of DNA. Furthermore, in only one manipulation, data from 36 individuals on 12 codominant markers were simultaneously collected. This genotyping tool was used to analyse populations of two tropical deer species. On one hand, populations of Vietnamese sika deer (Cervus nippon pseudaxis) reared in Vietnam for velvet production were studied. We notably assessed the impact of this traditional farming system on genetic diversity of this subspecies, which disappeared in the nature and was saved from extinction by traditional farming. On the other hand, genetic characterisation was performed on captive and wild populations of rusa deer (Cervus timorensis rusa) in Mauritius island and New Caledonia. In these islands, we estimated the founding effect, the genetic drift and the population dynamic to set up breeding schemes for the farmers. Finally, this molecular tool can be used for different purposes depending on the statistical analysis methods: population genetic structure, genealogy and products (velvet, meat) traceability to fight poaching. The most important aspect concerns the support to the management of the genetic resources. Knowledge on the genetic variability structure is the first step to set up management practices, which insure maintenance of genetic variability on the long run. This is notably a major issue to allow the populations to adapt to new pathogens. Moreover, since genotyping allows identifying paternity, it is a tool to control pedigree and improve either genetic resistance study or individuals selection.
Effects of Thin and Release Timber Management Practices on Abundance of Woodrats, Chipmunks, Mice, and Ticks within the Hoopa Valley Indian Reservation, Humboldt County, California (USA)

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Rodents and their ectoparasites often act as reservoirs and vectors for zoonotic disease agents. In California, dusky-footed woodrats are a reservoir for Borrelia burgdorferi sensu lato, the agents that cause Lyme disease, Ixodes pacificus is the primary vector to people, and Ixodes spinipalpis maintains this agent in enzootic cycles. However, the forested regions of Humboldt County differ from other areas studied, and the reservoirs and enzootic maintenance vectors of Lyme disease remain unknown. In addition, agents causing two other tick-borne diseases, human granulocytic ehrlichiosis and human monocytic ehrlichiosis, were recently recognized in Humboldt County. Once again the reservoirs and vectors for these disease agents are unknown for this area. However, woodrats have been suggested to be a likely reservoir.

This study reports a comparison of the relative abundances of three species of forest rodents among four types of forest stands: two ages of logged stands (20-25yrs and 35-40 yrs) and two types of silvicultural prescription (stands that have been thinned and released and those that have not been thinned and released) within the Hoopa Valley Indian Reservation in northwestern California (USA). Small mammals were live-trapped and sedated for the collection of ticks during the summers of 1999 and 2000 at two sites representing each stand type. Site-specific abundances of small mammals and ticks were correlated with vegetative structure. Closer examination of tick distribution and habitat associations may allow greater understanding of transmission cycles and of how timber management practices may impact those cycles.
Parasites of *Clarias gariepinus* (Burchell, 1822) (Pisces: clariidae) from a Small “Satellite” Lake, Lake Malimbe, Mwanza, Tanzania

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Lake Malimbe, about a kilometre square in surface area, is within the Lake Victoria basin along the Gulf of Mwanza. Direct connection to the main lake is blocked by floating macrophyte vegetational cover. A number of endemic species of fish currently known to be rare in Lake Victoria due to predation and/or environmental perturbation are represented in this small lake. Unfortunately, the parasite fauna of fish in this refuge have not been investigated. I conducted a survey of parasites in 100 catfish (*C. gariepinus*) in Lake Malimbe. My objective was to define the parasite fauna infecting *C. gariepinus* in this habitat, assess variations in parasite intensities (infection level) with sex and size of the host, assess the frequency distribution of helminth parasites in a population of *C. gariepinus*, and assess the quantitative effects of these parasites on the condition of an individual fish and/or the host population as a whole. About 10 Species of helminth parasites from different genera were identified.
Sero-Prevalence of Viral Infections in Captive and Free-living Birds of Prey in Spain - Implications for Conservation and Management of Wild and Captive Populations

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Wild birds, including waterfowl, passerines, birds of prey, and illegally imported wild birds are potential reservoirs for avian pathogens of importance to commercial poultry. On the other hand, scavenging wild animals are at risk of infection with poultry pathogens from dumped carcasses, especially in cases when mortalities caused by epizootics are disposed of illegally.

Since December 1997 a total of 700 sera of 32 different species of captive and free-living birds of prey from different regions of Spain were analysed for the presence of neutralising antibodies to avian paramyxovirus (aPMV) 1, 2 and 3 and Falcon herpesvirus. In addition a total of 163 sera was analysed for antibodies to the Aviadenovirus serotype 1 (Celo-virus), 30 sera were analysed for antibodies to avian Reovirus. 126 of 12 species of bird of prey were analysed for the presence of neutralising antibodies against Infectious Bursal Disease Virus (IBD). Methods employed include Haemaglutination inhibition (HI) test for the detection of antibodies to aPMV-1, -2 and -3, and virus neutralisation test (VNT) for all other viruses tested.

Out of 700 birds 120 tested positive for aPMV-1, while 10 birds had antibodies to aPMV-2 and only 4 birds tested positive against aPMV-3. Prevalence of antibodies against aPMV-1 was significantly higher in captive than in free-living birds of prey, and in Falconiformes than in Strigidae and Accipitridae. Sixty of 700 birds had antibodies to falcon herpesvirus and of 163 sera screened for aviadenovirus serotype 1, antibodies were detected in 33 birds with titres as high as 1:64. Thirty five birds of 126 were seropositive to IBDV with titres ranging from 1:2 to >256. Of 30 sera examined for antibodies to avian Reovirus, only one Griffon vulture (Gyps fulvus) was positive with a very low titre (1:4). For most pathogens, seroconversion was evident for wild and captive birds.

Several captive breeding projects for the endangered Iberian Imperial Eagle (Aquila adalberti) and other species such as the Bonelli's Eagle (Hieraaetus pennatus), peregrine falcon (Falco peregrinus), lesser kestrel (Falco naumanni) or the Montagu’s harrier (Circus pygargus) are under way in Spain. Implications of the results for wild endangered populations, for the management of captive breeding and evaluation of the offspring prior to reintroduction are discussed. Special reference is made to the management of rehabilitation centres and the importance of pre-release checks in rehabilitated birds.
VIRUSES
Epidemiological Features of African Swine Fever in Madagascar

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African swine fever (ASF) has recently made its appearance in Madagascar. It was first officially diagnosed in late 1998. Pig sera collected in 1996 were all found negative for anti-ASF virus antibodies. Genome comparison of an amplified fragment (VP73) of the ASF strains of the current epidemic with several African strains showed that the Malagasy strains are identical and related to a southern African strain ("Mozambique 1994" strain). ASF is therefore assumed to have entered Madagascar for the first time in 1997 from the African continent.

The mortality rate in affected areas was over 90% of animals during the peak of the epidemic between September 1998 and March 1999. Collected field data showed animal losses of over 60% in 1998-1999, as a result of mortality and preventive sales. Natural restocking was observed from mid-2000. Sporadic outbreaks in new herds, as well as in already affected ones, were reported from mid-1999. The disease is now considered to be enzootic in the absence of a stamping-out policy, with healthy carriers therefore occurring.

Tissue and serum samples were collected from almost all parts of the island and dispatched to the laboratory of the Pasteur Institute of Madagascar. Diagnosis of ASF was conducted using antibody, antigen and DNA detection (ELISA and PCR). Data from 1999 and 2000 indicated that 15% of the samples (n=459) were antigen-positive and 2.9% (n=939) antibody-positive. The correlation between PCR and the immunocapture test is under examination.

Ticks of the Ornithodoros moubata group, considered to be O. porcinus (Walton, 1962), were formerly known to occur in western Madagascar but seem to have disappeared from that region. However, recent studies have identified new infested sites in the humid and cool central highlands of Antananarivo province. A preliminary search for antibodies against tick saliva has been carried out using ELISA and seems to support the field data. These ticks are known to be efficient reservoirs and vectors of ASF virus and constitute a considerable complication for the control of the disease. Viral investigations on ticks using immunocapture and PCR are in progress. The presence of a species of African bushpig, Potamochoerus larvatus, as another potentially complicating factor is also discussed. The first viral and serological tests on bushpig samples were negative.

The greater part of Madagascar is presently endemic with ASF except for the province of Antsiranana (north) and an area around the town of Morondava (west, province of Toliara). Based on the concept of regionalisation, attempts to protect these zones from the introduction of the disease using animal movement restrictions and systematic sampling of animals are implemented. These regions could be used to re-establish pig production. The socio-economic impact of ASF is severe and the marketing chain is now disorganized. Pig keeping in Madagascar is mainly traditional with free-ranging animals. However, improved pig husbandry increased rapidly over the last few years before the occurrence of ASF. Projects to restore this modern pig sector by importation and/or artificial insemination are considered. The FAO and the French co-operation assisted the Malagasy veterinary services to strengthen their surveillance networks.
The Evolving Transmission Pattern of RVF in the Arabian Peninsula

Sahmsudeen Fagbo

Vector borne viruses and their vectors have repeatedly demonstrated that they have no respect for international boundaries. The newly reported outbreak of Rift Valley Fever (RVF) in Saudi Arabia this year clearly sends the message that once pathogens cross their known geographic limits they will tend to adapt to the local ecology and survive and maintain their transmission capabilities. This paper describes the various interplaying ecological factors that may contribute to the establishment of this viral haemorrhagic fever virus on the subcontinent.

