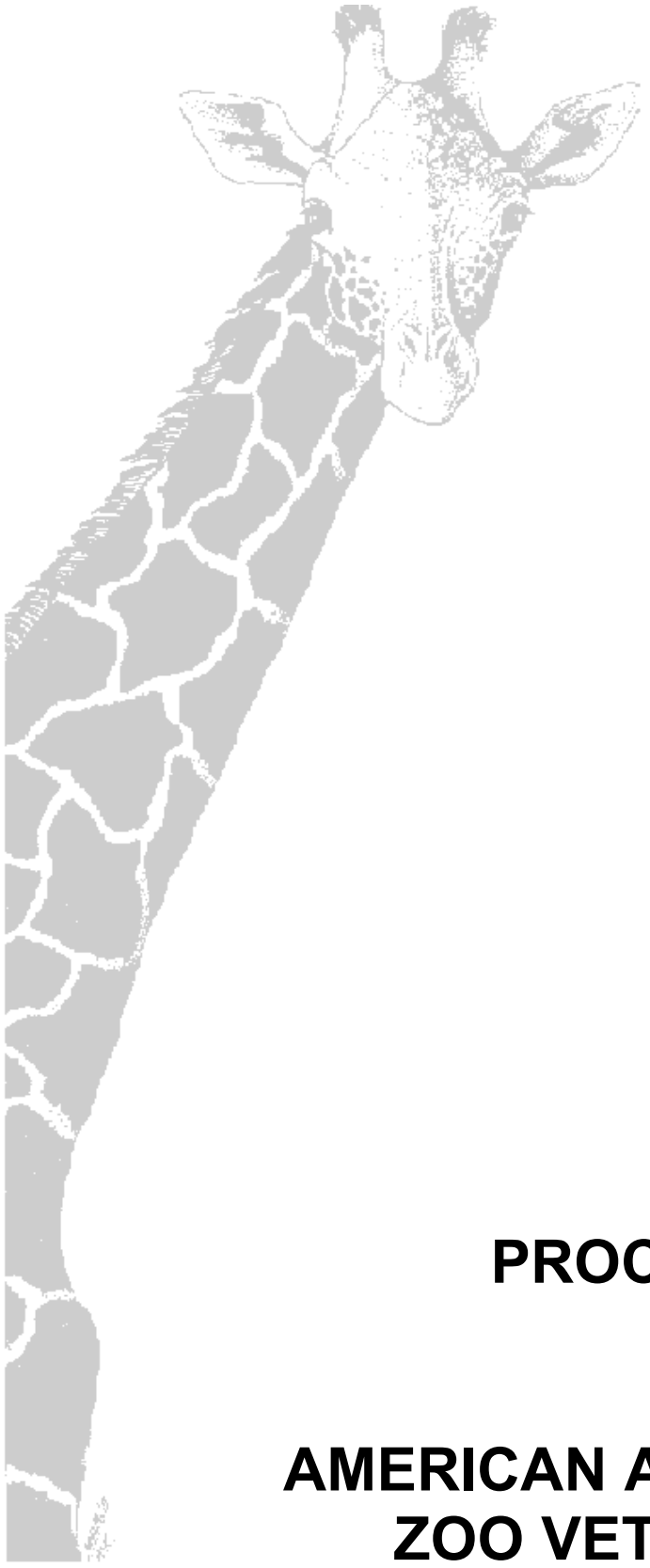
At this time, only abstracts for the Wildlife Disease Association portion of the conference are available.

Abstracts are available below from sessions on:

- DISEASE ECOLOGY AND EPIDEMIOLOGY
- TERRY AMUNDSON STUDENT PRESENTATIONS
- WEST NILE VIRUS AND OTHER VECTOR-BORNE DISEASES
- CHRONIC WASTING DISEASE
- DISEASE SURVEILLANCE AND DIAGNOSTICS I
- DISEASE SURVEILLANCE AND DIAGNOSTICS II
- ECOSYSTEM HEALTH



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AMERICAN ASSOCIATION OF ZOO VETERINARIANS

AMERICAN ASSOCIATION OF WILDLIFE VETERINARIANS

WILDLIFE DISEASE ASSOCIATION

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Joint Conference

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San Diego, California
August 28 – September 3, 2004

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AMERICAN ASSOCIATION OF ZOO VETERINARIANS

"Dedicated to wildlife health and conservation."

Dear Colleagues and Friends,

It is my pleasure to welcome you to this Joint Conference of the American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians, and the Wildlife Disease Association. It has been nine years since these organizations have met together, and I hope this meeting will rekindle professional relationships and friendships. I would also like to personally welcome you to San Diego, California.

There are many to thank for the planning of this very special meeting. The conference program is a reflection of the shared conservation mission and international scope of our organizations. Program chairs Joe Flanagan, Jonna Mazet, Pam Yochem, and David Jessup have planned a meeting that exemplifies our commitment to wildlife and the environment. Others to acknowledge for their tireless efforts include Charlotte Kirk Baer, Proceedings Editor, Dalen Agnew, CD-ROM Editor, and all of the session chairs. My colleagues at the San Diego Zoo and Wild Animal Park have been a great help in organizing many of the workshops and details of this meeting. You are sure to be inspired and exhausted by the end of the week!

The Zoological Society of San Diego (ZSSD) and the Local Host Committee deserve special recognition for the many months of planning. I would like to thank Doug Myers, Executive Director of the ZSSD, Robert McClure, Director of the San Diego Zoo's Wild Animal Park, and Richard Farrar, Director of the San Diego Zoo, for their commitment to making this endeavor a success. As local host, the ZSSD realizes the potential for reducing negative impact upon the environment by committing to green meeting practices. This conference will actively inform and encourage its participants to adopt similar practices and will serve as a model for future meetings hosted by the ZSSD. By incorporating a number of environmentally conscious choices into our meeting plans, we can all do something simple to support conservation in our daily lives. Throughout this conference, look for information and demonstrations on how your organization can make events and meetings "green."

I applaud Dave Jessup for his efforts in securing a $5000 grant from USDA to support student attendance. In addition, AAZV has increased their sponsorship of international attendees to $5000. Mazuri, Wildlife Pharmaceuticals, Morris Animal Foundation, Safe Capture, and Disney's Animal Programs continue to sponsor and support this conference and our association. I would like to acknowledge Sea World of San Diego and specifically, Dennis Burks, General Manager, and, Mike Scarpuzzi, Vice President of Zoological Operations, for their generous support. Continuing education credits are once again being sponsored by the American College of Zoological Medicine.

Finally, I would like to thank Wilbur Amand, AAZV Executive Director, and Julie Fazlollah, Conference Assistant, for making it all happen. I have worked with Wilbur and Julie for many years and they should be commended for their dedication and sense of humor.

I wish you a fun, educational, and memorable week. I hope that you do return home safely, eager to make a difference for conservation.

Kind regards,

Nadine Lamberski, DVM, Dipl. ACZM
President, American Association of Zoo Veterinarians 2003-2004

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July 12, 2004

To: Members of the American Association of Wildlife Veterinarians (AAWV) Wildlife Disease Association (WDA), American Association of Zoo Veterinarians (AAZV) and all other attendees at the joint annual meetings.

From: David A. Jessup, President AAWV

Re: WELCOME !!!

It has been 9 years since we last had a meeting of this size and scope in North America and since our 3 organizations last met together. Speaking for AAWV, its officers and members we are pleased and proud to have a part in this monumental undertaking and to welcome you to San Diego, California.

Meetings of this size and nature are not easy to put together. We have tried to balance an excellent, almost exhaustive, educational program with several opportunities to enjoy San Diego and its amenities. We have tried to balance the need for a large hotel venue with conservation efforts and the personal touch. This required hundreds of hours from the officers and members of three organizations, for which we are all grateful, and a willingness to compromise traditional activities and agendas with the recognition that the whole is more than the sum of its parts. Clearly we all share a dedication to and love of wild animals and their ecosystems as evidenced by the conservation theme of this meeting. Wilbur Amand and his staff have done an excellent job organizing the meeting. The San Diego Zoological Society has provided tremendous sponsorship and staff support.

A great deal of critical work is accomplished during working sessions at meetings like this and the education and professional advancement opportunities provided by pre-meeting workshops and wetlabs; the AAWV, WDA, and AAZV sessions; posters; exhibitors, as well as side meetings and interpersonal interactions are unparalleled. We are pleased to co-host a meeting that is so attractive to so many colleagues from around the world. Both WDA and AAZV supported international attendance and provided funding and opportunities for students to participate. We thank United States Department of Agriculture for a grant to all three organizations that allowed us to support more students than ever.

Welcome to San Diego, to California and its beautiful Pacific coast, and to the United States and have a great time.

We meet again!

On behalf of the Wildlife Disease Association (WDA), it is my great pleasure to welcome you to San Diego and the 53rd Annual Meeting of the WDA. Nine years ago the WDA also had a joint meeting with AAZV and AAWV. That meeting was a success, which many of us remember, and one of the reasons why the WDA with great anticipation accepted to have a joint meeting with AAZV and AAWV once more. Another reason is of course the location to San Diego with its wonderful surroundings, the San Diego Zoo and Wild Animal Park and other exciting places to visit.

Diseases of wild or non-domesticated animals are the main interest that puts our three organizations and us as scientists together. All diseases of wildlife, from the big pandemic emerging viral diseases of birds to the single case report of a malformation in an captive animal, improve our knowledge and makes us better in managing our wild animals, animal populations and ecosystems whether they are in the wild, in a zoo or in an animal park.

The organizers of this year's meeting have done an enormous job and the scientific program looks impressive and most interesting. Going through the program it is easy to see how much in common the three organizations have and that most papers are of interest for most people attending the conference, independent of the organization to which they belong.

Many thanks to all those in the organizing committee and everyone else who has spent many hours in arranging this conference. This includes the volunteers from all three organizations as well as the staff of the San Diego Zoological Society and a lot of others. I am convinced that this year's meeting will be a success and, from the perspective of the WDA, a most notable meeting.
I hope you all will enjoy the venue and the program and the fantastic opportunity to meet scientists with the same interests from all over the world.

Torsten Mörner
President
Wildlife Disease Association

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ABSTRACTS

Disease Ecology and Epidemiology

UNDERSTANDING THE ECOLOGY OF NIPAH VIRUS: AN EMERGING ZOONOTIC PARAMYXOVIRUS

Jonathan H. Epstein, DVM, MPH,[1]* Sohayati Abdul Rahman, BVSc,[2] Juliet Pulliam, BS,[3] Raina Plowright, BVSc,[4] Craig S. Smith, BSc,[5] Hume Field, BVSc, MVSc,[5] Peter Daszak, PhD,[1] and the Henipavirus Collaborative Research Group[6]

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Abstract

During the 1990s two novel, closely-related, paramyxoviruses emerged in Australia and Malaysia which resulted in the significant loss of human life.[1,2] Hendra virus (HeV) and Nipah virus (NiV) each moved from natural fruit bat reservoirs into intermediate domestic animal hosts (horses and pigs respectively) and then into humans, with fatal consequences. These two pathogens have been described as members of a new genus of paramyxovirus: *Henipavirus*. While the number of human cases of HeV was limited, (3

cases, 2 were fatal) in Malaysia there were 265 cases of NiV with a near 40% case fatality rate. Recurrent neurological infection has affected approximately 7.5% (n=160) of those who survived NiV infection.[3] Outbreaks of novel Nipah-like viruses have occurred within the past 4 years in South Asia, resulting in the loss of human lives, with the most recent outbreak occurring in Bangladesh in January, 2004.[4] The Henipavirus Collaborative Research Group, funded through the NIH Fogarty International Center, is working to understand the ecological and anthropogenic factors that drive the emergence of henipaviruses, as well as the mechanism for transmission between their wildlife hosts and humans. We are testing three main hypotheses in this study:

1) Did anthropogenic pressure on fruit bat habitat and populations via deforestation and hunting alter the distribution and movement patterns of fruit bats, bringing a higher than usual concentration of infected bats to the index farm prior to the 1998-9 outbreak?
2) Did climatic factors including the 1997 El Nino Southern Oscillation and land-use change, including the expansion of fruit orchards, alter the distribution of food availability for flying foxes, causing them to aggregate near the site of Nipah virus emergence and allow for an emergence in pigs to occur?
3) Did an expansion or intensification of pig farming in Malaysia provide the correct conditions for a change in host-pathogen dynamics that allowed a repeatedly introduced virus to become enzootic, then epidemic in pigs?

Pteropus vampyrus and *Pteropus hypomelanus* have been found to carry NiV-neutralizing antibodies at a significantly high prevalence, with virus having been isolated from *P. hypomelanus*.[5,6] These pteropid bats are considered the probable reservoirs for Nipah virus in Malaysia. We are using satellite telemetry (Microwave Technologies, Maryland) combined with ground-truthing to describe the distribution and long-range movement patterns of *P. vampyrus*, which has been located at the point of emergence of Nipah virus. We are also conducting disease distribution surveillance in *P. vampyrus* and both distributional and longitudinal disease surveillance in the Island flying fox, *P. hypomelanus*. To date, approximately 26.3% of *P. vampyrus* (n = 38) and 20% of *P. hypomelanus* (n = 157) have carried serum neutralizing antibodies to Nipah virus.[5,7] Computer models are being used to analyze the dynamics of NiV emergence in domestic swine and its spread between pig farms, with the goal being to identify a threshold density at which the infection can sustain itself long enough for an outbreak to occur. Laboratory studies are also underway to determine the mechanisms of transmission of henipaviruses between pteropid bats and between bats and other species.

Ultimately, our goal is to be able to prevent future outbreaks of known pathogens such as Hendra, Nipah, and other Nipah-like viruses, and by improving our understanding of the factors that drove their emergence, we also hope to prevent the emergence of new, potentially more lethal, paramyxoviruses.

Acknowledgements: This study is funded by an NIH/NSF "Ecology of Infectious Diseases" award via the John E. Fogarty International Center of NIH, TW05869 and by core funding to the Consortium for Conservation Medicine from the V. Kann Rasmussen

Foundation. We would like to thank the consultants to our group, Tom Ksiazek (CDC, Atlanta) and Sai Kit Lam (Univ. Malaya).

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PRESENT SITUATION OF WEST NILE VIRUS IN MEXICO

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Abstract

The initial outbreak of West Nile virus (WNV) in North America was recognized in New York City in August 1999, with deaths reported in humans, horses, and numerous species of birds.[8] Since then, the geographic distribution of WNV in North America has greatly increased, reaching Mexico in 2002, where a vast number of new potential hosts (avian, mammalian, reptilian) have been exposed to the disease. In Mexico, mosquito vectors are available throughout most of the year creating serious, long-term threats to human health, horses and vulnerable avian populations in the region.[9]

During the summer of 2002, the Agricultural Ministry of Mexico (SAGARPA) began to receive reports of encephalitis-like illness in horses from several different areas of Mexico, concurrent with reports of WNV encephalitis outbreaks in horses along the Texas border in the states of Coahuila, Tamaulipas and Chihuahua.[1,2] Other suspected cases were reported from several southern Mexican states.[2] In July 2002 antibodies to WNV were detected in horses in the

state of Yucatan.[8] The mode of entry of the virus into the Yucatan peninsula is unknown; however, the virus may have been brought in by migration because this area is a principal landfall of many species that migrate from the north-eastern and midwestern United States. Antibodies to WNV reported in certain species of migratory birds (gray catbird, rose-breasted grosbeak, and indigo bunting) supports this hypothesis.[3,8] There is even a report in mid 2001 that neutralizing antibodies to WNV were detected in a bovine in the southern state of Chiapas.[1,8]

The first evidence of WNV transmission among birds in northern Mexico, was in March 2003, 796 birds representing 70 species were captured and assayed for antibodies to WNV. Nine birds had flavivirus-specific antibodies by epitope-blocking enzyme-linked immunosorbent assay; four were confirmed to have antibody to WNV by plaque reduction neutralization test.[4] During 2003, several epizootics characterized by neurological disease occurred on farms housing *Crocodilus moreletii* and *C. acutus*. Crocodilians may serve as an amplification host for this virus. [5]

In early 2003, a nation-wide surveillance network for the detection and prevention of WNV in zoological institutions was formed in conjunction with the Wild Life National Agency (Dirección General de Vida Silvestre) incorporating 33 Institutions in the surveillance network and influencing a total of 85 zoos, aquariums and breeding facilities, from the Mexican Association of Zoological Parks and Aquariums (AZCARM). A specific protocol was put together and made available for these institutions in order to have a uniform approach to surveillance, diagnostics, case reporting and prevention of disease.

On May 5, 2003 a dead captive raven (*Corvus corax*) from a zoological park in the city of Villahermosa, Tabasco State, was analysed, and virus isolation of WNV was done on tissue samples at the CPA-SAGARPA biosafety level 3 facility in Mexico City.[2] Phylogenetic studies indicate that this isolate, the first from Mexico, is related to strains from the central United States but has a relatively high degree of sequence divergence.[2] Out of this isolate, a vaccine is being developed by the National Producer of Veterinary Biologics, mainly for the vaccination of horses in the army and for a cost-accessible product for mass vaccination (Hector Castell-Blanch personal communication).