Apprehension also exists for the potential spread from the Arabian Gulf to other continents. This, given the massive multidirectional human-animal movement that occurs within and across the subcontinent. The annual influx of over 2 million pilgrims for the Hajj pilgrimage as well as the large expatriate population in this region present a challenging agenda for public health authorities. The situation during the pilgrimage may create transient microcosms that are best understood and appreciated during that short time span. The potential risks within this period as well as other peculiar ecological factors are studied and discussed in this paper.
Experimental Vesicular Stomatitis in Horses

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Vesicular stomatitis is a disease of cattle, horses, and swine which is caused by related viruses in the genus Vesiculovirus, family Rhabdoviridae. Although recognized for at least 160 years, the epidemiology and pathogenesis of this disease remains undefined. In this study we infected horses with vesicular stomatitis virus New Jersey serotype (VSV-NJ) and Indiana serotype (VSV-I) by routes compatible with contact and vector transmission. These routes included intraepithelial inoculation of the tongue, oral inoculation via scarification of the lip, per os, and intradermal inoculation of the chin skin.

Horses infected with VSV-NJ developed lesions at the site of inoculation as early as post inoculation day (PID) 1 in animals inoculated in the tongue and as early as PID 2 in animals inoculated in the lip or chin. Secondary lesions occurred in some animals inoculated in the lip and chin by PID 4 and 5, respectively. Only one of three horses inoculated per os developed lesions. Virus shedding from the oral cavity occurred in all horses except one infected per os. This shedding occurred as early as PID 1 and lasted from 3 to 8 days in horses inoculated in the tongue, lip, or chin. Shedding occasionally occurred from the nasal cavity, but virus was never isolated from feces or blood. Serum neutralizing antibodies were first detected on PID 6 or 7. In general, results were similar in horses infected with VSV-I.

Horses can be infected with VSV-NJ and VSV-I by a number of different routes that simulate contact or vector transmission. Virus shedding is predominantly from the oral cavity and viremia cannot be detected. Serum neutralizing antibodies develop quickly resulting in the rapid termination of virus shedding.
Use of Sentinel Herds to Study the Epidemiology of Vesicular Stomatitis in Colorado, USA

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Outbreaks of vesicular stomatitis (VS) occur sporadically in the southwestern U.S. Three well documented outbreaks occurred in the 1990’s (1995, 1997, 1998). Although several epidemiological studies have been conducted in the U.S. and Central America, a complete understanding of the host, agent and environmental interactions of VS remain unknown. Questions such as why animals on the same premises are affected in different outbreak years remain unanswered. The objective of this study is to determine if viruses are persistence on the premises (endemic) or if virus is newly introduced at each outbreak of disease (epidemic).

Approximately 20 sentinel premises in Colorado are being visited quarterly during a 3-year prospective study to investigate the persistence of VS viruses in horses. Premises previously identified to house VS-positive horses were selected to identify potential factors associated with reoccurrence of VS in horses on these premises. A survey to assess management practices, health events, animal movements and environmental data is completed at each visit. Collection of serum samples and oral swabs along with a clinical examination are performed at each visit. Serum samples are tested by 1 or more of 4 available serological tests and oral swab samples submitted for attempts at virus isolation.

The data collected for years 1 and 2 (August 1998 to August 2000) is the focus of this abstract. The number of sentinel horses examined each quarter fluctuates between 160 and 175. The mean number of sentinel horses per premises is 8 (range 2-19) with a mean age of 10.1 years. The mean seroprevalence across all sites for the first 7 visits, as determined by the competitive ELISA, was 38.4% and 51.5% for the New Jersey and Indiana serotypes respectively. Most of these horses are seropositive due to the retention of antibodies from previous outbreaks. During this time period there was 1 and 8 horses positive based on capture IgM tests for serotypes New Jersey and Indiana respectively. The capture IgM indicates recent exposure to vesicular stomatitis viruses. These capture IgM positive tests were from 3 different premises and represented 5 different horses. Overall, there were 16 horses that were considered to have seroconverted during this time period. Virus isolation is in progress.

Survival analysis was used to allow for inclusion of censored horses, or those not seroconverting during the study period. Kaplan-Meier curves were generated for those premises with horses that seroconverted and the mean survival rate for all premises was 4.03 ± 2.5 quarters (range 1.0 – 7.0). Factors associated with premises differences in hazard and survival rates will be discussed. Seroconversions occurring during periods when no clinical disease is observed suggests the persistence of vesicular stomatitis viruses in the environment of the sentinel premises.
The Possible Role that Buffalo Played in the Recent Outbreaks of Foot and Mouth Disease in South Africa

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African buffalo (Syncerus caffer) act as maintenance hosts for foot and mouth disease (FMD) in southern Africa and most buffalo calves become subclinically infected with FMD virus as soon as maternal antibodies wane. These buffalo are called carriers of the disease. A single buffalo can become infected with all three of the endemic serotypes of FMD virus (SAT-1, SAT-2 and SAT-3) and pose a threat of infection to other susceptible cloven-hoofed animals. The recent outbreaks of FMD in Mpumalanga and Northern Province have been a reminder that this is an endemic disease in South Africa in areas where infected buffalo are found and that the disease can spill over into domestic livestock whenever control measures break down.

The floods of 2000 in southern Africa damaged the Kruger National Park (KNP) game fence extensively and there were several accounts of buffalo that had escaped from the park. Outbreaks of theileriosis were also recorded, indicating that buffalo had been in contact with cattle. Some of these buffalo were found in the FMD control areas where disease control relies on inspection only and animals are not vaccinated.

The VP1 gene, which codes for the major antigenic determinant of the FMD virus, has previously been used to determine phylogenetic relationships between different virus isolates and to trace possible origins of outbreaks. Virus isolates obtained from buffalo in the KNP have also been investigated using this methodology to determine possible relationships between buffalo virus isolates and historical outbreaks of FMD in cattle and impala. These databases were used to determine the possible origins of the recent outbreaks of FMD outside the boundaries of the KNP.

The outbreak in Mpumalanga was shown to have been caused by two different SAT-1 viruses that had a close phylogenetic relationship with buffalo viruses isolated previously from the south of the KNP. The SAT-2 virus that caused the outbreak in Northern Province was found to be related to buffalo strains previously isolated in the Orpen Gate region, close to the region where the outbreak occurred. These results demonstrate that buffalo were most probably the cause of the outbreaks, indicating that disease control using fencing as well as vaccination is extremely important to ensure that FMD does not become established in domestic livestock.
TICKS
Effect of the Association of Cattle and Rusa Deer (*Cervus timorensis russa*) on the Maintenance of a Viable Cattle Tick *Boophilus microplus* Population

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The wild population of Rusa deer in New Caledonia (South Pacific) is nearly as large as the cattle population (120 000 vs 150 000 head). The tick *Boophilus microplus* is widespread and occurs all the year round. There are divided opinions on the role of deer in the biological cycle of the tick: i) do they maintain a sustainable tick population which is secondarily available for cattle? ; or ii) do they contribute to decrease the infestation of the ecosystem by collecting larvae on the pasture but preventing their development until the engorged female stage? ;or iii) do they contribute to both situation? An experiment was conducted in 3 groups of pastures, each seeded with 500 000 larvae /ha and allowed to be grazed by a pure steers herd, a pure deer herd and a mixed deer/steers herd (deer representing 30 % the biomass), at approximately the same stocking rate (470-510 kg/ha). After 6 month of exposure, the tick burden per mass unit of host was 40 ticks /kg for the pure steers herd, and 0.2/kg for the pure deer herd. The steers in the “mixed group” harboured half as many as those of the pure group, and the deer in the “mixed group”, 47 times more (9.4/kg) than the pure deer. Three emergency acaricide treatments had to be performed in the pure steer group but none in the other groups. The long-term sustainability of a viable tick population on deer as well as the potential benefit resulting from the association of deer and susceptible cattle in the tick control of cattle are discussed.
Eradication of the Tropical Bont Tick in the Caribbean: Is the Caribbean Amblyomma Program in a Crisis?

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The progress and constraints in the Caribbean Amblyomma Program (CAP) are reviewed since its inception in 1995 when regional eradication activities for \textit{Amblyomma variegatum}, commonly known as the tropical Bont tick (TBT), were initiated. A general overview of technical progress shows a wide diversity between the islands in attaining eradication targets. One case study, that of St. Kitts, is presented as an example of a model program. However, one major concern that emerged during 2000 is that the elimination of the small remaining TBT “hot-spots” in both St. Kitts and St. Lucia remained elusive. Why is this so? - Egrets? Alternative residual hosts? Or is it mainly program management, both technical and administrative, and “fatigue”? On St. Kitts, for example, very low numbers of adult \textit{Amblyomma variegatum} have persisted in three sites for over two years. A second problem, of even greater concern, is the appearance of two, apparently new, foci: one in St. Croix (USVI) in the extreme north and another in St. Vincent in the extreme south. These findings extend the range of the TBT and the number of infested islands at a time when the chances of further spread should be diminishing.