Although WNV has already been detected in 13 states of Mexico, there is some difference in the impact of WNV in comparison with the USA experience, with a low rate of morbidity and mortality in both animals and humans. It has been hypothesized that extensive prior exposure to another *flavivirus* such as dengue (DEN), St. Louis encephalitis (SLEV), Venezuelan equine encephalitis (VEEV) or yellow fever virus, could attenuate the effects of WNV due to antigenic cross-reactivity of *Flavivirus* antibodies, especially after a second or sequential *Flavivirus* infection in the same host.[11]

For example, dengue and dengue hemorrhagic fever (DHF) have been present since 1982, when Mexico reported serotypes 1 and 2 and in 1995 serotypes 3 and 4 (hyperendemicity),[7] an outstanding increase of DEN-3 circulation was identified.[10] Risk factors include the numbers of infected and susceptible human hosts, size of mosquito population, (*Aedes aegypti*) feeding habits, and temperature (which affects vector distribution, size, feeding habits, and extrinsic incubation period).[6] There is also a Mexican isolate (200787/1983) which is antigenically unique by signature analysis with respect to all other dengue-2 topotype viruses. This strain is also unique in biological behaviour (neurotropism) and is of epidemiological significance in Mexico.[10]

The biological and epidemiological consequences of these mosquito-borne viruses co-circulating in the same ecosystem could either attenuate disease due to cross-protective antibodies or enhance disease due to immune enhancement. In the case of dengue, enhancement of virus replication by heterologous *Flavivirus* antibodies and T-cell activation are thought to occur in some patients during a second or sequential dengue infection, resulting in hemorrhagic fever or shock. In contrast, animal data indicate that prior infection with a heterologous *Flavivirus* reduces the severity of subsequent challenge with WNV. Results of experimental studies with rodents, monkeys, and pigs, suggest that heterologous *Flavivirus* antibodies protect against or modify subsequent infection with WNV.[11]

In the north-eastern region of the United States, the diagnosis of WNV infection has been relatively easy, since most people and animals were experiencing their first *Flavivirus* infection.[11] However, as WNV spreads into geographic regions where people and animals have other pre-existing *Flavivirus* antibodies, the interpretation of diagnostic tests becomes more difficult, and the prediction of the consequences are more challenging.

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COMPARING IMPLEMENTATION OF A LIVE TEST AND CULL PROGRAM FOR CHRONIC WASTING DISEASE IN WILDLAND AND URBAN SETTINGS

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Abstract

The mule deer (*Odocoileus hemionus*) population that winters in Estes Park, Colorado and the eastside of Rocky Mountain National Park, Colorado (RMNP) is currently sampled for chronic wasting disease (CWD) using a live tonsillar biopsy test. Utilizing the live test as a population level experimental management technique involves cooperation between federal and state field teams working in their respective jurisdictions. The Colorado Division of Wildlife (CDOW) works primarily in the town of Estes Park, Colorado and recently published a paper evaluating the feasibility of the live tonsillar biopsy strategy in this urban environment.[1] The costs and logistics associated with implementing this program in RMNP are considerably different. We compare and contrast the feasibility of implementing a live testing program for CWD in mule deer populations in urban versus wildland environments.

Costs associated with mule deer live testing are estimated on a per deer basis. The two major categories of expenditures are supplies/equipment and personnel services. Supplies and equipment are fixed costs and include wildlife pharmaceuticals, darts, ear tags, telemetry transmitters, vehicle, and lab fees. Supplies and equipment costs are similar between urban and wildland environments. Colorado Division of Wildlife reported a supplies/equipment cost range of $297 - $341 per animal dependent upon the drug combination used. Rocky Mountain National Park costs per deer are comparable. The real difference in feasibility between wildland and urban settings lies in personnel service costs. These costs are substantially higher per deer in a wildland setting. The time required to locate deer in wildland settings greatly increases personnel costs. In 28 field days, CDOW sampled 181 mule deer, averaging 6.5 deer per day. Rocky Mountain National Park sampled only 41 deer in 28 days, averaging 1.5 deer per day. These differences in efficiency between wildland and urban environments are important considerations for wildlife managers who may be considering using this technique.

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EPIDEMIOLOGIC ANALYSIS OF RISK FACTORS FOR MYOCARDITIS AND DILATED CARDIOMYOPATHY IN SOUTHERN SEA OTTERS (*ENHYDRA LUTRIS NEREIS*)

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Cardiac disease is an important cause of mortality for southern sea otters in California. This condition was newly described and had no known etiology in beachcast sea otters

necropsied from 1998 and 2001 at the California Department of Fish and Game's Marine Wildlife Veterinary Care and Research Center. The objectives of this study were to characterize cardiac lesions observed in southern sea otters and evaluate common sea otter pathogens and potential infectious, toxic and nutritional etiologies for their relationship with cardiac disease. Characterization of cardiac lesions by gross and microscopic necropsy findings has allowed the definition of two overlapping cardiac disease syndromes in otters: (1) myocarditis, characterized by lymphocytic inflammation of myocardium and (2) dilated cardiomyopathy (DCM), characterized by grossly enlarged atria and ventricles with concurrent myocarditis. Major risk factors associated with myocarditis included adult age, good body condition (likely as a result of an acute death), exposure to *Sarcocystis neurona*, and suspected exposure to domoic acid. Domoic acid, a marine toxin produced by *Pseudo-nitzschia australis*, is a common cause of mortality in sea lions and causes characteristic clinical signs involving the central nervous system. While there may be other factors associated with myocarditis that were not evaluated here, these findings suggest that *S. neurona* and domoic acid may both be important causes of myocarditis in sea otters. Myocarditis associated with exposure to *S. neurona* occurred predominantly in the northern part of the sea otter range, while domoic acid-related myocarditis occurred largely in the south, where domoic acid blooms were more frequent. A spatio-temporal cluster of DCM was identified in the southern aspect of the sea otter range in California from May to November 2000. Adult age and suspected previous exposure to domoic acid were associated with an increase risk of DCM. Also, otters with DCM had significantly lower concentrations of myocardial L-carnitine than controls and otters with myocarditis. Dilated cardiomyopathy may be an advanced stage of domoic acid-induced myocarditis in sea otters, possibly following chronic and repeated exposures to domoic acid blooms. While the relationship between domoic acid and myocardial L-carnitine concentrations requires further research, myocardial L-carnitine might play a key role in the progression of myocarditis to DCM in sea otters.

WILDLIFE AND THE ECOLOGY OF ANTIMICROBIAL RESISTANCE

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Antimicrobial agents are essential elements in the prevention and treatment of bacterial infections in humans, animals, and, to a lesser extent, in plant agriculture and aquaculture. In food animals, these agents are also used to promote growth and enhance feed efficiency. The increasing emergence of bacterial resistance to antimicrobials is of global public health concern, most notably due to the increasing incidence of multiple-drug resistant bacterial infections and higher rates in morbidity and mortality due to treatment failure. However, the impact of antimicrobial resistance may not be limited to human health. Because the emergence and spread of resistance is the result of complex

interactions between bacterial communities, antimicrobial agents, the host species, and the environment, animal health and environmental health effects are also likely. This paper explores the evidence for an impact on wildlife species and their potential role in the dissemination of antimicrobial resistance throughout ecosystems.

Potential routes of transmission of antimicrobial resistance within ecosystems include contacts between animals, animal products, humans, manure, soil, and surface water.[13, 19] Bacteria may acquire resistance to an antimicrobial agent as the result of a mutation or incorporation of transferable genetic resistance determinants via conjugation, transformation or transduction.[2, 15] Exposure of bacterial populations to antimicrobials may alter the bacterial populations through the elimination of susceptible bacteria and enables the survival and amplification of resistant bacteria, thus creating a selective pressure for resistance. Factors such as the method of administration, dosage, and frequency and duration of use are likely to impact the magnitude of this selective pressure.

Although they receive the most attention, it is important to note that pathogenic bacteria are not the only populations of concern with respect to antimicrobial resistance. Commensal bacteria comprise a large potential reservoir of resistance genes for bacterial pathogens. As the number of resistant commensal bacteria increases, the pool of genetic resistance determinants also increases, facilitating more frequent transfer of resistance to pathogenic bacteria. The indirect transmission of resistance via commensal bacteria and the environment may be as significant as direct transmission through the food chain or direct contact between humans and animals.[9] The high prevalence of antimicrobial resistance in commensal bacteria of humans probably reflects both the selective pressure exerted by antimicrobial usage in an environment as well as the potential for resistance in future infections in both humans and animals.[7, 8, 12, 20]

Although the high global prevalence of antimicrobial-resistant bacteria is widely attributed to use of antimicrobials in humans and domesticated animals, it is important to bear in mind that some bacterial populations are intrinsically resistant to certain antimicrobials, and others probably evolve resistance to certain of these drugs due to exposure to naturally produced antimicrobials in the environment. The extent to which the prevalence of resistance in bacteria from wildlife reflects such “natural” sources of resistance has not been well-characterized. For example, in a retrospective study of *Enterobacteriacae* isolated from wild mammals in Australia, Sherley et al. found that the rates of resistance were equal to rates seen in the natural, “antibiotic-free”, environment.[17] In contrast, in a Finnish study of wild moose, deer and vole, there was an almost complete absence of resistance in *Enterobacteriacae*, from which the authors concluded that resistance is not a universal characteristic of bacterial populations and most likely results from use of these drug classes in humans and animals.[14]

While studies exploring antimicrobial resistance in wildlife are limited in number, resistance has been demonstrated in multiple species of pathogenic and nonpathogenic bacteria within free-ranging and captive wild mammals,[5, 10, 17] birds,[4, 18, 21] reptiles,[1, 6] and aquatic species.[11] In particular, the spread of antimicrobial resistant bacteria and their

persistence in the environment may be enhanced by wild birds populations due to their mobility and distances traveled during migration. Wild birds have been implicated as a possible source of *Salmonella* infections in humans and farm animals.[3, 16] Antimicrobial-resistant *Salmonella spp*. have been observed in double-crested cormorants and common loons in Florida,[21] and *Salmonella typhimurium* were identified in black-headed gulls in the Czech Republic.[18] In a study in the United Kingdom, the range and serotypes of *Salmonella* carried by gulls was similar to the bacterial flora of human the population, which led the authors to suggest sewage as a possible source of infection.[4] Evidence for cycles of anthropozoonotic and zoonotic transfer of *Salmonella* infection demonstrates the potential for transfer of resistance determinants between animal, human and environmental sources.

Escherichia coli is a common component of the commensal fecal flora in humans and most animals. In one study of *E.coli* strains from captive mammals, birds, and reptiles in Trinidad, approximately 97% of the isolates tested demonstrated resistance to one or more of eight antimicrobials tested.[6] In a related study of *E.coli* isolates from both wild and captive mammals in Trinidad and Tobago, close to 96% of the isolates were resistant to one or more antimicrobial agents. In the second study, prevalence of resistance among the isolates from captive mammals was significantly higher for three of the antimicrobial agents tested. However, for ampicillin and cephalothin, the prevalence of resistance among the free-ranging animals was significantly higher than those from captive animals. The authors concluded that the high prevalence of resistance to antimicrobials among *E.coli* isolates within free-ranging and captive populations of wild animals may adversely affect treatment options available to veterinarians. In addition, the presence of resistant enteropathogenic serotypes among the isolates could pose a health hazard to consumers of wildlife meat.[1]

In an ongoing study in the United Kingdom of *E.coli* isolates in wood mice and bank voles, wood mice are significantly more likely to carry antimicrobial resistance than bank voles, even though they occupy the same habitat.[22] In an earlier related study in the U.K., vancomycin-resistant enterococci (VRE) were discovered to be part of the normal flora in wood mice, bank voles, and other species of wild mammals. However, while both wood mice and bank voles are reservoirs for VRE, the bank voles did not excrete VRE.[10] Wood mice tend to be omnivores and travel long distances in search of food and territory, whereas bank voles are herbivores and have limited territories. It was unclear how the animals acquired VRE and whether they were long-term carriers. Exposure to avoparcin (an antimicrobial related to vancomycin) was an initial consideration, but avoparcin had not been used in food animals in proximity to the study site and samples were collected after avoparcin was banned as a growth promoter.[10] In any case, it seems possible that host mammal species, ecological niche, and geographical location may influence the antimicrobial resistance profile of isolates and the potential for transfer and spread of resistance within the environment.

These and other studies suggest that free-ranging and captive wildlife are involved in the complex ecology of antimicrobial resistance. Both pathogenic and commensal **bacteria in wildlife may serve as reservoirs of antimicrobial resistance that may be selected**

for, amplified, and spread through the environment via various pathways. While the presence of antimicrobial resistance in wild mammals, birds, reptiles and aquatic species does not necessarily pose a risk to these populations (assuming they are not treated with antimicrobials), they may play an important role as reservoirs for the potential transfer of resistant bacteria and genetic determinants, potentially impacting the health of humans, companion animals, food animals and captive wildlife. Enhanced surveillance of pathogenic and commensal bacteria in wildlife is therefore warranted. Both the extent and significance of the risk to humans and nonhumans from antimicrobial resistance in wildlife should be evaluated, and, if deemed prudent, steps should be taken to mitigate the impact of resistance in wildlife populations.

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WILD BIRD SPECIES AND THE ECOLOGY OF VIRULENT AVIAN INFLUENZA

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Abstract

Avian influenza (AI) viruses have evolved little in 60 years in their natural hosts (wild waterfowl, shorebirds and gulls) that nearly always remain asymptomatic. They evolve much faster in aberrant hosts (pigs, chickens) in which they can become lethal. Although often blamed, wild birds are not significant in spreading virulent AI. Responses to AI outbreaks must be based on facts and not media hype. Poultry present the real danger.

INTRODUCTION

The two surface glycoproteins of influenza A viruses, haemagglutinin (H) and neuraminidase (N), are the most important for inducing immunity and therefore vary the most.[2] Few H and N subtypes have been isolated from mammals, but all 15 H and all 9 N subtypes have been isolated from migrating water-fowl and shorebirds worldwide. Natural influenza A infections have been reported in humans, pigs, horses, marine mammals, mustelids and birds.[23,3]

Although influenza viruses infect a wide variety of birds and mammals, the natural hosts are wild ducks, geese, swans, gulls and terns that intermittently transmit AI to other avian and even mammalian species (chickens, turkeys, pigs, horses, seals, whales, humans).[18,5] Avian influenza viruses evolve slowly in their natural hosts because of the brief avian lifespan and replication in their intestines and may be in evolutionary stasis.[8] The evolutionary rate accelerates rapidly in new (aberrant) host species due to selective pressures to adapt.[19]

Avian influenza is mostly asymptomatic in aquatic birds.[18,21] Viral replication in aberrant hosts is usually limited and overt disease is rare.[5] AI viruses bind preferentially to SAa2,3-galactose. Human strains preferentially bind to SAa2,6-galactose.[24] Thus, AI viruses do not replicate well in humans, and must reassort or adapt in an intermediate aberrant host before emerging in human populations. Pigs have receptors for both avian and human influenza viruses and are a likely intermediate host. The recent transmissions of avian H5N1 and H9N2 viruses directly to humans showed poultry can also be intermediate hosts.[21]

The first known direct transmission of virulent avian influenza (Hong Kong H5N1) from poultry to people killed 6 of 18 infected people in 1997 (thousands more people were exposed to infected chickens).[24] Human-to-human transmission was rare,[7] and no more human cases occurred after all poultry in Hong Kong were culled. H5N1 was evolving rapidly in the new chicken host and had acquired a number of amino acids that correlate with replication in humans. Eradicating 1.6 million chickens eliminated the immediate opportunity for H5N1 viruses to infect humans.[24]

Before depopulation, H5N1 virus was isolated from 20% of chickens and 5% of waterfowl in Hong Kong markets, but was not isolated from other birds, including other gallinaceous species, pigeons, and caged passerine and psittacine birds, or from wild birds. Chickens were the only clinically affected species in the live markets.[12] It is likely that H5N1 viruses are now widespread around Hong Kong. The multiplicity of H5N1 genotypes circulating in poultry in the wider region increases the opportunity for the emergence of pandemic strains by developing efficient human-to-human transmission through further reassortment.[5] Prior to 2003, wild ducks were not found to maintain virulent H5 influenza viruses.[14] H5 viruses can become highly pathogenic in domestic poultry but usually remain non-pathogenic in ducks.[1]

Avian influenza viruses are rarely isolated from passerines or psittacines. However, passerines are common near intensive poultry production worldwide and limited evidence supports the potential perpetuation and transmission of AI by passerines near intensive poultry production.[12] Most AI viruses from psittacines have been isolated during quarantine. The H9N2 viruses isolated from two ring-necked parakeets imported from Pakistan into Japan shared high sequence similarities with the 1997 H5N1 and 1999 H9N2 viruses transmitted directly from birds to humans.[12] The two H9N2 isolates identified a year apart were closely related, indicating they belong to the same lineage that must have been established in Pakistan for at least a year. These isolates were non-pathogenic in chickens and mice.[10] Although psittacines are not significant in the epidemiology of influenza A viruses, they can harbour and possibly transmit AI. This risk is greatest in countries with local, regional and international trading of wild birds.[12]

When tested with the 1997 H5N1 virus, seven gallinaceous spp. (chicken, turkey, Japanese quail, bobwhite quail, pearl guinea fowl, ringneck pheasant and chukar partridge) and zebra finches were the most susceptible (high morbidity, mortality >75%) with high viral re-isolation. Geese, emus, house finches and budgerigars were less susceptible and virus re-isolation was low.[12] Ducks, house sparrows and gulls showed

mild or no disease, and viral re-isolation was low to moderate. Pigeons, starlings, rats and rabbits resisted infection. This contrasts with previous experiments in which H7N7 virus killed all starlings and spread to contact starlings, but killed only 30% of sparrows and failed to spread to contact sparrows.[11] Thus, although the virulence of a single AI virus can vary substantially between avian species, including species within the same order, passerines and psittacines appear to play very minor roles in the natural epidemiology of AI.[12]

The pattern of spread of the virulent 2003/2004 H5N1 outbreak strongly suggests the virus was carried by smuggled poultry, a practice widespread in Southeast Asia. The genetic sequence of the virus isolated from a Vietnamese victim matched most closely one from Chinese poultry. Five of the eight genetic strands were almost identical to an H5N1 from duck meat smuggled from eastern China to Taiwan in 2003.[13] Some experts blamed migratory birds, but there is no direct evidence of wild birds spreading virulent AI. Wild birds were affected near big poultry outbreaks but regular monitoring of migratory birds in Thailand and elsewhere did not reveal the virus.[13]

The genetic diversity of AI viruses circulating in poultry in southeastern China has increased sharply since 2001. This shows H5 is circulating widely somewhere, under unusual selective pressures.[22] Asia's growing prosperity has caused a boom in intensive poultry production. Since 1997, many Chinese producers have vaccinated with inactivated H5N1. If a vaccine is a poor match, as is the case with the H5N1 strain that swept Asia, AI can still replicate in animals that show no disease. Intensive vaccination in south China ($>11 \times 10^6$) may have allowed the virus to spread widely unseen.[13] Vaccines that provide partial immunity and mask disease but allow hosts to continue to shed may speed viral evolution.[9] Vaccination may have led to the evolution of more virulent H5N1 strains that evaded vaccine protection.

After AI hit Bangkok, a special hotline received nearly 1,200 reports of "mysterious" bird deaths. However, the birds, mostly budgerigars and parrots, were dying from shock and starvation after being released by their fearful owners. Most callers were so panicky they demanded an immediate diagnosis over the telephone. Up to 128,091 caged birds were waiting to be tested for AI. Bangkok crows were sampled after the deaths of two crows at a zoo were linked to an H5 AI. In Thailand 500 migratory open-billed storks and another 300 birds died in wetlands. Only 30-40% of the dead storks were infected with AI. There were no reports of AI in Bangladesh from where Asian open-bill storks migrate. Thus, the storks most likely were infected in Thailand.[13]

In late 2002, H5N1 killed non-domestic birds in parks and a zoo in Hong Kong, including waterfowl, greater flamingos, gray herons and egrets. In February 2003, avian H5N1 was isolated from two humans, one of whom died. Despite high genetic homology (>99.0% in all genes), the human isolates showed a very different reactivity pattern compared with the H5N1 viruses isolated from the wild waterfowl. All H5N1 isolates from 1997 to 2001 were non-pathogenic in ducks but the H5N1 isolates from late 2002 were highly pathogenic in ducks. This is the first time since 1961 that influenza viruses are known to have killed waterfowl.[19] Despite the 2002 outbreak, there is little evidence the 2003/2004

H5N1 strain significantly affected wild bird populations or that wild birds spread it. Of 6000 wild birds tested in Hong Kong, one peregrine falcon was positive for the H5N1 strain. However, as the 2003/2004 H5N1 strain killed migratory wild birds, serological studies in wild birds across Asia are needed to determine whether it became established in wild populations.[4]

Despite a lack of evidence, governments in China, Thailand, Cambodia, Japan and Hong Kong were quick to implicate wild birds in the spread of the virulent 2003/2004 H5N1.[13] Responses ranged from the logical and effective to the fanciful and irresponsible. In China, authorities were required to monitor and disinfect the habitats of migratory birds, collect their excrement and sanitize it. Hong Kong closed parks and zoo exhibits but not poultry or wild bird markets. Despite the revelation that a chicken farm in Kyoto failed to report mass deaths due to H5N1 and continued to ship live chickens, eggs and meat while experiencing massive mortality, Japanese authorities supposed the virus was carried by migrating birds from Korea because there was no variation in the virus' sequences in Japan.[13]

The Thai Agriculture Ministry wanted to cull migratory birds because killing almost 30 million chickens on 40,000 farms had not controlled the epizootic. In Thai provinces where AI re-emerged, the infection was found mostly in fighting roosters. Authorities suspected infected fighting roosters smuggled out of red zones during the first outbreak then returned to the areas had probably re-kindled the infection. Some owners refused to slaughter their prized fighting roosters. Yet a spokesperson for the University's faculty of veterinary science said AI virus in yellow zones was due to the failure to eradicate all fowl and that birds in natural habitats should also be culled, not just chickens.[13]

Vaccination Trial

Virulent AI and SARS outbreaks in Hong Kong caused widespread fear and greatly reduced visitation to Ocean Park. Although H5N1 viruses are highly variable, there is much cross-protective immunity from H5N1 vaccines and non-pathogenic AI viruses such as H5N3.[6] To allay public fear and to protect collection birds, we tested a killed H5N3 vaccine (HK/goose/1999 H5 and A/duck/Germany/1215/73 N3). This allows differentiation between infected and vaccinated birds by testing for different neuraminidase antibodies. The number of birds with protective titres (>1:16) 28 days after a single vaccination are shown below:

Table 1. No. birds with protective titres (>1:16) 28 days after vaccination with experimental H5N3 vaccine

Titre	Ducks	Swans	Flamingos	Goose	Ibis	Heron	Crane
No. >1:16	28	7	23	1	0	2	1
No. <1:16	4	0	0	0	1	0	0
Mean titres	521	370	355	256	0	256	128

Vaccinations were repeated 28 days later (titres not yet available). Titres will also be determined 6 months after the second vaccination to see whether protective levels last for the whole influenza season (six months). The titre levels indicate very high protective

levels in most species. Three of 28 ducks and 3/7 swans maintained protective levels for over 12 months following single prototype vaccinations in 2003. Results for psittacines are pending also but after the single 2003 prototype vaccination, 2/5 parrots developed protective levels.

DISCUSSION AND RECOMMENDATIONS

Live-bird markets provide outstanding conditions for genetic mixing and spreading of AI viruses[22] and are are critically important in the perpetuation and transmission of AI viruses to other avian species and to mammals, including humans.[15] In contrast, free-ranging wild birds appear to play a much lesser if any significant role in the ecology and epidemiology of virulent AI. Intensively reared poultry provide excellent opportunities for AI viruses to increase in virulence due to:

1. Genetic uniformity in novel hosts providing intense advantage to mutant forms
2. High density, large populations
3. Confinement so that uninfected birds cannot avoid diseased birds or shed virus
4. Food and water readily available to prolong the lives of severely ill birds
5. Assisted transportation in vehicles to markets, farms and other businesses

Wild bird populations do not provide these opportunities due to:

1. High genetic diversity avoiding intense selection for a single new mutant strain[16]
2. Mostly low density, dispersed metapopulations
3. Freely able to avoid ill birds and low risk of contacting contaminated sources
4. Must work extremely hard for food under intense competition such that often even mildly affected individuals succumb rapidly, minimising pathogen spread
5. No assistance for dispersal. Ill migratory birds unlikely to travel far

Because all known influenza A subtypes exist in wild aquatic birds, avian influenza is not eradicable. Prevention and control are the only realistic goals.[22] Governments should strive to:

1. Monitor birds in live markets and exports/imports closely
2. Close live bird markets while virulent AI is circulating in the region
3. Improve biosecurity measures (eg prevent contact with wild aquatic spp.)
4. Separate land-based poultry, pigs and aquatic avian species in farms and markets
5. Monitor the movement of poultry between farms and markets closely
6. Only allow controlled effective vaccination in response to virulent outbreaks
7. Conduct serological and other epidemiological studies in wild birds across Asia to determine whether virulent AI became established in wild populations

Zoos and other organisations dealing with wild birds should:

1. Avoid birds from commercial sources such as markets and dealers, especially those trading internationally
2. Vaccinate at-risk or high profile birds with an effective vaccine prior to the risk period
3. Minimise the exposure of collection animals to wild birds, especially aquatic species.

4. Separate aquatic species from passerines, psittacines and other species.

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EPIDEMIOLOGIC INVESTIGATION OF *A MYCOBACTERIUM TUBERCULOSIS* INFECTION OF MULTIPLE ANIMAL SPECIES IN A METROPOLITAN ZOO

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Abstract

From 1997 to 2000, six cases of *Mycobacterium tuberculosis* (TB) infection were diagnosed in three species of animals at, or recently originating from, the Los Angeles Zoo. Restriction fragment length polymorphism (RFLP) analysis showed that five of six animal isolates shared an identical IS6110 pattern, with the sixth differing only by one additional band. A multi-institutional epidemiologic investigation was conducted to identify and interrupt possible transmission among the animal cases, and to screen personnel for active TB infection and TB skin-test conversion.

Animal Cases

In April and October of 1994, Asian elephant (*Elephas maximus*) #1 and Asian elephant #2 arrived at the Los Angeles Zoo from a private elephant facility where they had lived together. They were housed together at the zoo until November of 1996 when elephant #2 was returned to the facility for several months before transfer to another zoo. In the spring of 1997, Elephant #1 (30 years old) died of Salmonellosis, with *M. tuberculosis* found in granulomatous lymph node lesions from the thoracic and abdominal cavities, and Elephant #2 (30 years old) was found to have a positive trunk wash culture for *M. tuberculosis*. In July of 1998, one of a closed herd of three Rocky Mountain goats (*Oreamnos americanus*) consisting of a sire and two offspring, died of pulmonary *M.*

tuberculosis at 6 years of age. The goat's asymptomatic herdmates were screened and had negative chest radiographs and tracheal wash cultures, but one of the two goats was positive on tuberculin skin-test. In October of 1998, a clinically normal Black rhinocerus (*Diceros bicornis*) was diagnosed with *Mycobacerium tuberculosis* after a positive skin test and nasal wash culture. In the winter of 1998, the two remaining goats were evaluated again with negative chest radiographs and tracheal wash cultures. However, one year later, both were humanely euthanized at 8 and 12 years of age due to clinical evidence of tuberculosis on chest radiographs (both animals), and active clinical signs in one (neither were able to be orally treated). In January of 2001, a rhino was humanely euthanized after a protracted illness that was non-responsive to aggressive treatment. The rhino was found to have severe multifocal hemosiderosis and atypical mycobacterial infection in her lungs, with no *M. tuberculosis* cultured. This animal had been treated with oral Isoniazid and Rifampin for one year, cultured routinely, and was never culture positive again.

Epidemiologic Investigation

Investigators examined medical and location histories of the affected animals, animal handling practices, health-care procedures, and performed an infection control assessment of the animal compounds and health-care facilities (including measuring air flow in the compounds by smoke testing). We conducted a review of zoo employee medical records for evidence of TB symptoms, tuberculin skin-test results, and chest radiograph information. A list of current and former employees was cross-matched with reported TB cases in the California state registry from 1985 to 2000. As part of the annual occupational health screening in June of 2000, zoo employees underwent questioning regarding TB symptoms, received tuberculin skin tests, and completed a questionnaire on medical history, job type, and history of contact with the infected animals.

Epidemiologic Findings

No common cross-species contact outside the animal compounds and no contact with an infectious human were found. The distance at which the public was kept from the animals and the distance of the compounds from each other (the elephant compound was 27 meters from the rhino compound and the goat compound was 90 m from both) suggests that direct transmission was unlikely. No active TB cases in humans were found, and no matches were found in the database of reported cases. The RFLP analysis of this strain of *M. tuberculosis* matched that of three elephants with which #1 and #2 were housed at a private elephant facility from September of 1993-February of 1994.[1] We hypothesize that elephants #1 and #2 were infected at the private facility and were shipped with latent M. tuberculosis infection in 1994, subsequently infecting the black rhino and Mountain goats at the Los Angeles Zoo.

Of interest, animal caretaking and animal contact were not associated with a positive tuberculin skin-test, while groundskeepers were found to have an increased risk of tuberculin skin-test conversion compared with other job categories. Employees attending

the elephant necropsy and employees who trained elephants were more likely to have tuberculin skin-test conversion than those who did not.

Conclusion

This is the first documented human and veterinary epidemiologic investigation of *Mycobacterium tuberculosis* affecting multiple species in a zoo.[2] No evidence of transmission from humans to animals or active infections in humans were found. Genotyping evidence strongly suggests transmission from one species to another, although no evidence of transmission was discovered. Human tuberculin skin-test conversions associated with the elephants were most likely due to lack of respiratory protection for these employees when the risk of TB infection was not known. The finding that groundskeepers and not animal handlers were associated with a higher risk of tuberculin skin-test conversion was surprising, and we hypothesized that this may have to do with groundskeepers as a group being more likely to have been born outside of the United States.

Control measures to eliminate the spread of disease to people and animals were undertaken immediately and throughout this outbreak, and no further cases of *M. tuberculosis* have been diagnosed at the zoo in the past three years despite ongoing surveillance. Four elephants and three rhinos that had direct contact with the infected animals remain TB negative by trunk and nasal wash culture methods as outlined by the USDA for elephant TB surveillance. Methods of indirect transmission in mammalian zoo species and causes of variability in infection and morbidity within and among species warrant further investigation. Ongoing vigilance, occupational health programs and infection control measures in potentially exposed animals are recommended to prevent ongoing transmission of *M. tuberculosis* in zoo settings.

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The authors thank the Animal Care and Animal Health staff of the Los Angeles Zoo who cared so well for these animals, and the veterinarians (including consulting pathologists), technicians, and medical records staff who collected, analyzed, and organized the clinical data. We could not have performed this evaluation without Sue Thisdell, Safety Officer at the Los Angeles Zoo; Jothan Staley and Donna Workman-Malcom of the City of Los Angeles Occupational Health Services Division; Lee Borenstein, Elenor Lehnkering, Patrick Ryan, Jeanne Soukup, and Annette Nita of the Los Angeles County Department of Health Services; and Diana Whipple for her RFLP expertise.

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ZOONOTIC PATHOGENS RECENTLY FOUND IN WILD GOLDEN LION TAMARIN (*Leontopithecus rosalia*) AND COMMON MARMOSET (*Callithrix jacchus*) IN THE STATE OF RIO DE JANEIRO, BRAZIL, AND THEIR POTENTIAL FOR TRANSMISSION TO HUMANS

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A health evaluation study was conducted in eight wild groups of golden lion tamarins (GLT) and nine groups of common marmosets (CM) in a 1000 ha fragment of lowland Atlantic Rainforest in the State of Rio de Janeiro, Brazil. We analyzed 75 fecal samples from 35 individual GLT and 13 individual CM using the spontaneous sedimentation method. A total of 1676 parasite eggs were collected from the primates, representing four different parasitic helminthes. All four species of parasites were found in both GLT and CM. A comparison of parasites according to primate species showed neither morphometric nor statistical differences. The helminth eggs were classified as *Prosthenorchis elegans* (68,47 ± 4,36µm x 46,10 ± 15,89µm; *n* = 693); Ancylostomatidae (50.14 ± 0.804µm x 29.57 ± 9.34µm; *n* = 876); *Ascaris sp.* (64.33 ± 3.68µm x 52.46 ± 5.46µm; *n* = 68); and Oxiuridae (60.71 ± 4.76µm x 27.86 ± 2.11µm; *n* = 34).

Blood samples from 13 GLT and 55 CM were tested for the human serotype of *Tripanosoma cruzi*. Nine CM from four groups were positive by the indirect immunofluorescence assay (IFAT) with titers ranging from 1:20 to 1:320. Questions relating to the epidemiological position of these primates in the Chagas disease cycle and their potential susceptibility to disease caused by *Tripanosoma cruzi* are still unresolved. Studies are underway to identify the vector in the forest ecosystem and to evaluate the common marmoset as a possible reservoir.

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Terry Amundson Student Presentations

A NEWLY RECOGNIZED NEUROLOGIC DISEASE ASSOCIATED WITH *Parelaphostrongylus odocoilei* IN EXPERIMENTALLY INFECTED THINHORN SHEEP

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Abstract

Recently, the muscle-dwelling protostrongylid nematode *Parelaphostrongylus odocoilei* was discovered in Dall's sheep *(Ovis dalli dalli)* in the Mackenzie Mountains, Northwest Territories, Canada. Subsequently, a survey of thinhorn sheep (*Ovis dalli)* and mountain goats (*Oreamnos americanus)* in northern North America has revealed that this parasite is widely distributed in the Subarctic.