During 1998, there were major funding and administrative management problems. USDA resolved the acute funding crisis with a unique contribution of US$ 1.94 million that was in addition to their annual contribution. After three years of negotiation, the CAP has now secured an additional EURO 1.5m from the European Community/CARIFORUM program. Changes in administration in 1998 included the withdrawal of IICA from the program, and the transition during the decentralization of administrative and financial management from FAO headquarters in Rome, Italy to the Regional Office for Latin America and the Caribbean, based in Chile. A critical overview of the program has identified one outstanding constraint—an appropriate management support function at the regional level, that is the CAP Regional Coordination Unit, and in some countries, at the national level. Proposals for a revised management strategy, coupled with the proposal for a future technical strategy to succeed the CAP, namely a Caribbean Animal Resources Management (CARM) Program, are outlined.
Control and Eradication of Reptilian Tick Infestations, with Particular Reference to Vectors of Heartwater

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Following discovery that at least 11 exotic tick species were being imported into Florida on reptiles, including three species (Amblyomma marmoreum, A. sparsum and A. variegatum) known to be vectors of heartwater (Cowdria ruminantium infection), it became important to develop methods to control these ticks on reptiles and to eradicate established infestations. Since no acaricide is currently registered in the United States for use on reptiles, those acaricides registered for use on other domestic animals were chosen for initial study. A total of eight acaricides were tested for activity against A. marmoreum ticks, and they were amitraz, carbaryl, chlorpyrifos, cyfluthrin, fipronil, lindane, permethrin, and pyrethrins. The two best acaricides, killing all ticks at a 0.01% dilution within four hours, were cyfluthrin and permethrin. Further studies showed that one formulation of permethrin produced no signs of toxicity when used repeatedly on African spurred tortoises (Geochelone sulcata), rosy boa snakes (Lichanura trivirgata) and green iguana lizards (Iguana iguana) at 10 times the recommended dose. One established A. marmoreum infestation on the premises of a reptile breeder in Florida was eradicated successfully using permethrin on the tortoises and cyfluthrin as a premises spray.
Tick-borne Diseases (TBDS) of Dairy Cows in a Mediterranean Environment: A Clinical, Serological and Haematological Study

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Although *Babesia* spp., *Anaplasma marginale* and *Theileria* spp. have been recognised since the beginning of last century in Italy, there is still scanty information about the parasite species, their vectors, distribution, incidence and impact on livestock production. More recently, several reports indicate that *A. marginale*, *Babesia bigemina* and *Theileria buffeli* are widespread among dairy farms in Southern Italy. Lack of knowledge, however, exists concerning the clinical manifestation and epidemiology of the diseases in this environment; arthropod vectors that may be involved in their transmission are also not known. This paper describes the results of two longitudinal studies carried out with the purpose of gathering information on clinical aspects and the epidemiology of the diseases under field conditions.

**METHODS:** Two dairy farms (designated A and B) of about 140 Frisian and/or Brown Swiss animals each, located in Puglia Region and in which clinical cases due to *A. marginale*, *B. bigemina* and *T. buffeli* had been previously recognised, were chosen for the two longitudinal studies lasting from May 1999 to Dec. 2000. At the onset of the study and again about 12 months later all milking animals in the two herds were sampled for complete blood counts, and sera were screened for antibodies against *B. bigemina* and *A. marginale* using an ELISA (ILRI, Nairobi) based on recombinant antigens p200 and MSP5 of the two organisms, respectively. On each farm, ten calves, three months old or younger, kept in fenced areas, and ten heifers, 12 to 18 months old, with permanent access to pasture, were chosen at random, observed monthly for clinical signs and monitored haematologically and serologically as specified above. Ticks were also collected monthly from animals.

**RESULTS:** Farm A – The screening for TBDs on the whole herd at the beginning of the study revealed seroprevalences of 82% (64/78) and 86% (67/78) for *B. bigemina* and *A. marginale*, respectively. The ten calves remained healthy during 1999, but in July-August 2000, seven of them showed signs of babesiosis with packed cell volumes (PCVs) between 14 and 24%; in two of them *A. marginale* was also present and all were treated with diminazene and tetracyclines. Seven of the 10 heifers showed subclinical anaplasmosis during June-July 1999 with PCVs ranging between 16.7 and 20.9%; all of them recovered slowly without treatment. Between May 1999 and Dec. 2000, a total of 28 cases of TBDs were observed on Farm A, 18 of which were caused by *A. marginale*, 8 by *B. bigemina* and 2 by a mixed infection.

Farm B - At the start of the study the seroprevalences for *B. bigemina* and *A. marginale* were 57.5% (42/73) and 87.7% (64/73), respectively; 80% of blood smears were also positive for *Theileria* sp. In Farm B, as in Farm A, the ten calves remained healthy during 1999, but 5 of them showed marked symptoms of anaplasmosis with packed cell volumes (PCVs) between 14 and 22.3%; they required treatment with tetracyclines. Seven of the 10 heifers showed subclinical anaplasmosis during June-July 1999 with PCVs ranging between 16.7 and 20.9%; all of them recovered slowly without treatment. Between May 1999 and Dec. 2000, a total of 12 cases of TBDs were observed in the herd of Farm B, 8 of which were caused by *A. marginale*, 3 by *A. marginale* and *T. buffeli*, and 1 by *A. marginale*, *B. bigemina* and *T. buffeli*. On the basis of the above data the importance and the role of TBDs in Mediterranean Countries is discussed.
Ticks Associated with Armadillo *Euphractus sexcinctus* and Anteater *Myrmecophaga tridactyla* of Emas National Park, State of Goias, Brazil

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\(^1\)Universidade Estadual Paulista, Jaboticabal, SP, Brasil
\(^2\)Universidade de São Paulo, SP, Brasil

Introduction. Many species of wild animals, common in several regions of developing countries, have not been studied in relation to ticks and tick-borne pathogens that are associated with them. The present work describes the identification of ticks from anteater and armadillo of the Emas National Park, Goias State, Brazil, and is part of a more comprehensive study concerning established and emerging tick-host relationships and related pathological aspects.

Material and Methods. The study was conducted in October 1998 and November 1999 in the Emas National Park (131,868 ha), a savanna-type cerrado region situated in the far south of Goias State, Brazil, near the geographic center of South America (15°-23°S; 45°-55°W). The climate is tropical and the highly seasonal rains give about 250 mm rainfall between October and March. The greater part of the park is covered by scattered bushland. Animals were captured with the aid of nets and anaesthetized (15 mg/kg ketamine + 1 mg/kg xylasine) in order to collect ticks for identification and to establish laboratory colonies. They included: giant anteaters *Myrmecophaga tridactyla* (n=4) and armadillos *Euphractus sexcinctus* (n=6). Free-living ticks (larvae, nymphs and adults) were collected from the field by using a 1x2m flannel cloth.

Results and Conclusion. Free-living ticks were identified as *Amblyomma* sp., *A. cajennense* and *A. triste*. Adult ticks collected from anteaters were identified as *Amblyomma cajennense* and *A. nodosum* and from armadillo as *A. pseudoconcolor* and *A. nodosum*. The relevance of these host-tick relationships to possible mechanisms underlying emergence of tick-borne pathogens of public health importance will be discussed.

Financial support: FAPESP and CNPq.
Transmission of American Canine Hepatozoonosis by Ixodids

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Oklahoma State University, Stillwater, OK, USA

American canine hepatozoonosis (ACH) caused by *Hepatozoon americanum* is an emerging, often fatal, apicomplexan protozoal disease of dogs in the United States of America. In order to understand the invertebrate (definitive) host range of *H. americanum*, transmission experiments were carried out using four ixodids, viz., *Rhipicephalus sanguineus*, *Dermacentor variabilis*, *Amblyomma americanum*, and *A. maculatum*. Laboratory-reared nymphal ticks were acquisition fed on dogs that were either naturally or experimentally infected with *H. americanum*; when these ticks molted to the adult stage they were dissected and examined for the presence of oocysts. Experiments were repeated six times with *A. maculatum* and *R. sanguineus* feeding simultaneously on parasitemic dogs and four times with these two ticks feeding in the presence of each other and, additionally, *A. americanum* and *D. variabilis*. Mature *H. americanum* oocysts were found in *A. maculatum* in all attempts, while oocysts were not found in any of the other three species. This study confirms an earlier report that *A. maculatum* is an excellent host for *H. americanum* and suggests that this apicomplexan may have a narrow invertebrate host range, at least among ixodid ticks that are likely candidate vectors in the USA.
EMERGING INFECTIOUS DISEASES
Susceptibility of Wildlife Hosts in North America to West Nile Virus


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The introduction of the exotic West Nile virus (WNV) into the Western Hemisphere initiated a massive response and brought international attention to the events that followed. Following a human epidemic from the virus in the New York City (NYC) area in 1999, the American crow (Corvus brachyrhynchos) emerged as an indicator species of WNV activity in the northeastern United States because of its high susceptibility to infection with WNV. The virus caused extensive mortality in crows, and infected crows were likely responsible for the initial geographic expansion of WNV into adjoining counties and states. The increased intensity and further geographic expansion of WNV in the eastern U.S. in 2000 continued to involve crows, but many new wildlife species were affected as well. However, the lack of information on the susceptibility and pathogenesis of WNV in native wildlife species hindered efforts to predict persistence, intensity, and expansion of WNV transmission in the region.

Experimental studies were conducted at the USGS National Wildlife Health Center to determine the susceptibility of selected wildlife species to WNV infection and confirm their potential as competent reservoir host species. American crows, mallards (Anas platyrhynchos) and white-footed mice (Peromyscus leucopus) were inoculated with a 1999 New York strain of WNV in a Biosafety Level III containment facility. In two separate studies, all experimentally infected crows died within 4-8 days following inoculation; whereas, mallards and white-footed mice did not succumb to infection. In another study, direct transmission occurred between experimentally infected crows and un-inoculated crows sharing the same room, most likely by the oral route. All inoculated animals were viremic following inoculation, including crows, and high titers of virus were isolated from 8 different tissues collected aseptically from crows at necropsy.

Crows are highly susceptible to WNV infection and could continue to be a sensitive sentinel species for surveillance. The results indicate that crows could also contribute to local transmission of WNV prior to their death. The significance of the documented direct transmission among captive crows in an experimental setting to WNV natural transmission cycles is unknown at this time.
West Nile Virus Serologic Surveys of WCS New York City Zoological Collections

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¹Wildlife Health Sciences, Wildlife Conservation Society, Bronx, NY, USA; ²U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA

A West Nile virus (WNV) epornitic occurred in free ranging birds in New York City, New York, U.S.A. and surrounding areas in 1999. This was followed by human WNV encephalitis cases. Later, birds in the collections of the Wildlife Conservation Society (WCS) became infected.¹ WNV occurred in 4 states in the northeastern United States in 1999, and in the year 2000 it was found in 12 states and the District of Columbia.

A serologic survey of WCS collections (Bronx Zoo/Wildlife Conservation Park; Central Park, Queens, and Prospect Park Wildlife Centers; and the New York Aquarium) was performed by plaque reduction neutralization assay. Samples were obtained from 1 Jun 1999 to 14 Mar 2000. WNV specific antibodies were not detected in any mammal (n=4) or bird (n=43) housed indoors. Variable numbers of mammals (n=204) and birds (n=403) housed outdoors had WNV specific antibodies (Table 1). None of the archived samples (obtained from 27 Sep 1994 to 27 May 1999) from seropositive mammals (n=1) or birds (n=41) were positive. Clinical illness occurred in both mammals and birds, but only birds died of WNV during the outbreak. WNV infections were most often asymptomatic and most of the birds that recovered had only mild clinical signs.

Acknowledgments
We thank the WCS veterinary technicians and Animal Departments; and the USAMRIID Research Serology Branch and Pathology Division technical staff; for their assistance.

Table 1. 1999 WNV serologic results of outdoor housed mammals and birds at the WCS.

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An Epidemic of West Nile Fever in the South of France: Results of an Epidemiologic Survey on Wild Birds


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West Nile fever is a mosquito-borne viral disease, acquired from wild bird reservoirs. Many bird species may be carrying the virus. Man and horses, which sometimes may show mortal symptoms of encephalitis (dead-end infection), are accidental victims. Yet, since birds are most often asymptptomatically affected, they are the main indicators of the disease. The disease has recently been described in Algeria (1994), Morocco (1996), Rumania (1996), Poland (1996), the Czech Republic (1997), Italy (1998), Western Russia (1999) and Israel (1999-2000). In France, in August 2000, a few cases of equine encephalitis were observed in horses in the Petite Camargue area in the Herault département, not far from Montpellier, 40 km West of the Grande Camargue, a wetland where in 1962-63 the disease had already been described in the horse and man. *Culex modestus* and *culex pipiens* are the mosquitoes incriminated in the transmission of the virus in the Camargue.

Between September 4 and November 15, 2000, the laboratory of the Pasteur Institute confirmed 78 cases in horses among 141 suspected ones. Twenty of them died. No humans were found to be infected, although the mild symptoms of the flu caused by the West Nile Virus may have passed undetected. In birds, contrary to observations made in 1999 in the New York City area, no abnormal cases of mortality have been reported either in wild birds or in poultry farmed in this area.

At the request of the Ministry of Agriculture, in October 2000, ONCFS started a preliminary serological and virological survey to identify the wild bird species that are carrying the virus and thus are virus reservoirs. Since no cases of mortality have been observed, it was rather difficult to select the bird species that should be investigated. The migratory birds that are wintering in Africa are an interesting case since they may have brought the virus to France. Unfortunately they had already left the area when the study started. Thus for the first survey, we selected mostly sedentary species with large populations: the house sparrow (*Passer domesticus*), the yellow-legged herring gull (*Larus cachinnans*), the black-headed gull (*Larus ridibundus*) and the mallard (*Anas plathyrynchos*).

From each of the 440 birds captured or killed, a blood and brain sample were taken and, if possible, a few vector arthropods (ticks). The data will be useful in allowing us to know whether the avifauna is hosting the virus throughout the winter which will help orient our investigations in view of a much larger survey scheduled for the summer of 2001.
Comparative Pathology of Iridovirus Infections in Tadpoles, Frogs and Salamanders

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Amphibian mortality events attributed to iridoviruses (Family: Iridoviridae; Genus: Ranavirus) have been documented in Australia, Canada, Great Britain, the USA and the former Yugoslavia. At the US Geological Survey’s, National Wildlife Health Center, 28 amphibian mortality events attributed to iridoviruses (ranaviruses) have been investigated since 1996. In 2000, 10 iridovirus mortality events, involving 11 species of amphibians were investigated and confirmed by virus isolation.

In the western United States, casualties in seven viral epizootics (23%) were limited to tiger salamanders (Ambystoma tigrinum), although other species were present in 5 events. In the eastern United States, iridoviral mortality events involved ranid tadpoles only, or a mix of ranid tadpoles, newts, two species of larval ambystomid salamanders and occasionally, recently metamorphosed frogs. Molecular analyses of select iridoviruses (reported in 2000 at the WDA Annual Mtg by Docherty, et al) indicates an eastern iridovirus is closely related to Frog virus-3 (FV-3, the type species of Ranavirus), while western iridoviruses from tiger salamanders are more closely related to each other than to FV-3.

External abnormalities vary greatly in amphibians with lethal iridovirus infections. Larval and neotenic ambystomid salamanders showed hyperemia and petechiation of the ventral skin, and irregular deep ulcers, 1-35 mm in diameter. In tadpoles, hyperemia and petechia were present in the ventral skin. Occasionally, saprolegnia-like skin infections invade some skin ulcers in tadpoles. Tadpoles may present with few or no petechia, but instead have focal or generalized edema of the subcutis (lymphatic sacs) while severe edema with ballooning of the skin of the body and limbs occurs mostly in late stage tadpoles and recently metamorphosed frogs. Focal edema most often occurs in the ventral thoracic and gular region of larval salamanders.

Internal gross abnormalities range from subtle to marked. Mild to very severe hydrocoelom occurs in most tadpoles at some sites, but usually is mild in salamanders. Liver changes most consistently seen include mild to moderate enlargement, diffuse pallor or marked mottling, congested capsular vessels, and infrequent petechia or minute white foci of necrosis. Spleens may be atrophied or mildly enlarged and diffusely bright red. The mesonephroi (kidneys) are inconsistently affected; some show diffuse minute petechia due to necrosis and hemorrhage in glomeruli, while others show swelling and hemorrhage due to hemorrhagic necrosis of interstitial myeloid tissue.

Histologically, iridovirus infections are characterized by necrosis in the liver, pancreas, spleen, thymus and occasionally mesonephroi and gastric mucosa. The most consistent histologic changes are liver degeneration and necrosis, with multifocal necrosis of sinusoidal macrophages, endothelium, and macrophages of the pigmented macrophage aggregates (PMA). Iridoviruses induce a characteristic cytoplasmic inclusion body. The inclusion is usually spherical, paranuclear, about 25-40% of the size of the host cell nucleus, and is consistently homogenously basophilic (blue). The skin initially shows hydropic swelling of epithelium, followed by vesicle formation and ulceration; with careful search, blue cytoplasmic inclusions may be found in cells in the vesicles and at the margins of vesicles and ulcers. In tadpoles, vesiculating necrosis of the keratinized jaw sheaths ("beaks") and toothrows may be found.

These findings are compared to published descriptions of amphibian iridoviral mortalities in Europe, Canada, Latin America and Australia.
Diagnosis of Chronic Wasting Disease in a Captive Elk Herd in Montana

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¹USDA:APHIS:VS: National Animal Health Program Staff, Fort Collins, CO, USA; ²Colorado State University, Department of Pathology, Fort Collins, CO, USA; ³USDA:APHIS:VS: National Veterinary Services Laboratory, Ames, IA, USA; ⁴USDA:ARS Animal Disease Research Unit, Pullman, WA, USA; ⁵Montana State Veterinarian, Helena, MT, USA

Tissues were collected from 81 captive elk in a herd depopulated in December 1999. An adult cow elk in the herd had been diagnosed with chronic wasting disease (CWD) in October 1999. The herd, located in western Montana, USA, had been under surveillance because two elk that originated from the herd had been previously diagnosed with CWD in Oklahoma. At the time of the depopulation, eighty of the elk were clinically normal and one animal was found dead. Protease resistant prion protein (PrP⁰⁰) was demonstrated by immunohistochemical (IHC) staining of brainstem sections in the animal found dead and 7 additional animals. IHC staining in four of the seven clinically normal animals was minimal and confined only to the obex. Three of the eight PrP⁰⁰-positive animals were 5 to 6-month-old calves. Although detection of preclinical animals through the use of IHC staining has been previously reported, this was the first diagnosis of CWD in naturally exposed animals this young. Only five of the eight animals with positive IHC staining of the obex had concurrent positive staining of tonsil or other cranial lymphoid tissue. These findings will aid in development of CWD surveillance strategies. Sampling of animals euthanized in depopulation efforts should continue to be utilized to gain additional epidemiological information on CWD.
Use of Tonsillar Biopsies for Ante-Mortem Diagnosis of Chronic Wasting Disease in Captive Mule Deer

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¹Colorado Division of Wildlife, Fort Collins, CO, USA; ²Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, CO, USA

We investigated the usefulness of tonsillar biopsies collected ante-mortem from captive mule deer (Odocoileus hemionus) to diagnose the transmissible spongiform encephalopathy chronic wasting disease (CWD). Tonsillar biopsies were collected at 6-9 mo intervals between July 1996 and January 1999 from 40 captive mule deer maintained at a CWD-endemic facility. Tonsillar biopsies were examined with immunohistochemical (IHC) staining for PrP. Clinical health of study deer was monitored and post-mortem samples were collected through August 2000. Thirty-eight mule deer died during the study period. Thirty-four of these deer were diagnosed CWD-positive based on brain lesions or IHC staining for PrP in brain tissue. Preliminary results showed that at least 23 of these CWD-positive deer had IHC positive tonsillar biopsies ante-mortem. Results are pending on samples from the remaining deer. Positive tonsillar biopsy staining was detected >2 yr prior to onset of clinical signs of CWD. Tonsillar biopsy appears to be a promising technique for the ante-mortem diagnosis of CWD in mule deer. Application of this technique will aid in the clinical diagnosis of CWD in individual mule deer and also in study of the epidemiology of CWD in populations of mule deer.
BOMA DINNER
Ownership Begins Here! Theatre as a Communications Key in Wildlife and Livestock Management Systems

Nicholas Ellenbogen
Theatre for Africa, Cape Town, South Africa

Most top-down bureaucratic systems have been damaging to the sustainability of wildlife conservation and natural resource management. Solutions to the co-existence of wildlife and livestock need to be found and made “on the ground”, i.e. in the actual communities who live with and utilize these resources. The debate as to who should actually have ownership of those resources continues to be a fiery one, while poor communication and consultation between communities, stakeholders and decision-makers is the order of the day.