In thinhorn sheep both naturally and experimentally infected with *P. odocoilei,* eggs and larvae caused granulomatous interstitial pneumonia and lung hemorrhage, while adult nematodes were associated with localized myositis and muscle hemorrhage. Experimentally infected sheep showed a consistent pattern of weight loss and decreased muscle mass. At 2 wk prior to patency, two of five experimentally infected sheep

developed hind end ataxia, loss of conscious proprioception, hypermetria and an eosinophilic pleocytosis in cerebrospinal fluid. Antibody to *Parelaphostrongylus* spp. was detected in the cerebrospinal fluid and serum of infected, but not control, sheep. Neurologic signs stabilized at the time of patency and subsequently disappeared until recurrence following treatment with ivermectin. Uninfected control sheep showed no weight loss or clinical abnormalities at any time.

In five thinhorn sheep each experimentally infected with 200 third-stage larvae of *P. odocoilei,* pre-patent periods ranged from 68- 74 days. Shedding of first-stage larvae peaked at >10,000 larvae per gram of feces between 90 and 110 days post infection. The identity of first-stage larvae was confirmed by comparing sequence of the 1TS-2 region of nuclear DNA with known sequence for *P. odocoilei.* Adult *P. odocoilei* were recovered from three experimentally infected sheep, but not the two sheep that developed neurologic signs, which are currently being monitored.

While other researchers have recovered *P. odocoilei* adults from the epidural space of experimentally infected mule deer (*Odocoileus h. hemionus*), we did not find adult *P. odocoilei* in the spinal canals or cords of ten naturally infected or three experimentally infected thinhorn sheep. Therefore, this is the first evidence of a neural migration for *P. odocoilei* in experimentally infected thinhorn sheep, and the first description of a clinical neurologic syndrome caused by this parasite in any host species. These findings indicate that this host-parasite relationship is more complex than previously believed. Considering the susceptibility of protostrongylid life cycles and northern hosts to climate change, *P. odocoilei* may constitute a significant emerging disease risk for thinhorn sheep.

Frenkelia- LIKE ENTERIC COCCIDIA IN COOPERS HAWKS (*Accipiter cooperii*)

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Abstract

During a study of mortality in raptors, sporulated coccidian oocysts were noted in the lamina propria of the small intestine of 47 of 86 (54.7%) Cooper's hawks examined. No pathology was associated with the presence of these oocysts. On subsequent examination of fresh feces from seven birds, sporocysts with mean dimensions of 13.4 x 8.9 *.m,* a shape index of 1.3 (1.4-1.6), and diffuse residiuum were observed. These sporocysts were morphologically similar to *Frenkelia* and *Sarcocystis* spp. To determine the phylogenetic

relationship of this *Frenkelia* sp. to other *Frenkelia* and *Sarcocystis* spp., a fragment (~700-bp) of the 18S rRNA gene was amp1ified from three samples and sequenced. This *Frenkelia* sp. was most closely related to *F. buteonis (S. microti)* and *F. glareoli (S. glareoli),* both of which use hawks in the genus *Buteo* as definitive hosts and various rodents as intermediate hosts.

INFECTIOUS DISEASE EXPOSURE IN ENDANGERED ISLAND FOX (*UROCYON LITTORALIS*) POPULATIONS: IMPLICATIONS FOR SPECIES CONSERVATION MANAGEMENT

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The island fox (*Urocyon littoralis*) is only found on six Channel Islands off the coast of Southern California and is the largest terrestrial carnivore on the islands. Since 1994, island fox populations have experienced severe declines (up to 95%), which have resulted in the recent listing of the four most affected subspecies as federally endangered on March 4, 2004. An outbreak of canine distemper virus in 1999 is believed to be responsible for the dramatic decline of the Santa Catalina Island fox population, but little is known about current pathogen exposure in the entire fox population at risk. Prior to the decline, fox populations on all six islands had no evidence of exposure to canine distemper virus (CDV), while exposure to other canine viral pathogens, *Toxoplasma gondii* and *Leptospira interrogans* serovars varied among islands. Our study investigates what role infectious diseases like distemper may have had in the declines by estimating the exposure prevalence of infectious diseases in the post-decline island fox population. To date, 218 island fox serum samples collected from 2001 through 2003 on all six islands have been analyzed for exposure to canine distemper virus, canine adenovirus-1, canine parvovirus, canine coronavirus, canine herpes virus and six *Leptospira* serovars at Cornell University Veterinary Diagnostic Laboratory. Our results indicate that canine parvovirus and adenovirus exposure is still prevalent on most islands, and that Santa Catalina Island remains naive to adenovirus exposure. In contrast to pre-decline

serology, foxes on all six islands now have evidence of exposure to CDV (11.8% on San Miguel Island, 4.8% on Santa Rosa Island, 63.6% on Santa Cruz Island, 33.3% on Santa Catalina Island, 27.7% on San Clemente Island and 68.9% on San Nicolas Island). On islands where some or all of the current population is being held in captivity, stratification of CDV antibody titers by fox birth location (captivity vs. wild-born) reveals that titers are only present in wild-born foxes. These results suggest that wild fox populations on all six islands have been exposed to CDV or a closely related morbillivirus in the past, but it is not known why Santa Catalina Island foxes appeared to have high mortality while the fox population on San Nicolas Island has remained stable despite having the highest CDV antibody prevalence. These initial antibody titer results have been used by managers making decisions regarding vaccination programs for wild and captive island foxes and will be used to help assess the risk of moving island foxes to and from the mainland or between islands for captive breeding.

EXAMINING THE HEALTH RISKS TO WILDLIFE ASSOCIATED WITH INTRODUCTIONS OF DOMESTIC AND EXOTIC SPECIES IN THE NORTHWEST TERRITORIES, CANADA

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Abstract

The introduction and translocation of both domestic and wild animals are key factors in the emergence of infectious diseases. Throughout North America, interactions between domestic livestock and wildlife have often resulted in pathogen exchange and disease outbreaks; for example, contact between domestic sheep/goats and bighorn sheep has resulted in pneumonia epizootics and decimation of bighorn sheep populations. In the Northwest Territories (NT), large epizootics have not been reported and as yet there has been no contact between domestic sheep, goats, or llamas and wildlife such as thinhorn sheep and mountain goats. The equilibrium of the wildlife host-environment-pathogen system, particularly during this period of accelerating climate change, may be precarious and susceptible to additional stressors, such as contact with domestic animals, It is, therefore, very important to pro-actively assess and minimize these potential stressors.

In the Northwest Territories (NT), healthy populations of Dall's sheep, mountain woodland caribou, moose, and mountain goats are the foundation for subsistence hunting,

tourism, and outfitted sport hunting. At the same time, there is a growing movement towards promotion and development of an economically sustainable agricultural industry in the NT, including raising of domestic and exotic species for meat, milk, and wool/hair, Additionally, both domestic goats and llamas are becoming increasingly popular as pack animals for back-country expeditions.

To protect the wildlife of the NT while at the same time developing the agriculture industry, it is critical to understand: 1) the risk of disease introduction from domestic livestock and exotic species, 2) the risk of disease transmission between wild and domestic/exotic animals, and 3) how these risks can be mitigated with minimal impact on both sectors. To this end, we conducted a literature-based risk assessment and developed a framework for legislators, wildlife managers and domestic animal producers to pro-actively make informed decisions that minimize risks to wildlife health.

INFLUENCE OF HOST HABITAT ON THE HELMINTH COMMUNITIES IN BLUE-WINGED TEAL

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Abstract

Blue-winged teal are exposed to a wide array of habitat types during migration. The consequence of which is exposure to helminth species that may or may not be found on the breeding grounds. To learn more about the relationships between hosts, habitats, and helminths, we examined helminth communities of blue-winged teal collected from brackish and freshwater habitats. Thirty blue-winged teal were collected from each of the two habitat types. Blue-winged teal carcasses were placed on ice, viscera were fast frozen in the field, and both were stored in freezers. At necropsy, helminths were removed, identified, and counted. In blue-winged teal collected from brackish habitats, prevalence of trematodes, cestodes, nematodes, and acanthocephalans was 100, 100, 100, and 23%, respectively; whereas in hosts collected from freshwater habitats prevalence was 100, 90, 100, and 43%, respectively. At least one species, *Psilochasmus* sp., only occurred in birds collected from brackish habitats, whereas *Echinostoma* sp. occurred only in hosts collected from freshwater habitats. *Trichobilharzia* sp. occurred in all hosts examined. This study will aid in understanding how host habitat selection affects helminth communities in blue-winged teal.

AVIAN INDICATORS OF WEST NILE VIRUS IN GEORGIA IN 2002 AND 2003

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Abstract

West Nile virus (WNV) was first detected in the state of Georgia in the summer of 2001. Since then, dead bird surveillance, human and equine cases, and live bird serology have illustrated a nearly complete spread of WNV across the state. As amplifying hosts of WNV, avian species play an important role in the distribution and epidemiology of the virus. The objective of this study was to identify avian species that could serve as indicators for WNV over the physiographic and land use variation present in the southeastern United States.

A total of 6,750 avian serum samples from birds captured throughout Georgia during the summers of 2002 and 2003 were tested by plaque reduction neutralization test (PRNT) for antibodies to WNV and a closely related *Flavivirus,* St. Louis Encephalitis virus. Four hundred and fifty of these samples were found positive for antibodies to WNV. WNV antibody positive Northern Cardinals *(Cardinalis cardinalis)* and Northern Mockingbirds *(Mimus polyglottos)* were distributed across all land use types and physiographic regions, with wetland areas being least represented. Positive Rock Doves (*Columba Iivia)* had high antibody titers against WNV, however sampling sites for positive birds was not well distributed across all physiographic regions and land use types. Northern Cardinals appear to be the best indicators of WNV in the state of Georgia.

ASSESSING RELATIVE VULNERABILITY OF CHRONIC WASTING DISEASE INFECTED MULE DEER TO VEHICLE COLLISIONS

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ABSTRACT: Since 1996, tissue samples have been collected from deer killed in vehicle collisions throughout Colorado as part of a monitoring program for detecting chronic wasting disease (CWD) in free-ranging populations. We estimated CWD prevalence among vehicle-killed mule deer statewide and compared the estimate to CWD prevalence among the surrounding mule deer population to determine if CWD-infected mule deer are more vulnerable to vehicle collision. Overall prevalence was 66% higher in the vehicle-kill population; prevalence for vehicle-killed deer was 0.101 (95% confidence interval [CI] = 0.064–0.139) compared to 0.061 (95% CI = 0.051–0.072) for mule deer harvested or culled in the vicinity of vehicle-kills. The probability of detecting a CWD-infected, vehicle-killed deer, given that there is at least one other CWD-infected deer within a 3 km radius of the vehicle-kill site, was 16.67%. Our data suggest increased susceptibility of CWD-infected individuals to vehicle collisions. It follows that using vehicle-kill mule deer may be exploited in designing surveillance programs for detecting new foci of infection, but that this differential vulnerability also may bias estimates of CWD prevalence in natural populations. Evidence of increased susceptibility to vehicle collisions may aid in understanding vulnerability of CWD-infected individuals to other forms of death, particularly predation.

USE OF MAGNETIC RESONANCE IMAGING TO INVESTIGATE NEUROLOGIC DYSFUNCTION IN A SOUTHERN HAIRY -NOSED WOMBAT *(Lasiorhinus latifrons)*

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Abstract

A captive adult female Southern hairy-nosed wombat *(Lasiorhinus latifrons}* was discovered recumbent and unable to rise. Small patches of blood were present in her pelage and around her muzzle. She had appeared completely normal when last seen the previous evening. Physical examination revealed mild scrapes and lacerations, predominantly down the left side, consistent with convulsive or struggling self trauma. She also displayed rigid paralysis, an inability to raise herself or stand (with stimulation producing only uncontrollable rolling to the left), evenly dilated pupils which were responsive only to intense light, continuous nystagmus, tachypnoea and anxiety. Urine and CSF samples were normal and serial testing for toxoplasmosis proved negative. Blood samples revealed lymphocytosis and azotaemia. Supportive care was provided for the next 17 days during which the lymphocytosis and azotaemia resolved. Her neurologic condition improved slightly, however her physical condition deteriorated over this period.

Detailed neurologic examination at this point revealed mildly rigid paresis with significant muscle wastage, nearly absent postural reactions, depression (strong stimulation producing some mild paddling only), constant chewing, right head tilt, significant struggling if placed on left side, right side of head and ears cooler than left, profound blindness and deafness, and absent pupillary, menace and startle responses (to light, pain, movement and arousal) although pupils were even. Ophthalmoscopic examination, nystagmus, strabismus, cranial motor and sensory functions, olfaction and spinal reflexes all appeared normal. The problem could then be categorized as left central vestibular disturbance with possible partial involvement of cranial nerves II and VIII (producing visual and auditory deficits). However, there was still no indication of the cause of the disturbance and, thus, no way to determine treatment or prognosis.

It was decided to attempt magnetic resonance imaging (MRI) as this technique produces excellent soft tissue definition, particularly in the cranial region where soft tissue is difficult to visualise. Prior to transport to the MRI unit, the wombat was briefly anaesthetized (isoflurane in oxygen via a face mask) to place a catheter in each cephalic vein. At the unit, anaesthesia was induced using IV propofol. The animal was intubated and anaesthesia maintained using isoflurane in oxygen. As no metallic objects (or people) can remain inside the shielded MRI room during imaging, anaesthesia was maintained remotely using manual IPPV. The wombat was strapped into the MRI machine in dorsal recumbency with anaesthetic and oesophageal stethoscope extension tubing running into the next room. There was no visual contact with the wombat, so the anaesthetist relied on the oesophageal stethoscope alone to judge anaesthetic depth. A special MRI pulse oximeter, present in the MRI room, was used periodically to support the stethoscopic monitoring. A 20-min regular MRI series was followed by a further 20-min contrast MRI series. Had either of these series been interrupted due to anaesthetic difficulties it would have been necessary to repeat them from the beginning. The procedure and recovery went

extremely smoothly.

The MRI clearly revealed a focal oedematous lesion in the upper aspect of the cerebellum on the left side with a small area of extension to the left dorsolateral aspect of the brain stem. The lesion strictly respected the midline and vascular boundaries and was consistent with an area of subacute infarction in the region of the left superior cerebellar artery. The nature of the embolus was unknown. Nor was any embolic source ascertained on later cardiac ultrasound or blood culture. No other cranial abnormalities were detected and no cause for the deafness or blindness could be found. In humans and domestic species it is not uncommon that cerebellar infarction can occur spontaneously, unassociated with any event or underlying / pre-existing illness. As the wombat appeared normal the day before she became ill, this spontaneous sequence seemed quite feasible in her case.

Prognostically, many humans and domestic animals with spontaneous cerebellar infarction eventually recover to almost normal function. Once a plateau is reached during convalescence, it is likely that this will be the permanent state of the patient. There is no treatment other than supportive care. In the case of the wombat it was therefore decided to continue supportive care and monitor progression. After a further 2 wk the neurologic examination was repeated by a human neurologist producing findings consistent with multifocal CNS disease, bilateral optic atrophy and profound bilateral deafness. At this time her condition had plateaued at a level that was considered unacceptable in terms of quality of life and she was euthanatized.

Upon sectioning the fixed brain, a large malacic focus was identified within the left caudal cerebellum extending into the left dorsal region of the posterior colliculus. Histologically there was multifocally extensive encephalomalacia in the cerebellum and posterior colliculus, and mild, non-suppurative, focally eosinophilic encephalitis. The foci of malacia found within the cerebellum and posterior colliculus were consistent with the lesions seen on the MRI.

There is one further curious point that may or may not have had any bearing on part or all of this case. The wombat had recently recovered from a 2-mo period of profound sedation following administration of 20mg of flufenazine (1 mg/kg) some 3 mo prior to her illness.

MRl has very rarely been used on non-domestic species in Australia due to prohibitive costs, restraint / anaesthetic difficulties, lack of suitable facilities and ethical concerns regarding human waiting lists (there are no purely veterinary CT or MRI machines in Australia). As a result, cranial infarctions (and other cranial soft tissue lesions) have not previously been able to be confirmed ante mortem in these species. To our knowledge, this was the first wombat to undergo advanced imaging in this country. The diagnosis provided by MRI and the subsequent progression provided a solid foundation for treatment decisions and enough prognostic information to ultimately indicate the need for euthanasia.