Theatre has been proved to be an effective method of research and communication in community projects on sustainability and resource management. This paper outlines the methods used by Theatre for Africa from the first introduction into a community, through the processes of research, report back, rehearsal, and performance. It argues that theatre is unsurpassed as a means of channeling the voices of communities to policy-makers. At the same time, it can provide a powerful research tool from a social perspective into such age-old issues as competition between wildlife and livestock. It is a platform from which traditional wisdom can be incorporated into the management process.
SESSION 5

GENERAL SCIENTIFIC SESSIONS CONTINUED
TICK-BORNE DISEASES
Antigenic Variation of Anaplasma marginale in Persistently Infected Ticks

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Anaplasma marginale, an intraerythrocytic ehrlichial pathogen of cattle, establishes persistent infections in both vertebrate (cattle) and invertebrate (tick) hosts. The ability of A. marginale to persist in cattle has been shown to be due, in part, to major surface protein (MSP) 2 variants which are hypothesized to emerge in response to the bovine immune response. MSP2 antigenic variation has not been studied in persistently infected ticks. In this study we analyzed MSP2 in A. marginale populations from salivary glands of male Dermacentor variabilis persistently infected with A. marginale. After the ticks were infected by feeding on a bovine during acute parasitemia, they were removed and held in a humidity chamber and then allowed to feed successively on one susceptible bovine and three sheep. The salivary glands were dissected and pooled from twenty ticks collected from each host on days 3 and 7 of feeding, and the msp2 variants in each population were analyzed. New MSP2 variants appeared in each A. marginale population and sequence alignment of the MSP2 variants revealed multiple amino acid substitutions, insertions and deletions. As the multiplication progressed in tick salivary glands, more diversity was observed in msp2 sequences. The frequency of new MSP2 variants was higher in ticks collected during the first tick feeding, after which the frequency of new variants showed a tendency toward exponential decay. A shift in the predominant MSP2 variant types occurred in each population and was similar to the results of studies on A. marginale MSP2 from persistently infected cattle. These results suggest that selection pressure on MSP2 occurred in tick salivary glands independent of bovine immune response.
Cerebral Theileriosis in African Short-horn Zebu Cattle is caused by *Theileria taurotragi* and not by *Theileria parva*

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A fatal tick-borne disease complex among adult indigenous African short-horn zebu cattle, characterized by nervous symptoms and uncontrollable movements, has emerged over the past decades in a seasonal pattern in Northern Tanzania. The disease is called ‘Ormilo’ by the Maasai pastoralists, which reported the first cases in 1982 from Endulen division in Ngorongoro district. Maasai respondents consistently differentiate Ormilo from classical East Coast fever, which they record as Oltikana.

Despite reports that in East Africa cerebral theileriosis may be caused by *Theileria parva*, the use of reverse line blot (RLB) hybridization recently identified the disorder to be caused by the benign parasite *Theileria taurotragi*. Subversion to fatal infection of *T. taurotragi* is likely caused by a complexity of factors predisposing the expression of cerebral theileriosis. The presence of *Ehrlichia* organisms was demonstrated in a small proportion of samples but a direct link with clinical disease could not be made.

Bovine cerebral theileriosis has been reported from Tanzania as far back as the mid-eighties and was always contributed to *Theileria parva* infection. DNA extraction and RLB analysis of slides from cases in Ngorongoro District of Tanzania, as far back as 1987, have equally demonstrated the presence of *T. taurotragi* instead of *T. parva*.

The value of the oral history of disease occurrence in Maasai culture, the conscientious collection and storage of field samples in the past, and the development of new and powerful diagnostic tools have given us the opportunity to start re-writing the textbook chapter on cerebral theileriosis. However, although the results clearly demonstrate that the BCT cases from past and recent studies were/are caused by *T. taurotragi*, the subversion to fatal infection of the usually benign *T. taurotragi* parasite, as well as the increasing incidence reported from the field is likely to be caused by a complexity of factors. Identifying these predisposing factors will require studies on the entire tick-borne disease complex as part of the pastoral cattle management - wildlife interaction system.
Pan Mediterranean Comparison for the Molecular Detection of *Theileria annulata*


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Seven laboratories decided to compare their molecular diagnostic techniques to identify Mediterranean theileriosis due to *Theileria annulata*. Each laboratory used either PCR or PCR and reverse line blot hybridisation (RLB) to identify this tick-borne pathogen responsible for major economical losses in the Mediterranean area. Each laboratory sent its own samples to one participating group to be re-coded and passed to at least two other laboratories. A total of 120 blood samples were analysed during this study.

One laboratory sent only *T. annulata* infected samples and all the laboratories detected the infected. One laboratory sent only negative samples from a Mediterranean area where *T. annulata* was unknown and two laboratories found a few positive results for different samples. For the remaining samples detection performance was variable.

Percentage of unmatching results for each laboratory was recorded and it ranged from 0.8% to 9.3%. Laboratories using RLB got more matching results and found more positive samples than those using only PCR.

This paper describes the methodological parameters that could explain this variation.
An Assay to Evaluate the Role of Anaplasma Marginale Major Surface Proteins 1a and 1b in Infection of Cultured Tick Cells

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Previous studies have demonstrated differential adhesion of major surface proteins (MSPs) 1a and 1b of the ehrlichial cattle pathogen Anaplasma marginale in which MSP1a and MSP1b were both found to be adhesins for bovine erythrocytes, while only MSP1a adhered to native and cultured tick cells. In these studies, the Anaplasma marginale-Ixodes scapularis culture system was used to determine the role of MSP1a and MSP1b in infection of cultured tick cells by testing whether various antisera would neutralize infectivity of A. marginale. The Virginia isolate of A. marginale was used to inoculate monolayers of IDE8 cells grown to confluency in 24 well plates. An indirect ELISA test was developed to determine parasite densities in the cultured tick cells. Antisera tested for their ability to neutralize A. marginale infection included high titer bovine polyclonal antisera from acute and persistently infected cattle, sera from cattle immunized with purified erythrocytic A. marginale, and monospecific rabbit sera against MSP1a, MSP1b or both combined. In a series of trials monolayers in replicate wells were exposed to each antisera and inoculum for 60 minutes, and then washed to remove extracellular ehrlichiae. After incubation for 4 days, the monolayers were harvested and tested by indirect ELISA for A. marginale infection. Additional wells were collected to determine intracellular colony counts by light microscopy. Controls cultures included inoculum exposed to naive serum, untreated inoculum and uninfected tick cells. Polyclonal bovine sera, either from experimentally infected, naturally infected or immunized cattle did not effect neutralization of A. marginale in cell culture. When antisera to MSP1a, MSP1b or both combined were incubated with A. marginale, a neutralizing effect was observed but the most pronounced effect was with MSP1b or with MSP1a/MSP1b combined. These results support the role of the MSP1 complex in the adhesion of A. marginale to tick cells. The relevance of these results to naturally occurring A. marginale infections will be discussed. Additional studies are underway to determine neutralizing effects of bovine serum from cattle immunized with purified MSP1a and MSP1b. The cell culture system can, therefore, be used to determine the role of individual MSPs in infection of A. marginale in tick cells.
Comparison of Specificity and Sensitivity of the MAP 1B recombinant protein with a synthetic MAP 1B peptide for diagnosis of *Cowdria ruminantium* infection

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Abstract: Heartwater or *Cowdria ruminantium* infection causes severe economic constraints to livestock farmers in sub-Saharan Africa. Control is hindered because of a lack of reliable diagnostic assays. The MAP 1B antigen of *Cowdria ruminantium* has been proposed for serological diagnosis of heartwater using an indirect ELISA. This immunogenic region spans amino acids 47-92 of mature MAP 1 and is considered to be more specific for detection of *C. ruminantium*-specific antibodies than any other antigen of *C. ruminantium* so far tested. Due to difficulties cloning this immunogenic region, a recombinant MAP 1B that contains 60 additional amino acids (i.e. a.a. 47-152) is typically used in diagnostic assays. We investigated whether a synthetic peptide containing just the immunogenic region could replace the longer recombinant protein. A synthetic MAP 1B antigen of Crystal Springs strain (Zimbabwe) was compared with the *Escherichia coli* produced recombinant MAP 1B antigen of the Senegal strain for the detection of *C. ruminantium* antibodies in an ELISA format. The results of these analyses demonstrate that the Crystal Springs synthetic MAP 1B antigen can be utilized in this assay instead of an *E. coli* produced antigen without any detrimental effect on the sensitivity and specificity of the assay. Adaptation of this ELISA with region specific MAP-1B antigen(s) would possibly improve sensitivity of the assay and optimize the detection of *C. ruminantium*-specific antibodies. Additionally, a synthetic peptide-based ELISA may be more convenient for implementation by regulatory agencies concerned with control of heartwater disease.
Diagnostic Tests for the Detection of *Theileria* spp. Carrier Infections and their Implications for Translocation of African Buffalo (*Syncerus caffer*) in South Africa

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A myriad of diagnostic techniques is available for the detection of tick-borne disease pathogens, including *Theileria* spp., in carrier animals. These range from the rather simple less sensitive and less specific microscopic examination of stained blood films, through the more sensitive serological techniques, such as the indirect fluorescent antibody test and enzyme-linked immunosorbent assay, to the molecular techniques employing the polymerase chain reaction and oligonucleotide probes, the latter test promising unprecedented levels of detection and discrimination between *Theileria* spp. Although all these techniques may be used to confirm a diagnosis of clinical infection in a host, they are more frequently used in epidemiological studies or surveys in which disease distribution, prevalence and incidence are to be determined and not to certify the presence or absence of carrier infections in individual animals.