WEST NILE VIRUS DETECTION IN A VIAN CARCASSES IN THE WEST CENTRAL UNITED STATES, 2003

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Abstract

We tested 343 dead birds of 58 species for West Nile virus (WNV) infection from mid-July to September 2003 at the Centers for Disease Control and Prevention in Fort Collins, Colorado. The vast majority of bird carcasses tested was from Colorado (19 counties), with a relatively small number of birds from Wyoming (2 counties) and Nebraska (3 counties). The sample set consisted of dead birds found by the public, as well as birds that died or were euthanatized at wildlife rehabilitation centers. The gold standard test in our study was TaqMan RT-PCR of heart samples with two sets of primers. Additional tests included VecTest@ WNV Antigen Assay of oral swabs and plaque assay of heart samples. Thirty-two percent of birds tested positive by TaqMan RT -PCR of heart; concurrent experimental evaluation of VecTest@ WNV Antigen Assay of oral swab indicated an overall sensitivity of 70%. When birds were separated into groups, VecTest@ sensitivities were 85% (n = 60) for corvids, 44% (n = 27) for raptors, and 62% (n = 26) for other (non-corvid, non-raptor) bird species. The sensitivity of plaque assay of heart tissue was 89% as compared to TaqMan RT -PCR of heart tissue, with 88% sensitivity for corvids, 89% for raptors, and 92% for other species. We recognize that none of these tests are 100% sensitive for WNV, but based on our results, we believe that VecTest@ WNV Antigen Assay of oral swab is a reasonable test for use in corvids, and plaque assay is a relatively useful test in all bird species to detect the presence of WNV when RT -PCR is not available. Our results may be useful for WNV surveillance as well as diagnostic purposes.

BATS, RATS AND CIVET CATS: POPULATION DYNAMICS, MAINTENANCE AND RESERVOIR CHARACTERISTICS OF TWO EMERGING INFECTIOUS DISEASES

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Abstract
Hendra virus (HeV) is a recently emerged paramyxovirus in the genus *henipavirus*. HeV came to our attention in 1994 and 1999 when three separate outbreaks caused the death of fifteen horses and two people. The fruit bat (genus *pteropus*) has been identified as the natural wildlife reservoir of HeV. All four mainland species of Australian pteropid bats have antibodies to HeV but the seroprevalence differs significantly between species.

In this study we use mathematical models to investigate the maintenance strategies of HeV in populations of *Pteropus poliocephalus*, *P. scapulatus*, *P. conspicillatus*, and *P. alecto*. We identify mechanisms that may lead to species differences in seroprevalence and explore how these differences could affect the comparative risk of spillover from pteropid species to domestic animals and humans.

Our results indicate that HeV cannot be maintained in bat populations with simple SIRS-like dynamics. However, incorporating metapopulation dynamics, latency or loss of resistance into the model led to maintenance of infection over time. Furthermore, our model indicated that *Pteropus scapulatus* is the species most likely to act as a reservoir for HeV in wild populations.

We contrast the dynamics of HeV with that of Sudden Acute Respiratory Syndrome (SARS) in proposed wildlife hosts. The dynamics of this coronavirus are vastly different to that of HeV and we reflect on the different reservoir characteristics required to maintain these 2 viruses.

Thyroid histology and hormone concentrations in the bowhead whale interpreted with respect to histological, seasonal and contaminant-related factors.

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Abstract

Thyroid activity was investigated in the bowhead whale (Balaena mysticetus) during the spring and fall phases of their annual migration from the Bering Sea to the Beaufort Sea. Histological sections from thyroid glands (n=27) were examined in conjunction with serological thyroid hormone analyses (n=50). Serum was assayed for triiodothyronine (total {tT3}, free {fT3}) and thyroxine (total {tT4} and free {fT4}) via radioimmunoassay. Thyroid tissue was assessed via light microscopy and the utilization of an epithelial-follicular index (EFI, via methodology developed by Sorkin, 1971). Results show no effect of age, sex or season on serum hormones. However, a seasonal effect was noted on the epithelial follicular index (EFI), with a significant difference in the height of the follicular lining being noted in spring (versus fall) samples. All results were compared to additional data collected in these whales, including vitamin A and E and organochlorine concentrations in the liver, serum and blubber.

EVALUATION OF THE WESTERN IMMUNOBLOT FOR USE IN DIAGNOSING *Brucella abortus* INFECTIONS IN ELK

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Abstract

As the prevalence of wildlife and cattle brucellosis in the United States continues to fall, the effects of false positive serology become increasing detrimental to both wildlife and agricultural managers. Cross reactions between commensal bacteria that share surface proteins with Brucella on standard serology have continued to frustrate the efforts of regulatory agencies to control Brucella and increase the need for more reliable methods of diagnosing infection. Because of these difficulties, the western immunoblot was evaluated for use in diagnosing *Brucella abortus* infections in elk. 133 samples encompassing 4 different elk herds were tested using management standard buffered antigen and CARD serology as well as western immunoblots. Samples were analyzed from Wyoming animals experimentally challenged with *Brucella abortus* strain 2308 and animals naturally infected with *B. abortus* biovar 1 as positive controls. For negative controls, sera from two different CA elk herds were used. Negative serologic tests, negative cultures, and no history of herd infection or disease support non-infected status. By comparing sensitivities and specificities among the tests used, the western immunoblot indicates higher reliability than standard serology in establishing disease status in elk.

PREVALENCE AND ANTIBIOTIC SENSITIVITY OF *CAMPYLOBACTER* AND *SALMONELLA* SPP. FROM THE GASTROINTESTINAL TRACT OF WILD AND STRANDED NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*)

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Salmonella and *Campylobacter* spp. are zoonotic, pathogenic bacteria that can cause gastrointestinal disease. *Salmonella* has previously been reported in multiple marine mammal species, although not in northern elephant seals (*Mirounga angustirostris*). *Campylobacter* has not previously been reported in marine mammals. The Centers for Disease Control is finding increasing antibiotic resistance in both of these bacterial species.[1] Increasing antibiotic resistance in both of these bacterial genera is a concern for human and veterinary medicine because it causes an increase in mortality, morbidity, and cost of treatment. There are also increasing reports of antibiotic resistance in marine wildlife.[2-3] The source of the resistance in marine wildlife is not known, however they may be exposed to bacteria and/or antibiotics when near high population coastal areas which may be contaminating the marine environment. Once these marine animals are exposed, they can continue to shed antibiotic resistant bacteria in the environment. The

prevalence and antibiotic sensitivity of *Salmonella* and *Campylobacter* have not been previously established in northern elephant seals.

In this study, *Campylobacter* and *Salmonella* species were isolated from juvenile wild northern elephant seals at two different colonies in California, Point Reyes and Año Nuevo, and from seals presenting for rehabilitation at The Marine Mammal Center (TMMC) in the months of February through June in 2003. Rectal swabs were performed on the seals, selective culture techniques were used, and isolates were then identified as *Campylobacter* and *Salmonella* through standard identification techniques. Antibiotic sensitivities were obtained by either broth microdilution or agar dilution methods.

In the Point Reyes colony, *Campylobacter* was detected in only one animal (n=32) with no evidence of *Salmonella* infections. In the Año Nuevo colony, *Salmonella* prevalence was 8.8% and *Campylobacter* prevalence was 32.4% (n=34). The difference in *Campylobacter* prevalence between the two colonies was found to be statistically significant. In elephant seals stranded and admitted to rehabilitation, the prevalence of *Salmonella* spp. was 37.3% (n=102), *Campylobacter jejuni* was 33.7% (n=101), *Campylobacter lari* was 6.9% (n=101) and a novel *Campylobacter* sp. was 11.9% (n=101). *Salmonella* serotypes were Typhimurium, Newport, Saint-Paul, Montevideo, or Reading. Seals which were stranded and being admitted to TMMC showed a higher prevalence of both *Salmonella* and *Campylobacter* when compared to wild seals which was statistically significant. *Salmonella* and *Campylobacter jejuni* isolates were sensitive to all antibiotics tested for, with a few exceptions in stranded seals. This study demonstrates that *Campylobacter* and *Salmonella* are common in seals that are in relatively close association with humans, and that prevalence of antibiotic resistance exists but is low in these seals.

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SPATIAL ANALYSIS OF THE DISTRIBUTION OF *Ehrlichia chaffeensis*, CAUSATIVE AGENT OF HUMAN MONOCYTOTROPIC EHRLICHIOSIS, ACROSS A MULTI-STATE REGION

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Abstract

Ehrlichia chaffeensis, the causative agent of human monocytotropic ehrlichiosis (HME), is maintained in a zoonotic cycle involving white-tailed deer (WTD; *Odocoileus virginianus)* as a vertebrate reservoir and the lone star tick *(Amblyomma americanum)* as the principal biologic vector. Using data from a prototypic white-tailed deer *Ehrlichia chaffeensis* surveillance system, we modeled the probability *of E. chaffeensis* occurrence using geostatistic analyses (kriging) and logistic regression. The analyses included the *E. chaffeensis* serostatus of 563 counties from 18 south-central and southeastern states. Cross-validation showed that kriging accurately predicted counties with high HME risk (87%). Large clusters of negative counties were accurately identified, but negative counties surrounded by large numbers of positive counties tended to be misclassified as high risk. Logistic regression modeling of the entire region and three subregions detected climatic and land cover variables significantly associated with *E. chaffeensis* occurrence. The accuracy of each subregion model (78-85%) was higher than the regional model (75%). Use of subregions also greatly increased the specificity from 39% for the regional model to 48-68% for the subregional models. The predicted *E. chaffeensis* distribution had good concordance with human case data. The integration of a WTD surveillance system with geostatistic and logistic regression analyses was useful in developing HME risk maps.

West Nile Virus and Other Vector-borne Diseases

PREVALENCE OF WEST NILE VIRUS IN MIGRATORY BIRDS DURING SPRING AND FALL MIGRATION

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Abstract

Since the discovery of West Nile virus (WNV) in New York in 1999, this disease has spread throughout 44 of the lower 48 states and to Canada and the Caribbean and Mexico. Migrating birds are often thought of as the principle mechanism for the dissemination of this virus. We investigated the prevalence of WNV and antibodies to WNV in birds during the spring and fall migrations at 8-10 sites in the Atlantic flyway during 2001-2003 and 5 sites on the Mississippi flyway during 2002 and 2003. We obtained blood samples from 13,402 birds captured in mist-nets, representing 135 species. Seroprevalence each season was low (<5%) at most sites but was as high as 18.4% (Memphis, Tennessee; fall 2002). In the Atlantic flyway, gray catbirds (*Dumetella carolinensis*) and northern cardinals (*Cardinalis cardinalis*) were most commonly found with antibody to WNV, as well as the first and third most commonly sampled species. In the Mississippi flyway, antibody to WNV was most commonly detected in northern cardinals, the most commonly sampled species. Additionally, two birds in this flyway had detectable WNV viremias, an indigo bunting (*Passerina cyanea*) and downy woodpecker (*Picoides pubescens*). Both individuals were sampled in fall 2002 at Mark Twain National Wildlife Refuge, Illinois. No viremic birds were detected in the Atlantic flyway.

AVIAN WEST NILE VIRUS SURVEILLANCE AT THE NWHC: A 5-YEAR SUMMARY

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Abstract

West Nile virus (WNV) emerged in the New York City region in 1999 and has rapidly spread across the North American continent in the short course of five years. At this time, much remains unknown about the ecology of WNV in North America. There are two unusual characteristics of the North American epidemic: high avian mortality associated with the human and equine epidemic and the increasing number of species in which WNV has been detected. Mortality rates have been particularly high in corvids (crows, jays) and raptors, and 226 avian species have been reported by state and federal public health, veterinary and wildlife agencies. The USGS National Wildlife Health Center (NWHC) has been actively involved in testing dead birds submitted through state

and federal WNV surveillance programs since 1999. Due to changing surveillance programs, variation in the data collected by each state, and the constantly evolving role of the NWHC in surveillance programs, the wild bird data are impossible to interpret epidemiologically. However, the surveillance testing does serve as an indicator of the avian mortality that has occurred since 1999. Although WNV has been a major cause of avian mortality during this time period, the data shows that other uninvestigated, and therefore unknown, causes of death have probably contributed to the avian mortality, even in those species found to be particularly susceptible to fatal WNV infection.

OUTBREAK OF WEST NILE VIRUS IN RAPTORS FROM VIRGINIA DURING 2003: CLINICAL, DIAGNOSTIC AND EPIDEMIOLOGICAL FINDINGS

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ABSTRACT

SINCE ITS INTRODUCTION TO NORTH AMERICA IN 1999, WEST NILE VIRUS (WNV) HAS IMPACTED A BROAD RANGE OF ANIMALS AND HUMANS.[6] REPORTS OF RAPTORS INFECTED WITH WNV ARE LIMITED IN THE SCIENTIFIC LITERATURE; HOWEVER, THERE IS EVIDENCE OF REGIONAL INCREASES IN RAPTOR MORTALITY.[3] ALTHOUGH IMPACT ON AVIAN POPULATIONS SECONDARY TO WNV INFECTION IS UNKNOWN, ANECDOTAL REPORTS SUGGEST A LIKELY NEGATIVE EFFECT IN THE FORTHCOMING YEARS.[1,3]

AS PART OF AVIAN SURVEILLANCE FOR WNV IN VIRGINIA (2003), CLOACAL AND/OR OROPHARYNGEAL SWABS COLLECTED FROM 61 LIVE RAPTORS ADMITTED TO THE WILDLIFE CENTER OF VIRGINIA (WCV) WERE TESTED AT VIRGINIA DEPARTMENT OF CONSOLIDATED LABORATORY SERVICES (DCLS) FOR WNV BY REAL TIME REVERSE-TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) USING FAM- AND TAMRA-LABELED PROBES AND PRIMERS THAT PREVIOUSLY HAVE BEEN REPORTED.[2,5] FORTY RAPTORS, INCLUDING NINE SPECIES, WERE POSITIVE FOR WNV BY RT-PCR ON OROPHARYNGEAL AND/OR CLOACAL SWABS (TABLE 1) WITH RED-TAILED HAWKS (*BUTEO JAMAICENSIS*) (RTH) AND GREAT-HORNED OWLS (*BUBO VIRGINIANUS*) (GHO) OVER-REPRESENTED (15/40; 37.5% AND 16/40; 40%, RESPECTIVELY). SEVENTEEN OF 32 BIRDS (53%) TESTED ONLY WITH

OROPHARYNGEAL SWABS WERE POSITIVE. IN ADDITION, TWENTY-THREE OF TWENTY-NINE BIRDS (75.8%) TESTED POSITIVE WITH COMBINED OROPHARYNGEAL AND CLOACAL SWABS. FOUR BIRDS (TWO GHOS AND TWO RTHS) (4/29; 13.8%) WERE POSITIVE ON OROPHARYNGEAL SWABS BUT NEGATIVE ON CLOACAL SWABS. TWO RTHS (2/29; 6.9%) WERE POSITIVE ON CLOACAL SWABS BUT NEGATIVE ON OROPHARYNGEAL SWABS.

PHYSICAL EXAMINATION, HEMATOLOGY, SERUM CHEMISTRY PROFILE, AND RADIOGRAPHS WERE PERFORMED ON WNV INFECTED BIRDS. CLINICAL PRESENTATION VARIED BETWEEN SPECIES. THE MOST COMMON FINDINGS ON PHYSICAL EXAMINATION IN ALL SPECIES WERE NON-SPECIFIC SIGNS OF ILLNESS INCLUDING DEPRESSION, DEHYDRATION AND EMACIATION. THE MAIN PRESENTING SIGNS IN GHOS INCLUDED HEAD BOBBLING, HEAD TREMORS, AND ATAXIA. HEMATOLOGY (N = 10) SHOWED A MODERATE ANEMIA, MARKED LEUKOCYTOSIS, HETEROPHILIA WITH LEFT SHIFT AND A MONOCYTOSIS. CHEMISTRY RESULTS ($N = 4$) SUGGESTED DEHYDRATION. ON RADIOGRAPHY ($N = 7$), GHOS HAD A MILD TO MODERATE INTERSTITIAL LUNG PATTERN (4/7). ONE GHO HAD MILD SPLENOMEGALY AND ONE HAD HEPATOMEGALY. IN CONTRAST, RTHS PRESENTED WITH NON-SPECIFIC SIGNS OF ILLNESS WITH MINIMAL NEUROLOGIC SIGNS. HEMATOLOGY ($N = 9$) SHOWED A MODERATE TO MARKED ANEMIA, MODERATE LEUKOCYTOSIS AND HETEROPHILIA WITH LEFT SHIFT. NO CONSISTENT CHEMISTRY ($N = 3$) FINDINGS WERE NOTED. ON RADIOGRAPHY, RTHS WERE EMACIATED (4/5) WITH DECREASED SPLENIC MASS (3/5). TWO OF FIVE RTHS ALSO HAD FRACTURED LONG BONES.
THE MEAN MONTHLY NUMBERS OF RAPTORS ADMITTED TO WCV FOR THE PREVIOUS 10 YEARS WERE COMPARED TO THE MONTHLY NUMBER OF RAPTOR ADMISSIONS FOR 2003. THERE WAS A DECREASE IN THE NUMBER OF NESTLINGS RECEIVED DURING MAY AND JUNE 2003. THE NUMBER OF CASES IN 2003 SHOWED A MARKED INCREASE DURING AUGUST AND SEPTEMBER FOLLOWED BY A MARKED DECREASE IN ADMISSIONS FOR OCTOBER TO DECEMBER COMPARED WITH THE PREVIOUS 10 YEARS. RETROSPECTIVE REVIEW OF MEDICAL RECORDS FROM 2002 SUGGESTED A SIMILAR EPIDEMIOLOGIC PATTERN AND CLINICAL PRESENTATION, ALTHOUGH LESS MARKED; HOWEVER, NO WNV CASES WERE CONFIRMED.

Of great concern is the impact of West Nile virus infection on threatened species and those of ecological importance. For example, two bald eagles (*Haliaeetus leucocephalus*) were identified as WNV positive by RT-PCR, which were euthanized due to poor prognosis for recovery. In addition, four juvenile peregrine falcons (*Falco peregrinus*) were positive for WNV detected by RT-PCR, including three juveniles that were submitted directly to DCLS by Virginia Department of Game and Inland Fisheries (VDGIF). These falcons were part of the VDGIF Peregrine Falcon Restoration Project: Falcon Trak (http://www.dgif.state.va.us/wildlife/falcontrak/index.html).

Early detection of clinical cases with accurate and rapid diagnosis will aid in monitoring the spread of WNV. This is the first clinical description of WNV in red-tailed hawks and will assist in recognition of this disease. The clinical presentation of great-horned owls is consistent with previous reports of WNV infection in owls.[3] Oropharyngeal and cloacal swabs for WNV RT-PCR provided a reliable ante-mortem diagnosis of current infection in field samples and is consistent with the findings of Komar et al (2002).[4] Due to the difference in RT-PCR results in 6 birds, testing of both oropharyngeal and cloacal swabs is recommended. RT-PCR of oropharyngeal and cloacal swabs correlated well with clinical presentation of WNV in great-horned owls and red-tailed hawks. The epidemiologic findings are consistent with outbreaks of WNV infection in raptors from Virginia during 2002 and 2003. In addition, the change in the monthly distribution of raptor admissions may indicate declines in local populations and provides evidence to support that WNV is having a negative impact on local raptor populations. The apparent increased number of WNV infected raptors during 2003 is consistent with the generalized spread of WNV in Virginia compared with previous years (D.N. Gaines, pers.communication). Studies are urgently needed to determine if the decline in the number of raptor admissions during spring and winter represents more widespread raptor population declines.

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Table 1. Raptors from the Wildlife Center of Virginia positive for West Nile virus by RT-PCR using oropharyngeal and/or cloacal swabs during 2003.

Species	Scientific name	Number Positive	Percent Positive
Great-horned owl	***Bubo virginianus***	**16**	**40.0**
Red-tailed hawk	*Buteo jamaicensis*	15	37.5
Broad wing hawk	*Buteo platypterus*	2	5.0
Bald eagle	*Haliaeetus leucocephalus*	2	5.0
Sharp-shinned hawk	*Accipiter striatus*	1	2.5
Peregrine falcon	*Falco peregrinus*	1	2.5
American kestrel	*Falco sparverius*	1	2.5
Barn owl	*Tyto alba*	1	2.5
Black vulture	*Coragyps atratus*	1	2.5
Total		**40**	**100 %**

DETECTION OF WEST NILE VIRUS FROM ORAL SWABS OF NESTLING CLIFF SWALLOWS: POTENTIAL USE AS AN EARLY SURVEILLANCE METHOD

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Abstract

We report early seasonal activity of West Nile (WN) virus infection in cliff swallow nestlings from the Fort Collins, Colorado area. Using TaqMan reverse transcription-PCR we were able to detect WN virus in oral swab samples taken from nestling cliff swallows. The timing of virus activity in the nestling population predates the general human activity

of WN in the Fort Collins area by five weeks. West Nile virus activity in nestlings corresponded spatially to case reports of viral infection in humans. This surveillance method may prove useful in designing a sensitive, spatially-explicit, early-detection monitoring system that can predict risk to human populations and thus help guide mosquito control efforts.

Serosurvey for antibodies to flaviviruses in wild mammals, Central and eastern United States

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Abstract

ELISA techniques were used to detect antibodies to flaviviruses and West Nile virus (WNV) in wild mammals. Two different monoclonal antibodies (6B6C-1 and 3.1112G) were used. More than 500 serum samples from over twenty mammal species captured in five states (CO, LA, NY, OH, PA) were screened for flaviviruses, and those which were flavivirus positive were screened for WNV. Antibodies to flaviviruses were detected in multiple species. This number was significantly reduced for WNV as was the overall prevalence of antibodies, indicating that multiple flaviviruses may have been present at some study sites. High prevalence rates were noted for select species in both assays. Future work will employ plaque reduction neutralization tests to detect neutralizing antibodies to WNV and other flaviviruses.

EVIDENCE OF INFECTIONS BY *ANAPLASMA PHAGOCYTOPHILUM* AND *BORRELIA BURGDORFERI* SL IN AMERICAN BLACK BEARS, WOODRATS, AND DOMESTIC DOGS FROM INLAND FOREST HABITATS OF NORTHERN CALIFORNIA

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Abstract

Transmission cycles of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sl appear parallel with reservoirs in wild rodents and transmission by *Ixodes* spp. ticks. Dusky-footed woodrats are the putative reservoir hosts of both of these organisms in California. We report high seropositivity to both organisms in woodrats, American black bears, and domestic dogs sampled in inland forest habitats, and DNA of *A. Phagocytophilum* was PCR-amplified from 10% of woodrats and 3.8 % of bears, but none of the dogs, suggesting greater risk to humans than is appreciated locally. However, the sequence of DNA typed as *A. phagocytophilum* from woodrats appears slightly different from reported sequences. Moreover, current evidence suggests that dusky-footed woodrats are the reservoir for *Borrelia bissettii* (a genospecies closely related to *B. burgdorferi* within the *B. burgdorferi* sl complex) but they may not be the most important reservoir of *B. burgdorferi* ss, the primary pathogen associated with human Lyme disease in the western US. Thus, the reservoir(s) of both of these important zoonotic pathogens remains unclear, at least in northern California.

THE ROLE OF HOST BIODIVERSITY, DENSITY AND TRANSMISSION ROUTES IN GENERATING NON-LINEARITIES IN TICK BORNE INFECTIONS

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Abstract

The dynamics of tick-borne infections incorporates a series of non-linear phenomena operating in the transmission processes between ticks, hosts and pathogens. Ticks feed on a diverse range of hosts that vary in their competence to transmit the pathogen and vary in the routes of transmission.
We explore the consequence of variations in the relative abundance of the two host species (deer and rodents) and the interaction with the transmission routes on the persistence and success of Lyme disease and Tick-borne encephalitis in Trentino (northern Italy). More generally we wish to explore the consequences of host abundance

and non-linearities in the transmission processes on the likelihood of tick borne diseases emerging as significant threats to human and wildlife health.

We develop a general model on tick borne infections,[1] predict the relative conditions that would lead to disease persistence (R_0>1) and then test the model against surveillance data we have collected from northern Italy.

REFERENCES

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Chronic Wasting Disease

ECOLOGY AND MANAGEMENT OF CHRONIC WASTING DISEASE IN NORTHERN COLORADO MULE DEER

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Abstract

Chronic wasting disease (CWD) has been endemic in northern Colorado for over two decades. We analyzed prevalence data from mule deer (*Odocoileus hemionus*) populations in Larimer County to discern the likely influences of temporal, spatial, and demographic factors on patterns observed in naturally infected populations and to look for evidence that recent management actions have affected temporal trends. We observed spatial heterogeneity among wintering mule deer subpopulations, marked difference in CWD prevalence by sex and age groups, and clear local trends of increasing prevalence over a 7-yr period that largely preceded management intervention. For both sexes, prevalence peaked in the 4–6-yr old age class, with the largest increase occurring between the 2–3-yr-old and 4–6-yr-old age classes. This differential was larger for males (5.9% among 2–3-yr-olds vs. 19.4% among 4–6-yr-olds; P = 0.0002). Demographic, spatial, and temporal factors all appear to contribute to the marked heterogeneity in CWD prevalence in endemic portions of northcentral Colorado. These factors likely combine in various ways to influence epidemic dynamics and responses to management on both local and broad geographic scales.

Comparison of genetic variability in North American ruminant prion protein (PrP) sequences

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ABSTRACT

We obtained genomic DNA from members of ten species and subspecies of wild North American ruminants and two Old World cervids and determined the DNA sequence of the prion protein (PrP) genes to investigate the degree of similarity between them. We also assessed genetic variability of this gene in the different species by ascertaining the number and location of any commonly recurring polymorphisms. The species sampled included the three natural hosts for chronic wasting disease (CWD), a transmissible spongiform encephalopathy seen in *Cervus elaphus*, *Odocoileus hemionus*, and *Odocoileus virginianus*. We also sampled *Alces alces shirasi*, *Rangifer tarandus*, *Odocoileus hemionus columbianus*, *Odocoileus hemionus sitkensis*, *Antilocapra americana*, *Bison bison*, *Ovis canadensis*, *Dama dama*, and *Capreolus capreolus*. We compared the locations of interspecific substitutions and of polymorphic loci relative to the other species and to the elements of secondary structure of the normal cellular protein.

Our findings are consistent with previous, large-scale comparisons of PrP protein sequences across a wide array of taxa that show conservation of amino acid sequence in the two beta-sheet and three alpha helix regions of mammalian prion proteins. The interspecific differences and twelve to fifteen intraspecific polymorphisms of the mature polypeptides in the ruminant species examined fell in the carboxy terminal two thirds of the protein. Most substitutions occurred outside the secondary structure features of cellular PrP, although in addition to the well-known substitution in beta-1 of leucine for methionine at codon 132 which occurs at low frequency in *Cervus elaphus nelsoni*, we noted an isoleucine-for-methionine substitution at 209 in *Alces alces shirasi*; this falls in alpha helix 3. In our samples (n = 1 to >300) the number of non-rare, fixed polymorphic loci per species varies from 0 to 3, and none exhibits more than two alleles per locus. None of the polymorphisms are the same in any two species. About half of the 11 interspecific differences fall outside elements of secondary structure, while two each occur within the second and third alpha helices. In addition, bison, bovines and pronghorn carry a sixth copy of the eight-amino acid repeated region in the amino terminus of the protein. Overall, we found amino acid identities within these species to vary between 96% and 100% as compared to *Cervus elaphus* PrP.

INVESTIGATION OF CHRONIC WASTING DISEASE STRAIN VARIATION USING FERRETS (*Mustela putorius furo*) AS A MODEL

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Abstract

We investigated evidence for strain variation in the chronic wasting disease (CWD)-associated prion (PrP^{CWD}) of deer (*Odocoileus* spp.), as well as the utility of domestic ferrets (*Mustela putorius furo*) as a common host for such studies. Ferrets (n = 3/group) were inoculated intracerebrally with brain material from single natural cases of CWD from northeastern Colorado that had occurred in a) a captive mule deer (*O. hemionus*) prior to 1985, b) a captive mule deer in 2000, c) a free-ranging mule deer, d) a captive white-tailed deer (*O. virginianus*) in 1999, and e) a free-ranging white-tailed deer; two additional groups of ferrets (uninoculated and inoculated with CWD-negative mule deer brain) were maintained as controls. Clinical signs and postmortem findings consistent with CWD in ferrets were observed in four of five groups inoculated with tissue from infected deer, but not in the free-ranging white-tailed deer or control groups. Incidence and incubation periods were consistent among affected groups. Moreover, Western blots (WB) revealed no apparent differences in glycosylation patterns among WB-positive ferrets. No strain variation in PrP^{CWD} was evident among these representative cases of CWD in captive mule deer and white-tailed deer and free-ranging mule deer from northeastern Colorado; however, no variation was expected among the three groups inoculated with materials from the same captive facility. Based on our experiences, domestic ferrets have limited utility as a laboratory model for studying CWD. Despite our findings, further investigation of potential strain variation among more geographically and epidemiologically distant cases of CWD in deer still appears warranted.

CHRONIC WASTING DISEASE VACCINE RESEARCH

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Abstract

Chronic wasting disease (CWD) in deer and elk is a transmissible spongiform encephalopathy (TSE) purportedly caused by prions. Studies by other research groups have elucidated key contact sites on normal cellular prion protein that are needed for duplication of the abnormal, disease-causing prion form. When these sites are blocked with antibodies, the course of the disease may be altered. We are investigating vaccines that include peptide sequences found within these sites that may be used to elicit a protective, active immune response. These vaccines tested in rabbits have elicited an antibody response. The next phase of research will investigate vaccine efficacy in the face of disease challenge using a mouse scrapie model.

Disease Surveillance and Diagnostics I

INFECTIOUS DISEASE SURVEY OF SAGE-GROUSE IN NEVADA AND OREGON

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Abstract:

The U. S. Fish and Wildlife Service has received numerous petitions for listing sage-grouse (*Centrocercus urophasianus*) as a threatened or endangered species. The role of infectious diseases in reduced productivity and population declines in sage-grouse over their range is not known. Information on diseases of sage-grouse is limited. Therefore, to determine if there were a high prevalence of disease in sage-grouse in portions of their range, we surveyed sage-grouse *(n* = 40) from southeastern Oregon in April, 2003, for

serologic evidence to selected disease agents including: *Salmonella typhimurium*, *S. pullorum, Mycoplasma gallisepticum, M. synoviae*, avian influenza, Newcastle disease, *Chlamydophilla psittaci* (n = 36), and West Nile virus (n = 27). All were negative. We also surveyed the same sage-grouse from SE Oregon (n = 40) and additional (n= 37 to 38) sage-grouse from northwestern Nevada for serologic evidence of exposure to avian infectious bronchitis virus (AIBV; Arkansas 99, Massachusetts 41, and Connecticut types) using the hemagglutination-inhibition test. Avian infectious bronchitis virus causes early chick mortality in domestic poultry, and we had observed unexplained early sage-grouse chick mortality in southeastern Oregon. We found 46% (36/78) had positive titers ($\exists$1:16) for AIBV Arkansas 99 type, 8% (6/77) for Massachusetts 41 type, and 53% (20/38) for Connecticut type. During October, 2003, attempts to isolate AIBV from Nevada sage-grouse tracheal and cloacal swabs (n = 21) by egg-culture and fluorescent antibody techniques were unsuccessful. This is the first known published report that sage-grouse have positive antibody titers to AIBV. The effects AIBV may have on sage-grouse populations are unknown. The importance of surveys for parasites and diseases in sage-grouse cannot be overemphasized. Only with such knowledge, can proper management of this dwindling species be accomplished.

AN EPIDEMIC OF AVIAN POX VIRUS IN LARKS (*CALANDRELLA RUFESCENS*) AND PIPITS (*ANTHUS BERTELOTTI*) IN THE CANARY ISLANDS, SPAIN

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Abstract

Over the past 2 years on the islands of Fuerteventura and Lanzarote in the Canary Islands off the west coast of Africa, ongoing ecological studies of desert passerines have uncovered the occurrence of an apparent epidemic of health problems in two species, the short-toed lark, *Calandrella rufescens,* and Berthelot's pipit, *Anthus bertelotti.* Two other native passerine species which are found in the same steppe habitats associated with dairy goat farming, the Spanish sparrow, *Passer hispaniolensi,s* and the trumpeter finch, *Bucanetes githagineus,* have been studied simultaneously. Over 800 birds from the four species have been trapped and ringed over the past two years in April, July and November. Of 465 individuals from the two affected species studied over the various collection periods, 28% to 49% (mean 42.5%) of the birds had clinically obvious pox-like lesions involving the legs, feet and, occasionally face. Of a similar number of trumpeter finches and sparrows trapped at the same locations at the same time, none showed

evidence of infection. Histopathology and electron microscopy have confirmed the presence of poxvirus in the lesions, whereas serology using standard, fowl and pigeon poxvirus-based diagnostic agar gel immunodiffusion techniques yielded negative results. Serology was not diagnostic in this case because of the limited (74.6%, pipit; 74.9% lark) similarity between the viruses in our species and fowlpox virus on which the serological tests are based. Using a 575 base pair DNA fragment from the 4b core gene of a fowlpox virus strain, the virus isolated from dried lesions of *C. rufescens* has only 80.5% similarity with the virus isolated from *A. berthelotti*, and 91.3% similarity with canarypox, whereas *A. berthelotti* poxvirus has 80% similarity with canarypox, which indicates that these are two distinct, and possibly new avian poxviruses.

The conservation implications of this epidemic of avian pox among birds in the Canary Islands are considerable. We have discovered a high prevalence of disease similar to that described in well studied, native passerines in Hawaii that are known to be threatened, endangered, or even extinct, at least in part due to poxvirus infection. Of the species we have studied, all except the sparrow are designated threatened. Other globally endangered species exist in the same habitats in the Canary Islands of Fuerteventura and Lanzarote, such as the houbara bustard, *Chlamydotis undulata,* and the stone curlew, *Burhinus oedicnemus*. Subspecies of these birds on the mainland have been diagnosed with poxvirus infections. It will be essential to investigate environmental and biological factors that are contributing to the increased disease susceptibility of these isolated populations of vulnerable bird species, in order to reverse this alarming trend in disease occurrence.