As one of the so-called "Big Five" dangerous big game species of Africa, African buffalo (*Syncerus caffer*) are amongst the most sought after game species for introduction into newly developed small game reserves, game farms and larger game conservancies in South Africa. However, as reservoir hosts of a number of important cattle diseases, amongst which is *Theileria parva*, the cause of Corridor disease in cattle, the movement and translocation of these animals are strictly controlled. Certification of the *Theileria* carrier status of buffalo is difficult due to limitations of sensitivity and specificity of the available diagnostic tests. Of particular relevance to South Africa is the use of these diagnostic techniques to certify the disease status of buffalo prior to translocation, particularly that of animals produced in breeding programmes aimed at breeding disease-free buffalo from infected parent stock obtained from *Theileria parva*-endemic areas.

Inevitably a certain amount of risk is involved when relying on a diagnostic technique to certify the disease status of individual animals, but particularly in the case of buffalo in South Africa, the financial and socio-economic implications of introducing an infected buffalo in an otherwise disease-free herd can be tremendous. Every effort should therefore be made to develop, standardize and fully validate those techniques which show the greatest potential for sensitivity, specificity and reliability, but for the present and foreseeable future, no single technique adequately complies with these requirements, thus necessitating the application of a battery of diagnostic techniques.
The Innate Resistance of Kenana Cattle to Tropical Theileriosis (Theileria annulata) Infection in the Sudan

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In Sudan, the Kenana zebu breed of cattle is well recognized for its high milk production potential and an ability to tolerate diverse environmental and nutritional conditions. A study was carried out to assess the innate resistance of the breed to tropical theileriosis, Theileria annulata infection. Nine susceptible Kenana calves were obtained from an area free from Hyalomma anatolicum anatolicum and tropical theileriosis and found negative to T. annulata antibodies in the indirect fluorescent antibody test. They were infected by inoculation of 1.0 ml T. annulata stablate (equivalent of 10 ticks, 96.2% infection rate, 200 infected acini per tick). Three Friesian calves were also infected and served as negative controls. The prepatent period to lymph node enlargement, body temperature, first appearance of schizonts and piroplasm parasitaemia were the same in all animals. However, the percent of schizont parasitaemia (Macroscopic Index, MSI) in the Kenana cattle was reduced by 70% compared to the Friesian calves. The percent of piroplasm parasitaemia was also significantly low in the Kenana calves (7.1 compared to 12.6). The rate of the white blood cells reduction was significantly greater in the Friesian calves. These differences were attributed to the high rate of schizont multiplication in the control cattle. Seventy-eight percent (7/9) of the Kenana cattle had recovered spontaneously and only 22% required treatment compared to the 100% mortality in the Friesian controls. All of the Kenana cattle developed high antibody titres to T. annulata parasitaemia by day 12 after infection. It is concluded that the ability of the Kenana cattle to limit the MSI, resulting in less severe damage of the lymphoid tissue during the acute phase of the disease, is the probable basis of their innate resistance to tropical theileriosis.
TOOLS FOR MANAGEMENT
Collaborative Research Initiatives in Botswana

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Oregon State University (OSU), the University of Botswana (UB), Conservation International (Cl) and the NW Consortium for Wildlife Conservation Research (CWCR) are currently collaborating on several research initiatives in the northern Botswana-Zimbabwe-Zambia region. The CWCR is a cooperative effort among regional zoo, wildlife, and educational institutions, based in the NW region of the USA. The Harry Oppenheimer Okavango Research Center (HOO RC) is UB’s field station on the southern border of the Okavango Delta. A recent USAID-ALO grant is facilitating research and training activities between OSU, the CWCR, and the HOO RC. Cl is evolving its landscape level approach to conservation by implementing biodiversity corridor strategies. By working together on multi-disciplinary research projects, using migratory and key species to identify ecological corridors and needs, we hope to promote cooperative management schemes among neighboring countries and thereby protect viable ecosystems which are otherwise vulnerable to disruption by limitations imposed by political borders.

Research efforts are focusing on increasing our understanding of the biology and ecology of the hippopotamus (*Hippopotamus amphibius*) and African elephant (*Loxodonta africana*). Threats to these species primarily relate to habitat loss and/or fragmentation associated with expansion of human populations and activities such as fishing, farming, livestock grazing, poaching, and tourism. As keystone species, disappearance of either species would have a cascading effect within the ecosystem. Our general study area will encompass the proposed Okavango—Upper Zambezi Transfrontier Conservation Area (OUZTFCA), including the Hwange and Zambezi National Parks in Zimbabwe, the Sioma Ngwezi and Kafue National Parks in Zambia, the Chobe and Makgadikgadi Nxa Pan National Parks and Moremi Game Reserve in Botswana, as well as conservancies in the Eastern Caprivi. A team of 20 scientists will be working with hippos to develop reliable census and tracking techniques to monitor population dynamics, and learn about hippo geomorphologic/hydrologic environmental impact, social behavior, nutritional requirements, reproductive biology, and biophysical ecology (e.g., thermoregulation, energetics). Elephants in this region (representing the largest contiguous population remaining on the African continent) will be studied to determine abundance and distribution, local, regional and transboundary movements, and habitat associations. Both projects will also genetically characterize subpopulations, and determine the extent and causality of hippo- and elephant-human conflicts.

The area where Botswana, Namibia, Zambia and Zimbabwe meet is a biological unit dominated by huge wetlands like the Okavango Delta, and Linyanti and Barotse floodplains with related rivers. Wise management of this region should be based on an understanding of the ecological impacts and needs of key species, and requires implementation through international cooperation. The HOO RC is playing an important role in coordination of both projects through its collaborations with the National Conservation Strategy Agency in Botswana to develop a management plan for the Okavango Delta (the Ramsar Plan), as well as the Department of Wildlife and National Parks. When formally established the OUZTFCA will represent the largest contiguous wilderness, wetland and wildlife area in southern Africa.
Mapping Wildlife and Human Disease as a Conservation Medicine Tool: The Yellowstone to Yukon Project with Implications to Africa

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The occurrence and distribution of disease on a bioregional scale provides an important indicator of ecosystem health. The knowledge of disease incidence has important implications for management involving translocation of species or for re-establishing habitat corridors between isolated populations. Described in this presentation is the Yellowstone to Yukon (Y2Y) Wildlife Disease Project. This project is an innovative endeavor that is part of a larger effort focused on reconnecting fragmented habitats for all wildlife, including migratory species and large carnivores that historically range beyond present day political and structural boundaries created by highways and human habitation. Using Geographical Information System (GIS) mapping techniques, we provide map layers of wildlife and human health over an environmental landscape. A comprehensive database was developed using disease information from state, federal, and provincial agencies and academic and private research institutions. Evaluating disease on a regional scale will provide wildlife and public health managers a tool to analyze environmental health within an ecological context and beyond jurisdictional boundaries.

The main objectives of our project include: 1) provide a baseline map of important wildlife diseases encompassing an area spanning the northern Rocky Mountains of the United States to the northern Canadian Rockies and, 2) provide corresponding map layers of associated human diseases transmitted directly or indirectly from wildlife or other vectors. The information will be collated and provided in a common GIS format to be distributed back to wildlife resource and public health agencies through an electronic information system. By looking at health within an ecological landscape we can address important issues in this region that will provide a database designed to minimize public health impacts of emerging diseases. Our database will set the stage for development of surveillance methods to identify public health threats from cross-species disease transmission. Diseases selected for analysis were based on biological, zoonotic, and socioeconomic importance to the region. Diseases included in the database and identified through the literature, professional symposia and agency contacts include brucellosis, bovine tuberculosis, chronic wasting disease, sylvatic plague, and Hantavirus infection. By working with agency personnel we can gain a better understanding of the issues affecting management of individual wildlife populations and the effects of disease on those populations. We also set the stage for the management of wildlife diseases across international borders which will provide wildlife resource and public health agencies a tool to implement collaborative strategic planning for wildlife populations, habitat, and human health objectives on a larger bioregion scale.

Many of the disease dynamics and political and jurisdictional boundary issues in the Y2Y region are similar in other regions of the world, and may be especially relevant to Africa. We will discuss how the ideas and techniques of the Y2Y project may be used to address similar problems in an African context.
Occupational Health Programs for Primate Fieldworkers

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The potential for disease transmission among humans and nonhuman primates has long been recognized and addressed in captive settings. Occupational health programs have been developed for those working closely with primates, and include prophylactic vaccination and regular screening for infectious diseases such as tuberculosis. However, such programs are generally not part of field research projects. Visiting researchers and tourists may be current on vaccinations because of traveler’s health recommendations, but many researchers and field assistants from developing countries are lacking primary immunization series or boosters. Although World Health Organization initiatives provide vaccines for children and women of reproductive age, access and compliance are often limited, particularly in the remote or rugged areas where many primate research projects are located. Beyond childhood, vaccines are not generally provided for men.

Field projects need to develop and consistently implement occupational health programs for their researchers, employees, support staff, and associated families. Those programs should consist of several components, including: (1) a pre-employment health review, (2) baseline bloodwork and screening/testing for specific diseases (e.g. tuberculosis, gastrointestinal parasites), (3) prophylactic vaccination, (4) maintenance of medical records and, ideally, a reference serum bank, (5) development and implementation of standard operating procedures, and (6) periodic health evaluation and continuing education. It is the responsibility of those working with primates in the field to take appropriate actions to protect their health as well as habitat.