LONG-TERM NEUROLOGIC EFFECTS OF EXPOSURE TO DOMOIC ACID IN STRANDED CALIFORNIA SEA LIONS

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Abstract

Domoic acid is an excitatory neurotoxin that is produced by a number of marine algae, including the diatom *Pseudonitzschia australis*. Acute domoic acid toxicity can result in a number of neurologic signs in affected California sea lions (*Zalophus californianus*) such as ataxia, disorientation, seizure, and possibly death. However, the long-term, sublethal effects of domoic acid toxicity have not been fully investigated. This study describes the neurologic lesions associated with the long-term effects of acute exposure to domoic acid in stranded sea lions, and investigates the survival of animals with these lesions using satellite-linked telemetry. Animals in the study were suspected of having long term effects of domoic acid toxicity if they exhibited neurologic signs typical of domoic acid toxicity yet stranded during a time of no known domoic acid producing algal blooms, restranded after initial treatment for domoic acid toxicity, or continued to exhibit neurologic signs after multiple courses of anti-convulsant therapy. The animals were then screened for other causes of neurologic disease by performing complete blood counts, serum biochemistry analysis, serology for *Toxoplasma* sp. and *Sarcocystis* sp., radiographs, cerebrospinal fluid evaluation, and magnetic resonance imaging (MRI). If the animals showed no clinical signs of neurologic disease for at least 10 days after the end of anti-convulsant therapy, they were fitted with a satellite-linked transmitter and released.

Ten animals (2 male, 8 female) were suspected of having long-term effects of domoic acid toxicity and entered into the study. Eosinophilia was noted in eight animals. Radiographs and cerebrospinal fluid evaluation did not reveal any significant abnormalities. Markedly elevated and rising *Toxoplasma* sp. titers were detected using an immunofluorescent antibody test in one animal, while weak positive titers were found in two additional animals. Evaluation of MRI studies in the animals revealed a variety of lesions. The most common MRI findings were varying degrees of unilateral and bilateral hippocampal atrophy found in all 10 animals. Additional lesions suggestive of cerebritis in two animals and cerebral hemorrhage in one animal that were seen with MRI and confirmed with histopathology were not considered to be typical of domoic acid toxicity. Histopathology from the other three animals that either died or were euthanized revealed neuronal necrosis and gliosis in the limbic system considered typical of California sea lions naturally exposed to high doses of domoic acid. One animal was released without a satellite-linked transmitter. Five animals were released with satellite-linked transmitters and monitored for up to 106 days post-release. Of these five, two animals restranded and were euthanized, one animal had questionable success after release due to premature failure of the telemetry system, one animal displayed normal behavior after release, and it is still too early to evaluate the success of the last animal. This study highlights the difficulties encountered with the diagnosis of long-term effects due to acute domoic acid toxicity in stranded California sea lions. The variable post-release success of animals diagnosed with long-term effects of domoic acid toxicity suggests that diagnosis of the severity of lesions must be improved in order to better evaluate the prognosis for affected California sea lions.

AN UNUSUAL GENOTYPE OF *TOXOPLASMA GONDII* IS COMMON IN CALIFORNIA SEA OTTERS *(Enhydra lutris nereis*) AND IS ASSOCIATED WITH MORTALITY

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Abstract:

Toxoplasma gondii-associated meningoencephalitis is a significant disease of California sea otters (*Enhydra lutris nereis*), responsible for 16% of total mortality in fresh, beachcast carcasses. *Toxoplasma gondii* isolates were obtained from 35 California otters necropsied between 1998 and 2002. Based on multi-locus PCR-RFLP and DNA sequencing at conserved genes (*18s rDNA, ITS-1*) and polymorphic genes (*B1, SAG1*, *SAG3* and *GRA6*), two distinct genotypes were identified: type II and a novel genotype, here called type x, that possessed distinct alleles at three of the four polymorphic loci sequenced. The majority (60%) of sea otter *T. gondii* infections were of genotype x, with the remaining 40% being of genotype II. No type I or type III genotypes were identified. Epidemiological methods were used to examine the relationship between isolated *T. gondii* genotype(s) and spatial and demographic risk factors, such as otter stranding location and gender, as well as specific outcomes related to pathogenicity, such as severity of brain inflammation on histopathology and *T. gondii*-associated mortality. Differences were identified with respect to *T. gondii* genotype and sea otter gender and stranding location along the California coast. Localized spatial clustering was detected for both type II (centered within Monterey Bay) and type x (centered near Morro Bay) - infected otters. The Morro Bay cluster of type x-infected otters overlaps previously reported high-risk areas for sea otter infection and mortality due to *T. gondii*. Nine of twelve otters that had *T. gondii*-associated meningoencephalitis as a primary cause of death were infected with type x parasites.

Disease Surveillance and Diagnostics II

PARESIS AND DEATH IN ELK (*Cervus Elaphus*) DUE TO PRESUMPTIVE LICHEN TOXICOSIS IN THE RED RIM HABITAT AREA OF SOUTH CENTRAL WYOMING

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Abstract

In February and March 2004, 304 elk (*Cervus elaphus*) developed paresis in the Red Rim habitat area southwest of Rawlins, Wyoming. Elk were found in sternal recumbency, alert and responsive, but unable to rise. Bright red discolored urine was adjacent to many affected elk. Many elk progressed to lateral recumbency followed by dehydration, obtundation, and death. Several elk provided feed and water remained alive and responsive but never became ambulatory. The majority of elk were euthanized due to the poor prognosis for survival and return to normal function. Postmortem examinations were performed on 12 elk from the field, and at necropsy these animals were in fair to good body condition. Most of the elk that were recumbent for a day or more demonstrated gross evidence of myopathy, with pallor and streaking in skeletal muscles, particularly the semimembranosus, semitendinosus, and gastrocnemius muscles. Microscopic examination of tissues from the majority of elk was unremarkable, with significant lesions most consistently observed in skeletal muscles. In affected muscles, there were degenerative lesions of varying duration, severity, and distribution, some with early mineralization and attempts at regeneration and some associated with degenerating protozoal cysts (*Sarcocystis* sp.). Sporadic lesions were observed in other tissues from a small number of elk, including mild tubular degeneration/necrosis in kidneys, mild fibrinoid degeneration/change in small blood vessels of adrenal glands and a few other organs, and mild hepatocellular degeneration or less frequently apoptosis/necrosis.

Common infectious, inflammatory, toxic, and traumatic causes of weakness, paresis, and recumbency were ruled out via histopathology, virus isolation and associated tests, bacterial culture, parasitology analyses, and toxicology analyses. During field investigations, large quantities of ground lichen (*Xanthoparmelia chlorochroa*) were observed in the area where affected elk were found. This lichen was found in the rumen

contents of several elk. Approximately 50 kg of lichen was collected and fed to captive research elk. Three elk initially were offered a diet of 100% lichen for 7 days. After 7 days, elk were offered free choice alfalfa hay and lichen. After 7 days on this diet, one elk became sternally recumbent and was unable to rise. After 10 days on the diet, a second elk went down in a similar manner. Both elk were euthanized and necropsied. Gross and microscopic lesions were consistent with lesions from the affected elk in the field and red discolored urine was noted in the pens where the elk had been housed.

Our preliminary conclusion is that this lichen was responsible for recumbency and death. Interestingly, cattle, horses, mule deer, and pronghorn also were observed on Red Rim habitat area during the elk mortality event, with access to the lichen, but were unaffected. The toxic compound of the lichen has not yet been identified. We plan to analyze the lichen for toxic compounds and analyze the diets of the other herbivores in the area to determine if they ate the lichen.

AN INTERAGENCY INVESTIGATION INTO CAUSES OF BALD EAGLE *(Haliaeetus leucucephalus)* AND GOLDEN EAGLE *(Aquila chrysaetos)* MORTALITY IN MARYLAND 1988-2004

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Abstract

Eagles are protected under the Endangered Species Act (ESA), Bald and Golden Eagle Protection Act, the Lacey Act, as well as the Migratory Bird Treaty Act. States also protect eagles through state threatened and endangered species laws. National efforts to protect eagles have focused on habitat conservation, minimizing contaminant effects, monitoring wild bird nests, and coordinating captive propagation and release programs. Bald eagle *(Haliaeetus leucocephalus)* and golden eagle *(Aquila chrysaetos)* populations are growing in the U.S. The Chesapeake Bay is home to hundreds of nesting pairs of eagles. Currently, eagles are listed as threatened in Maryland.

Four organizations work collaboratively on eagle morbidity and mortality in our region. The Maryland Department of Natural Resources (MD DNR) and U.S. Fish & Wildlife Service (FWS) serve as regulating and permitting agencies. Tri-State Bird Rescue and Research and the Baltimore Zoo are the two facilities permitted to conduct treatment and rehabilitation for eagles. Carcasses of eagles that die or are found dead in Maryland are transferred to FWS and frozen for eventual shipment either to the National Eagle Repository or the FWS Forensics Laboratory.

To examine eagle mortality in Maryland, databases from the four organizations were reviewed. Minimal information existed on cause of death for Maryland eagles prior to 1988, so the period examined begins with January 1988 and extends through March 2004. A total of 220 eagles were found dead or died in Maryland during the study period with an average of approximately 14 deaths per year (range 2 - 29). For all eagles, causes of death included 63 traumatic injuries: collision (34), other physical trauma (29). Other causes of death included electrocution (32), poisoning (23), drowning (9), disease (5), entanglement (3), gunshot (5), and unknown causes (80). Little is known about eagles in the unknown category other than the date and location of the incident. Legal cases are still open, and no information is available until the cases are settled. However, the majority of eagles in the unknown category had no cause of death information listed.

The databases were reviewed for temporal and spatial significance. Three separate years (1988/ 28, 1997/ 22, 2003/ 29) accounted for nearly one-third of the total number of eagle mortalities. No other clusters of mortalities were found in the period examined; however, an overall increasing trend in mortalities was noted. From a geographic perspective, eagles were reported dead in 21 of 23 counties in Maryland. The majority came from three counties: Charles (34), Harford (34) and Dorchester (31) accounting for nearly half of all mortalities reported (99/220). Since these counties are located in different regions of the state, the mortalities did not represent a geographic concentration. Additionally, 124 eagles were identified as adults, 72 as immature and 24 were not identified by age.

The primary objective in this study was to examine the causes of eagle mortality in Maryland. Results indicated that eagles died throughout most counties of the state over the entire study period for a variety of reasons. However, the majority died from unknown causes. Through our cooperative efforts, we hope to better identify causes of eagle mortality that will lead to identification of threats and impacts to Maryland populations. This collaboration will serve as a model for future wildlife investigations and conservation efforts in our region.

DISEASE MONITORING IN CAPTIVELY PROPAGATED AND REINTRODUCED RIPARIAN BRUSH RABBITS (*Sylvilagus bachmani riparius*) IN CALIFORNIA

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Abstract

The riparian brush rabbit (*Sylvilagus bachmani riparius*) is a state- and federally-listed endangered species. It is native to riparian communities in the northern San Joaquin Valley of California. Riparian habitat in the San Joaquin Valley has been reduced to less than 1% of its historical extent, primarily due to clearing of natural vegetation, irrigated agriculture, livestock grazing, impoundment of rivers, and stream channelization. At the time of state and federal listing of the species, there were only two known remnant populations of riparian brush rabbits in California, one in Caswell State Park along the Stanislaus River, and another along a overflow channel of the San Joaquin River (Paradise Cut). The size of both populations was too low to provide sufficient captures to estimate population sizes with capture-recapture population estimator models. To recover riparian brush rabbits, the US Fish and Wildlife Service set a goal of establishing three or more self-sustaining populations outside of Caswell Memorial State Park within the historical range of the species. Because the extant populations at Caswell State Park and Paradise Cut were isolated from other suitable sites that are currently uninhabited, it was determined that reintroduction of individuals derived from existing populations would be required to achieve this goal. The USFWS contracted with California State University Stanislaus' Endangered Species Recovery Program to design and implement a controlled propagation and reintroduction program (plan available at www.esrp.csustan.edu).

The UC Davis Wildlife Health Center (WHC) drafted guidelines for monitoring and maintaining the health of the captive and reintroduced riparian brush rabbit populations, and has provided veterinary input on all aspects of the program since its inception. Veterinary oversight has generally been in the form of:

- Health screening of all founding adult breeders captured at Paradise Cut before translocation into the controlled propagation pens at the start of the breeding season
- Health screening of all progeny born in the controlled propagation pens before reintroduction into San Joaquin National Wildlife Refuge

- Disease monitoring in the captive and reintroduced populations via complete necropsies and histopathologic evaluations of all dead rabbits for which sufficient remains are recovered and by serologic surveys for select pathogens
- Disease screening of other lagomorph species at the reintroduction site prior to reintroduction of riparian brush rabbits
- Opportunistic disease screening in sympatric mammals
- Individual animal treatment and care as needed

Health screens typically consist of a physical examination under gas anesthesia, and blood collection for complete blood count, serum chemistry, and serum banking. Ectoparasites are collected opportunistically. Fecal analysis for gastrointestinal parasites in live rabbits is not performed routinely, except when disease due to parasite infections is suspected in clinically ill rabbits. Additional diagnostics performed on several rabbits have included radiography, ultrasonagraphy, ophthalmologic examinations, and cytology and biopsies of superficial masses

As of the end of 2003, 26 rabbits have been brought into captivity to serve as founding breeders; 340 offspring have been produced; and 243 rabbits have been reintroduced to the wild at San Joaquin National Wildlife Refuge. As of March 15, 2004, 63 necropsies have been performed on rabbits for which sufficient remains were available. Major causes of mortality have included: predation; parasitic encephalitis (presumed *Baylisascaris*), necrotizing typhlitis, trap-related trauma (including conspecific aggression), bacterial sepsis, inanition/starvation (in neonates) and lymphoproliferative disease. Principle causes of morbidity which have required therapeutic intervention have included: ocular disease (keratitis, uveitis, conjuncitivits), wounds related to radiocollars, and miscellaneous traumatic injuries, wounds and abscesses.

Thirty rabbits from the 2003 breeding season were screened for antibodies to *Encephalitozoon cuniculi* and *Treponema cuniculi*. All 30 rabbits were seronegative for *Treponema*, and one rabbit was weakly seropositive for *Encephalitozoon*. This rabbit was trapped in the wild at Paradise Cut for screening as a founding breeder, treated for a subcutaneous mass, and returned to the wild prior to testing. We have not seen clinical or pathologic evidence of disease due to either pathogen in the captive or reintroduced populations.

ANTIGEN RECOGNITION BY SERUM ANTIBODIES IN WHITE-TAILED DEER (*Odocoileus virginianus*) EXPERIMENTALLY INFECTED WITH *MYCOBACTERIUM BOVIS*

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Abstract

White-tailed deer (*Odocoileus virginianus*) have emerged as reservoirs of bovine tuberculosis (TB) in Northern America. For TB surveillance of deer, antibody-based assays are particularly attractive because deer are handled only once, and immediate processing of the sample is not required. Sera collected sequentially from 25 *Mycobacterium bovis*-infected and 7 non-infected deer were evaluated by ELISA, immunoblotting, and Multi-Antigen Print Immunoassay (MAPIA) for immunoglobulin specific to *M. bovis* antigens. Various routes of experimental *M. bovis* infection, such as intratonsilar inoculation (n = 11), aerosol (n = 6), and exposure to infected deer (in contact, n = 8) were studied. Upon infection, specific bands of reactivity at ~24-26 kDa, ~33 kDa, ~42 kDa and ~75 kDa to *M. bovis* whole cell sonicate were detected by immunoblot. Lipoarabinomannan-specific immunoglobulin was detected as early as 36 days postchallenge, and responses were detected for 94% of intratonsilar and in contact infected deer. In MAPIA, sera were tested with 12 native and recombinant antigens coated on nitrocellulose. All "in contact" infected (8/8) and 10/11 intratonsilarly-infected deer produced antibody reactive with one or more of the recombinant/native antigens. Responses were boosted by injection of tuberculin for intradermal tuberculin skin testing. Additionally, 3/6 deer receiving a very low dose of *M. bovis* via aerosol exposure produced antibody specific to one or more recombinant proteins. *Mycobacterium bovis* was isolated from 1/3 non-responding aerosol-challenged deer. Of the 12 antigens tested, the most immunodominant protein was MPB83; however, a highly sensitive serodiagnostic test will likely require use of multiple antigens.

RATES AND CHARACTERIZATION OF SAMPLES FROM *Salmonella* spp. ISOLATED FROM WILD AND CAPTIVE BROAD-SNOUTED CAIMAN (*Caiman latirostris*) IN SÃO PAULO STATE, BRAZIL.