Additional opportunity exists to collaborate with regional medical personnel to improve health care delivery for people living in or adjacent to primate habitat. Support can range from the sponsorship of regular health and hygiene education seminars or the loan of project equipment to provision of salary for a nurse or physicians assistant at a local clinic. Such programs can increase the benefit that local people derive from primate habitats and associated research projects, and thus strengthen their support for both. Improving the health of people who live and work in proximity to primates benefits not only the people but the other primates as well, by decreasing the risk of interspecies disease transmission.
Parenteral Delivery of Vaccines to Free-ranging Bison in Yellowstone National Park

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Yellowstone National Park bison have been affected by bovine brucellosis for at least 80-plus years. How to deal with this economically important cattle disease in wildlife has been controversial, and the subject of numerous interagency negotiations. One element that has widespread support is use of a safe, effective and deliverable brucellosis vaccine in free-ranging wildlife. Deliverability has received little attention even though current brucellosis vaccine technology is most effective by the parenteral route. Such a route of delivery will be logistically difficult in free-ranging wildlife.

Ballistically-delivered S19 has been used for years on fed elk populations in the southern Greater Yellowstone Ecosystem. This system uses a hydroxypropyl cellulose biodegradable bullet (biobullet) propelled by compressed air to parenterally deliver encased, lyophilized vaccine. We used an updated version of this technology to assess its effectiveness at penetrating and delivering vaccine to free-ranging bison. We used a new Ballistic Technologies, Inc, stainless steel gun with rifled barrel, 4x telescopic site, and braced on a monopod for shooting. A high pressure regulator (1500psi) was attached to improve penetration. Bullet size was 25 caliber, 1.69cm (0.667 inches), weighing an average of 761mg with payload. All animals were shot so that the biobullet was observed to strike at or near the caudal thigh. Misses were re-shot.

We delivered mock vaccine-laden bullets to 26 bison calves and yearlings at mean distances of 20 (n=9), 35 (n=9) and 50 (n=8) yards. Four animals at 20 yards were double dosed so that total bullets that impacted bison was 13. We chose iophenoxic acid (IPA), a safe parenteral serum biomarker in bison (Sweeney, et al. 2000) to avoid using vaccine whose detection depends on bison immuno-response. Detection of IPA in blood is solely dependent upon parenteral penetration and dissolution of the biobullet and has a half-life in bison of about 51 weeks.

Individual bison were handled in chutes and identified by ear and pit tag, blood sampled and randomly assigned to a shooting distance. We shot bison with IPA biobullets by allowing 3 at a time to free-range in an enclosure. Each animal was identified by ear tag and shot at a distance close to its assigned distance. Actual distance was determined by laser range-finder, and fell within 2 yards of assigned distance. Post-shooting blood was sampled at 21, 49 and 78 days.

Pre-shooting IPA blood levels were below detection, as were 4 bison shot with blank biobullets. We observed bounce-offs and shattered biobullets during shooting. IPA was detected in sera of 8/9 (89%), 4/9 (44%) and 1/8 (13%) of bison at 20, 35, and 50 yards, respectively, and provides a maximum measure of effectiveness of the system at these distances. Of the 4 bison at 20 yards that were shot twice, 1 animal, and possibly a second, received both doses of IPA. We missed shots at both 35 (1) and 50 (4) yards. Overall delivery success was 77% (10/13), 40% (4/10) and 8% (1/12) at 20, 35 and 50 yards.
Evaluation of *Brucella abortus* Strain RB51 and Strain 19 in Pronghorn Antelope

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Free roaming elk and bison in The Greater Yellowstone Area remain the only wildlife reservoirs for *Brucella abortus* in the United States, and the large number of animals and a lack of holding facilities make it unreasonable to individually vaccinate each animal. Therefore, oral delivery is being proposed as a possible option to vaccinate these wild ungulates. One of the main problems associated with oral vaccination is the potential exposure of non-target species to the vaccines. The purpose of this study was to determine the effects of two *Brucella* vaccines, Strain 19 (S19) and the rough strain RB51 (SRB51), in pregnant pronghorn antelope. Thirty animals per group were orally exposed to either 1x10¹⁰ colony forming units (cfu) of S19 or SRB51 or saline. Acute respiratory disease and chronic endemic foot-rot necessitated euthanasia of large numbers of antelope in all three treatment groups. Acute mortality was due to capture-related problems that included stress, heat, pasturella pneumonia, and trauma. Foot-rot and gram negative pneumonia contributed to chronic mortality. Despite the high mortality rate, it was possible to determine vaccine colonization rates and the vaccination effects on maintenance of pregnancy. All of the animals remained pregnant throughout the experiment regardless of vaccine status. None of the animals' reproductive tracts or fetal membranes showed any pathological lesions associated with brucellosis. Only two dams were culture positive during early gestation; one with S19 (1-2 cfu) and one with SRB51 (3 cfu). In mid-gestation two animals' fetal tissues were S19 (1-2 cfu) culture positive. Therefore, we conclude that S19 and SRB51 rarely colonize maternal and fetal tissues of pregnant pronghorn and were not associated with fetal death. Oral delivery of either vaccine at this dose appears to be non-hazardous to pregnant pronghorn.
A Vaccination Trial Using an Experimental Recombinant Canine Distemper Vaccine in Island Foxes (*Urocyon littoralis*)

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In 1999, free-ranging island foxes (*Urocyon littoralis*) on Santa Catalina Island (California, USA) underwent a severe population decline. One of the foxes found dead had evidence of canine distemper virus (CDV) in the pulmonary tissues by histologic and immunohistochemical stains, suggesting that an outbreak of canine distemper may have occurred. In order to determine the extent of the apparent fox mortalities, trapping transects were run across the island. Only 4 island foxes were captured on the eastern 75% of the island (0.4% trap success), while 55 were captured on the remaining 25% of the island (37% trap success). Two of the 4 clinically normal island foxes from the heavily impacted eastern portion of the island had antibody titers to CDV of 1:128 and 1:256. Neutralizing antibody titers from island foxes on the western end of the island (*n* = 37) were considered negative, indicating a probable lack of exposure of this group to CDV.

In order to protect the remaining island foxes (estimated at 150-200) from another possible outbreak of canine distemper, a vaccination program against this disease was considered, although no commercially available vaccine safe for island foxes was available at the time. In February 2000, using an experimental monovalent canary pox vectored canine distemper vaccine (CPV-CDV) provided by Merial, Ltd. with a protocol developed by one of the co-authors (RJM), a preliminary vaccination trial was initiated with six captive island foxes. Four of the foxes were inoculated with an initial intramuscular injection and revaccinated two weeks later. A fifth fox was given a single dose of the vaccine, and the sixth was used as a control and given only sterile water injections. Blood samples were taken from these animals just prior to and at two weeks and six weeks after the initial administration of the CPC-CDV. All of the vaccinated foxes seroconverted with titers ranging from 1:32 to 1:128. The control animal remained seronegative throughout the trial. None of the animals showed any untoward local or systemic effects from the vaccine.

With the promising results of this preliminary trial, the California Department of Fish and Game granted permission to use the experimental vaccine on the free-ranging foxes remaining on the island. In October 2000 the vaccination program was initiated as follows: On first capture all of the island foxes were bled for CDV titers and 70% received a single intramuscular dose of CPC-CDV; 10% were given two doses, and 20% served as controls receiving only sterile water injections. Booster vaccinations following the same protocol were given after a minimum of 21 days.

As island foxes are easy to capture and tolerate handling well, we expect that >90% of the foxes will be included in the trial. In March 2001, attempts will be made to recapture as many foxes as possible to obtain sera for six month post-vaccination titers. This new vaccine may provide a safe method for conserving carnivore species that are sensitive to canine distemper virus.
Restoration of Elk (*Cervus elaphus*) in Ontario, Canada

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Efforts are currently underway in Ontario, Canada, to restore a species that was once native to that Province. The Ontario Elk Restoration program is a collaborative effort that includes 14 partner organizations. Wild elk for the Ontario restoration program are acquired from Elk Island National Park (EINP), Alberta. Following live-capture of elk using corral traps at EINP, animals are tested for diseases such as bovine tuberculosis and brucellosis, treated for liver flukes and other parasites, administered agents for the prevention of capture myopathy, fitted with radio-collars (VHF and GPS), ear-tagged for identification, and shipped to Ontario in goose-neck trailers. To date (February 2001), about 465 elk have been shipped to Ontario for release into the wild.

Six release sites in Ontario have been selected to receive elk. Those locations were determined through the use of a habitat suitability model. To date (2001), elk have been released in four areas of the Province. Restoration of elk at the other two sites will be dependent on the results of a program assessment to be initiated in the near future.

Cumulative mortality of restored elk in Ontario was about 26% (n=86/336) during 1998-2000. Causes of mortality included: emaciation (21%), wolf predation (20%), bacterial infections (10%), injuries (10%), shooting/poaching (8%), drowning (7%) and road-kills (5%). Mortality was higher in female calves than in adults.

About 55 calves produced by the restored elk were observed during 1998-2000. Efforts are underway to determine the causes of calf mortality and low productivity.

Research programs at post-secondary institutions have been initiated at three of the release sites. Programs are focusing on the dynamics of restored elk populations, potential for competition between elk and other ungulates such as white-tailed deer, impact of predation by wolves and bears, prevalence of infectious diseases in restored elk, and development of an elk population and dispersion model using GPS technology. There is also concern over the impact of escaped ranch elk transmitting infectious diseases to wild elk in Ontario.
Wildlife Rabies Control in North America

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There are several variants of rabies present in terrestrial wildlife species in North America. They include: the Arctic fox strain found primarily in the northern regions of the continent; two skunk strains, one in the Prairie Provinces of Canada and the U.S. Midwest, and the other in the south central U.S.; coyote and gray fox strains in Texas; and the raccoon strain in the eastern U.S. and Canada. Control of rabies in the vector species involves the use of three different tactics: (1) population reduction; (2) live-trapping, vaccination by injection, and release of the animal; (3) oral vaccination using vaccine baits.

Control of the Arctic fox strain of rabies in Ontario, Canada, has been accomplished through the aerial distribution of baits containing ERA modified live-virus rabies vaccine. More than 11 million vaccine baits were aerially distributed in Ontario during 1989-2000. As a result, the number of Arctic fox strain cases in Ontario has declined from an average of 2,000 cases annually during the 1980’s to fewer than 100 cases per year during late 1990’s. Economic savings to Ontario due to control of the disease is about $3 million/yr Cdn. As well, human post exposure treatments have declined dramatically.

The raccoon variant of rabies is enzootic over a one million sq km area from Florida north to Ontario, Canada. Raccoon rabies associated costs approach $300 million annually. Control of that strain of rabies has been initiated in several U.S. states including Ohio, Florida, Massachusetts, Vermont and New York using oral rabies vaccine baits (Merial V-RG recombinant vaccine). In Ontario, Canada, Point Infection Control methodologies are being used to eradicate the disease. This involves the population reduction of vector species (raccoons, striped skunks) in a core zone around any case locations. Outside of the population reduction zone, vaccination of vector species is accomplished via live-trapping, intramuscular injection of Imrab (Merial) inactivated rabies vaccine, and release at the point of capture. Oral rabies vaccine baits (Merial V-RG recombinant vaccine) are then distributed around the perimeter of the control zone. During 2000, more than 5000 raccoons were euthanized, about 10,000 raccoons were vaccinated by injection, and more than 800,000 V-RG vaccine baits were aerially distributed in eastern Ontario to control raccoon rabies. Since July, 1999, only 50 cases of raccoon rabies have been reported in the control zone. In U.S. States where raccoons control programs were not implemented following the initial report of the disease, several hundred cases of raccoon rabies were reported during the following year. Proactive raccoon rabies control programs in Ontario are saving that Province about $12 million Cdn/year.

More than 13 million oral rabies vaccine baits have been aerially distributed in Texas since 1995 to control coyote and gray fox rabies. The disease has been virtually eliminated in coyotes due to intensive control efforts. The key to international control of rabies in North American wildlife populations will be through the coordination of efforts at the regional level.
ANTIGENS & VACCINES
Use of the Mannan Receptor to Selectively Target Vaccine Antigens for Processing and Antigen Presentation through the MHC Class I and II Pathways

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Extensive studies have shown that synthetic and recombinant vaccines developed against hemoparasites have not been as effective in eliciting protective immunity as whole parasites or crude membrane fractions. A possible reason is that synthetic vaccines are not being presented in a form that induces the appropriate immune response. We have developed a bovine model system to evaluate the ability of adjuvant compounds to induce an immune response to peptide antigens dominated by a cytokine profile with a Type 1- (cell-mediated) or Type 2- (humoral) bias. In the initial testing of this system, we have found that the mRNA expression of certain cytokines (IL-1β, IL-6, IL-12, IL-15, GM-CSF, iNOS, and TNF-α) is enhanced when monocyte-derived macrophages are stimulated with peptide antigen conjugated with mannan under oxidizing conditions compared to peptide conjugated with reduced mannan. The data suggest this model will be useful in identifying adjuvant systems that selectively modulate the cytokine profile of antigen presenting cells at the time of antigen presentation and the consequent downstream maturation of naïve T cells to effector cells with Type 1 or Type 2 cytokine bias.
Contributions of MHCII, TLR4 and NRAMP1 Genes in Conferring Cellular Immunity to Ehrlichia chaffeensis

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Human monocytic ehrlichiosis (HME) is an emerging tick-borne disease caused by the rickettsia, Ehrlichia chaffeensis. In this study, we investigated the role of macrophages and T-cells for their contributions to cellular immunity against E. chaffeensis using wild-type mice and mice lacking a functional macrophage activation gene (Tlr4), natural resistance associated macrophage protein 1 gene (Nrampt), and the MHCII genes. All E. chaffeensis-infected mice developed transient hepatic inflammation within the first three weeks of infection. The decline of liver inflammation was not correlated with the rickettsial clearance. Wild-type mice stimulated an effective immune response resulting in the clearance of infection within two weeks. The response is associated with the synthesis of E. chaffeensis-specific IgG synthesis, predominantly IgG2a subclass antibodies. Mice with Nrampt deficiency had no impact in the E. chaffeensis clearance, but influenced the IgG isotype expression from a predominant IgG2a to IgG3. Mice lacking the functional macrophage activation gene, Tlr4, impacted the nitric oxide and IL6 production and resulted in the short-term persistent infection for 30 days. All MHCII−/− mice had persistent infections throughout the three months evaluation period. Two MHCII−/− mouse strains also had E. chaffeensis-specific transient IgG response suggesting that B-cell class-switching can occur independently of CD4+ T-cells. However, the synthesis of E. chaffeensis-specific antibodies in MHCII−/− mice did not completely eliminate the infection. Together, these data suggest that macrophage activation is important for early clearance, while the long-term persistence in MHCII−/− mice suggests that CD4+ T-cell-mediated immune response is critical for conferring immunity to E. chaffeensis.
Function and Evolution of Major Surface Protein 1A of the Ehrlichial Pathogen *Anaplasma marginale*

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The major surface protein (MSP) 1a of the MSP1 surface protein complex of the ehrlichial cattle pathogen *Anaplasma marginale*, encoded by a single-copy gene *msp1α*, has been shown to be an adhesin for bovine erythrocytes and tick cells. The molecular weight of MSP1a varies among geographic isolates of *A. marginale* because of varying numbers of a tandemly repeated 28-29 amino acid peptides. However, *msp1α* has been found to be a stable genetic marker of *A. marginale* isolate identity throughout the pathogen's development in acutely and persistently infected cattle and in ticks. Phylogenetic analyses of MSP1a in North American isolates with regional tick species have provided biogeographic information and suggested tick-parasite co-evolution. Furthermore, MSP1a was shown to be a marker for transmissibility of *A. marginale* isolates by ticks because this protein is the adhesion protein required for infection of tick cells. The MSP1a domain responsible for adhesion to tick cells was localized to the amino terminal of the protein, including the sequence of the tandem repeats. The MSP1a of a non-tick transmissible isolate (a recent Florida isolate of *A. marginale*) proved to be nonadherent to tick cells and thus confirms the key role of MSP1a in the ability of *A. marginale* to infect and be transmitted by ticks. Variation in the tandem repeats may have resulted from evolutionary pressures exerted by ligand-receptor and tick-parasite interactions. Markers of tick transmissibility of ehrlichial pathogens as demonstrated herein are important to characterize and will influence control strategies and the design of effective subunit vaccines.
Tick Cell Culture Derived Anaplasma marginale as an Immunogen for Cattle

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Vaccines marketed for control of anaplasmosis have included both live and inactivated products made with Anaplasma marginale antigen derived from bovine erythrocytes. Blood-derived anaplasmosis vaccines were difficult to standardize, expensive to produce and were at risk of being contaminated with bovine cell stroma or other hemoparasites and viruses that often persistently infect cattle. In addition, geographic isolates of A. marginale were not cross-protective when used as vaccine strains. We recently developed a cell culture system which allows for propagation of A. marginale in a continuous cell line derived from embryos of Ixodes scapularis ticks. In this study we tested the cell culture-derived A. marginale as an immunogen for cattle. The Virginia isolate identity of the cell culture-derived A. marginale was confirmed by Pvull restriction analysis of the msp1a gene amplified by polymerase chain reaction. Eleven yearling holstein cattle were immunized subcutaneously with the cell culture-derived A. marginale and eleven cattle were non-vaccinated contact controls. Each vaccine dose contained $1.89 \times 10^{10}$ A. marginale in an oil-based adjuvant. Immunizations were administered four weeks apart and the cattle were challenge-exposed 10 weeks after the second immunization with $1 \times 10^5$ infected erythrocytes (Virginia isolate) administered intravenously. Antibody titers were determined by an A. marginale competitive ELISA using a monoclonal antibody specific for MSP5. Maximum antibody titers were observed two weeks after the last immunization. The specific antibody response against MSP1a and MSP1b was also determined and immunized cattle demonstrated a preferential recognition for MSP1b which has been shown to be an A. marginale adhesin for bovine erythrocytes. Cattle immunized with the cell culture-derived A. marginale had a significantly lower percent reduction in the packed cell volume ($P<0.05$) as compared with the controls and did not display clinical anaplasmosis. The cell culture derived antigen appears to be useful as an antigen for vaccine development that will be safe, easily standardized and will be free of contaminating bovine cells or pathogens. Several isolates of A. marginale propagated in cell culture may be combined to insure vaccine efficacy in diverse geographic areas.
**Cowdria ruminantium** Antigens of Around 15 kda are Potent Inducers of IFN-γ

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Three creole goats were immunized with inactivated *C. ruminantium* (Welgevonden strain) mixed in ISA50. In two of them, cellular responses induced by the vaccination were confirmed by IFN-γ production (ELISA) and IL-2 receptor expression (flow cytometry). Both CD4+ and CD8+ T cells but not γδ T cells showed a substantial increase in cell surface expression of IL-2 receptor molecules in response to whole *Cowdria* lysate but not to non-infected endothelial cell lysate.

*C. ruminantium* proteins were fractionated using continuous-flow electrophoresis and tested for their ability to induce IFN-γ production by PBMC collected on two different occasions: Three weeks after the first inoculation and one week after the booster injection. Pooled fractions of 15.2 to 15.6 and 22 and 24.4 kDa were found to induce significant (above medium + 3 standard error) IFN-γ production in both animals on one of the two occasions. Antigens of 15.2 to 15.6 kDa induced substantially higher IFN-γ production than any other fractions in both animals.

These pilot experiments pave the way towards the identification of proteins/genes that have potential for the development of a recombinant vaccine against heartwater.