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Abstract

Salmonella sp. is an important worldwide zoonotic agent, frequently isolated among reptile microbiota. This genus has ever-growing importance to public and animal health, as well as food hygiene. In the present study, cloacal swabs were collected from *Caiman latirostris* individuals; 103 samples from animals captive in two different colonies and 12 from animals in the wild. All animals were sexed, and total length was measured at sampling. Swabs were inoculated in Tetrationate broth and were cultured in MacConkey and XLT4 agars, and incubated at 37°C, for the isolation of *Salmonella* spp. Samples isolated were characterized according to their biochemical profile using API 20E (BioMérieux). Serotyping was performed according to the Kauffman-White method, and the pattern of susceptibility to antibiotics was verified using the disk diffusion method. Presence of four virulence genes (*inv*A, *spv*C, *sef*C e *pef*) was assessed using Multiplex-PCR. Correlations between the presence of *Salmonella* spp., gender and total length were also analyzed. Frequency of animals positive for *Salmonella* spp. among captive animals was 30% and 48.38%, respectively. The agent was detected in 50% of the animals in the wild. There were significantly more positive males than females in one of the captivity sites and in the other, a significant correlation between the presence of the bacteria, and total length was observed. There were no significant differences in incidence of *Salmonella* spp. between captive and wild *Caiman latirostris*. A total of 45 *Salmonella* spp. samples from 15 different serotypes were isolated: *S.* Infantis, *S.* Typhimurium, *S.* Grumpensis, *S.* Cerro, *S.* Anatum, *S.* Agona, *S. enterica* subsp. *enterica*. (O: 13, 23), *S.* Coeln, *S. enterica* subsp. *enterica* (rough strain), *S.* Enteritidis, *S.* Newport, *S.* Minnesota, *S. enterica* subsp. *enterica* (O: 6, 8: ch: -), S. *enterica* subsp. *enterica* (O: 4, 12: ch: -), *S.* Schwarzengrund. All of them were in the subspecies *Salmonella enterica* subsp. *enterica* (Group I). In general, strains were sensitive to all antibiotics tested, but resistances were observed for cotrimoxazole, chloranfenicol, neomycin, gentamicin and tetracycline in five samples of *Salmonella* spp. isolated from captivity. There were also three multiresistant strains in captivity (*S.* Infantis, *S.* Typhimurium and *S.* Grumpensis). In relation to virulence genes, all samples presented the *inv*A gene, *S.* Enteritidis presented all genes studied and in *S. enterica* subsp. *enterica* (rough strain) was detected the genes *inv*A and *sef*C.

NONPLAGUE YERSINIAE IN A ZOO

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Abstract

Following the death of a hooded pitta *(Pitta sordida)* from the zoonotic bacterium, *Yersinia pseudotuberculosis,* at the Sequoia Park Zoo, Eureka, California, USA, in December 2001, we surveyed cloacal swabs and feces of 44 birds and 31 mammals, January through May 2002; four soil samples also were evaluated. Cold storage (4°C) incubation for 11-12 months in trypticase soy broth was followed by isolation on Yersinia Selective Agar/Antimicrobic Supplement CN. Suspect strains were identified with an API 20E (bioMerieux) system and biotyping confirmed by the methods of Wauters et al.[2-3] Eight strains were evaluated by pulsed-field gel electrophoresis.[1] Serotyping of one strain was attempted by slide agglutination.

No *Yersinia pseudotuberculosis* was found, including in eight samples of rat feces, supporting the notion that no enzootic focus for this bacterium is present at the zoo. *Yersinia enterocolitica* biotype 1A was isolated from a sacred ibis *(Threskiornis aethiopicus),* a cedar waxwing *(Bombycilla cedrorum),* an unidentified bird in the aviary, a nyala *(Tragelaphus angasii),* a black bear *(Ursus americanus),* and one soil sample; this was a 7.6% prevalence for all samples taken. One biotype 1A strain (ibis) tested by slide agglutination was too rough for serotyping; other strains were not serotyped. Based on a pulsed-field gel electrophoresis of eight biotype 1A strains, we observed 5 distinct profiles of *Y. enterocolitica* – evidence for several sources of the bacteria rather than a clone stemming from a single introduction. In addition, *Yersinia frederiksenii* was isolated from a nyala and *Y. mollaretii* was collected from an Argus pheasant *(Arguslanus argus).* There were no apparent patterns in *Yersinia* spp. isolations related to host class or geographic location within the zoo. *Serratia* spp. (10% of all samples) and *Providencia* spp. (5 %) also were isolated from both birds and mammals.

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A REVIEW OF PSEUDOTUBERCULOSIS AT A EUROPEAN ZOO: EPIDEMIOLOGY AND APPROACHES TO CONTROL

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Abstract

Epidemiological trends of pseudotuberculosis outbreaks in callitrichids and Rodrigues' fruit bats (*Pteropus rodricensis*) due to *Yersinia pseudotuberculosis* (*Y. pstb*) at Jersey Zoo are analysed from 1981-2000. The organism appears persistent in the population with peaks of disease in winter months. Sub-adult bats (2-3 yr) appear especially susceptible as do young (< 2 yr) and old (> 9 yr) callitrichids. Control of yersiniosis through vaccination and through monitoring techniques have not been effective at Jersey; however, improved husbandry methods are employed with apparent success to date.

PLAGUE INFECTION IN CANADIAN LYNX REINTRODUCED TO COLORADO: OCCURRENCE AND RESULTS OF A PILOT PLAGUE VACCINE TRIAL

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Abstract:

Plague appears to be a significant obstacle to successful reestablishment of lynx (*Lynx canadensis*) in Colorado. *Yersinia pestis* infections have been confirmed in 6 Canadian lynx reintroduced into Colorado as part of an ongoing species recovery program. Since 1999, plague was the primary cause in 4/15 natural deaths, possibly contributed to 1/6 hit-by-vehicle deaths, and killed at least 1 kitten. In an attempt to minimize these impacts in future restoration efforts, we evaluated a recombinant capsular F1-V fusion protein vaccine that is safe and effective in black-footed ferrets. During January–April 2004, we vaccinated and serially bled 10 captive female lynx held in southwestern Colorado prior to their release in April 2004; 10 unvaccinated lynx served as controls. We observed no adverse effects of either the primary vaccine or booster doses on captive lynx. As of 15 March 2004, our study is still underway and serology results are pending. Based on observations to date, F1-V vaccine appears to be a safe vaccine in lynx; serologic responses and efficacy in reducing plague-related mortality remain to be determined.

Ecosystem Health

ASSESSING CORAL REEF HEALTH IN AMERICAN SAMOA

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Abstract

We surveyed corals in American Samoa for presence of lesions. We did 19 SCUBA and additional snorkel dives on 6 and 7 sites on Tuituila and Ofu-Olosega, American Samoa. We photographed and took 70 samples from 49 corals comprising 29 species. Corals were fixed, decalcified, and sectioned on microscope slide to examine cellular architecture. Grossly, the most common lesions in corals were bleaching, growth anomalies, and tissue necrosis. On histology, depletion of zooxanthellae from coral tissue was most often seen followed by tissue necrosis associated with algae or fungi, hyperplasia of gastrovascular canals, or uncomplicated tissue necrosis. Two grossly bleached corals had evidence of pathologic lesions associated with invasion by ciliates (protozoa). One coral had evidence of primary infection with a fungus that manifested grossly as growth anomaly. One coral had evidence of skeletal enlargement associated with polychaete infestation. Incidental lesions included presence of bacterial aggregates

or crustacea in normal tissues of several coral species. A gross diagnosis (e.g. bleaching) could have several different causes. This phenomenon underlines the importance of conducting microscopic exams on coral lesions to better define what the underlying causes of grossly visible changes. This study also provided the first baseline survey of corals in this region for pathogens and the first evidence that ciliates may, in some instances, be responsible for bleaching of selected coral colonies. This study also extended the documented range of growth anomalies in Acroporid corals. Future surveys should concentrate on systematically evaluating the spatial distribution of major lesions to allow for better comparisons among sites.

HOOKWORM ENTERITIS/BACTEREMIA COMPLEX IN CALIFORNIA SEA LIONS AND NORTHERN FUR SEALS, SAN MIGUEL ISLAND: A POPULATION DENSITY DISEASE

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Abstract:

During an investigation of high mortality of California sea lion (*Zalophus californianus*) and Northern fur seal (*Callorhinus ursinus*) pups on San Miguel Island in southern California, hookworm (*Uncinaria* spp.) enteritis with secondary bacteremia was found in 65% of the 225 pups examined. Ages ranged from 2 weeks to 9 months. Lesions found in these pups included parasitic enteritis, peritonitis, myocarditis, hepatitis, encephalitis, nephritis, pneumonia, and arthritis. Adult parasites including eggs were even found within the peritoneal cavity causing peritonitis. This severe epizootic hookworm infection is having an effect on the population of these two species of marine mammals. Over the last 30 years or so the populations of California sea lions and fur seals have steadily increased causing the rookery to become fairly crowded, thus this recent problem with hookworms is considered to be a density dependent disease.

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DEMOGRAPHICS, ECOLOGY, AND SEROSURVEY OF DOMESTIC DOGS IN THE ISOSO OF BOLIVIA

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Abstract

Disease is increasingly recognized as a threat to the conservation of wildlife, and in many cases the source of disease outbreaks in wild carnivores is the domestic dog. For disease to spill over from a domestic to a wild population, three conditions must be satisfied: susceptibility of the wild species, presence of the disease agent in the domestic population, and contact between the two populations of interest. This study investigated the potential for disease spillover from the domestic dog population to the wild carnivore population in the Isoso of Bolivia, an area of tropical dry forest contiguous with a national park. Using questionnaires, data were gathered on the demographics of dogs, including adult and neonatal mortality, litter size, and hunting frequency. A large (6475 hunts) dataset containing self-recorded hunting information from 1996 to 2002 was analyzed to determine the extent of dog participation in hunting and duration, success, and frequency of hunting trips. Blood samples were taken from 98 Isoceno dogs for a serosurvey of canine pathogens of conservation concern, including canine distemper virus, canine parvovirus, canine herpesvirus, canine coronavirus, canine adenovirus, leptospirosis, toxoplasmosis, canine brucellosis, heartworm disease, and the sarcoptic mange mite.

Results from the demographic portion of the study indicate that the number of dogs present in the Isoso is remarkably high. The ratio of people to dogs is approximately 1.5:1, and each household has an average of 3.8 dogs. This is equivalent to more than 500 dogs in a village with a human population of 760. The average age of dogs is relatively low (3-4 years), and the average litter size is 4.1 pups. These values, along with high neonatal (80%) and adult (38%) mortality rates, indicate that the population turnover among dogs is quite high, suggesting that the population is large enough to support diseases endemically by providing a constant source of susceptible hosts. Most (86%) dogs participate in hunting, and of these dogs, 82% hunt weekly or more often. The vast majority (97%) of hunts include dogs. The average hunt is 10 hours long and

involves 2.8 dogs. Based on the average number of dogs participating in hunts, and the frequency and duration of hunts, the forest surrounding the Isoseño communities is subjected to an average of 30,000 dog-hours each week. Results of the serosurvey demonstrate a high seroprevalence of canine distemper virus (95%), parvovirus (95%), herpesvirus (68%), and sarcoptic mange (63%). These findings, as well as the high population turnover of dogs and frequent opportunities for contact between domestic and wild carnivores, indicate that domestic dogs represent a disease risk for wildlife in the Bolivian Isoso.

EXPERIMENTAL LEPTOSPIROSIS IN CAPYBARAS (*Hydrochaeris hydrochaeris)*

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Abstract

The objective of the present trial was to characterize the periods of seroconversion, leptospiremia and leptospiruria in capybaras *(Hydrochaeris hydrochaeris).* In order to achieve this aim, six animals were infected intravenously, using *Leptospira interrogans* serovar pomona. The capybaras were anesthetized using intramuscular injections of ketamine (Vetaset, Fort Dodge; 1.5 mg/kg) and xylazine (Rompum, Bayer; 0.5 mg/kg). After the experimental infection, blood and urine collections were performed for culture of *Leptospira sp.*, as well as serologic testing and polymerase chain reaction (PCR). The animal sera were tested by microscopic agglutination test using a collection of 24 serovars. The samples for culture were inoculated in semi-solid modified EMJH medium with 5 fluorouracil (300 mg/l) and nalidixic acid (20 mg/l) and after 24 hours, transferred to both Fletcher and modified semi-solid EMJH media without antibiotics. These media were incubated at 28ºC and examined weekly for 8 weeks. The capybaras were euthanised after the experiment, and kidney and liver were collected for culture and PCR. Anti-*Leptospira* agglutinins started to be detected between day 2 and 10, and peak was reached between the 9th and the 27th day, coinciding with the 83rd day after infection (AI). Leptospiremia was detected until the period between days 12 and 14 AI.

Leptospiruria was first detected between days 6 and 10 AI and was detected until the 43rd day AI. The culture of the tissues was negative. The control animal was negative to all diagnostics tests. Results of this study indicate that capybaras may have a role as *Leptospira* reservoirs and may contribute to the maintenance of this infection in rural and wild environments. This is the first description of experimental infection in capybaras with *Leptospira.*

WINTER MORTALITY OF BALD EAGLES ALONG THE LOWER WISCONSIN RIVER

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Abstract

Unusual morbidity and mortality of bald eagles (*Haliaeetus leucocephalus*) wintering in two counties along the lower Wisconsin River, Wisconsin, began in 1994-1995 with the deaths of at least 14 eagles. Nine eagles were found dead, five were collected alive but died within 2 days, and two additional birds were found sick, then rehabilitated and released after 2.5 months. Bald eagles at roosts from 10-65 km upriver and 10-150 km downriver from the affected region and elsewhere in the state were not found sick or dead. Beginning in 2000-2001, after a hiatus of 4 years, during which eagle populations in the region were carefully monitored, similar bald eagle morbidity and mortality has recurred each winter. The area of concern has expanded to eight counties, with mortality events occurring primarily in January and February, and infrequent cases found from late November to early April. Of 85 bald eagles that have died in the target area within the appropriate time frame, 63 have been necropsied; some evaluations are still in progress. Sick eagles present in good body condition, with weakness, incoordination, tremors, vomiting and seizures. Snow or litter around dead eagles is often disturbed, consistent with observations of terminal seizures. Eagles brought into veterinary and rehabilitation facilities frequently have repetitive seizures, refractory to medication, over hours or days before death or euthanasia. No other avian or mammalian species have been involved. At gross necropsy, no consistent abnormalities have been found. By light microscopy, a minimum of 40 affected eagles had mild to severe multifocal to diffuse hepatocellular cytoplasmic vacuolation. Special stains revealed the presence of lipid in the vacuoles of a subset of affected livers. Vasculitis and microhemorrhages in the brain have been noted; it is unclear if the hemorrhages are a consequence of the seizures. This suite of lesions has not been seen in more than 4000 bald eagles from throughout the United

States. The characteristic lesions of avian vacuolar myelinopathy, initially noted in 1994-1995 in Arkansas, have not been seen in Wisconsin bald eagles.

Extensive laboratory investigations on dead and sick birds have been inconclusive. Investigations have focused on agrochemical and veterinary drug use, contaminants associated with the Badger Munitions Plant, livestock mortality events, fish kills, and forage fish species available to eagles. Toxicological tests have ruled out heavy metals, organophosphorus and carbamate pesticides, organochlorines, 4-aminopyridine, white phosphorus, strychnine, anticoagulants, and barbiturates as causative agents. Additional compounds, including sodium fluoroacetate and cyanide, have not been detected in a limited number of samples tested. Aerobic and anaerobic cultures, fungal cultures, viral cultures, assays for exposure to viruses and protozoal parasites, and tests for biotoxins have not found an etiology. Agricultural fields, where eagles feed on pig and duck carcasses, often with crows and turkey vultures, have been examined, farmers interviewed, and samples of pigs and ducks collected for evaluation. In 1995, a mortality event in the same area involving rock doves was investigated; the birds had severe, nonsuppurative encephalitis caused by pigeon paramyxovirus 1. There is no correlation with fish kills, and heavy metals and organochlorines in fish were below toxic concentrations. Forage fish have been tested for levels of thiaminase, with one species, gizzard shad, testing very high. The hypothesis that the syndrome is caused by a severe thiamine deficiency as a result of feeding largely on gizzard shad remains to be adequately tested. Evaluation of test results continues, and repeated multi-agency workshops allow for generation of new hypotheses.

EXPOSURE OF DESERT BIGHORN SHEEP TO SELECTED DISEASE AGENTS IN CENTRAL ARIZONA

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Abstract

Twenty adult desert bighorn sheep (*Ovis canadensis mexicana*) were sampled from a population in the Mazatzal Mountains of central Arizona during multiple captures between June 2000 and October 2002. Serologic, nasal, pharyngeal, and cervical swab, fecal, and ectoparasite samples were examined for evidence of pathogen exposures during drought conditions prior to and following removal and exclusion of domestic livestock from the study area. Evidence of bacterial and viral activity persisted throughout the study, and was unremarkable in comparison to seroprevalence and antibody titers against disease agents reported for other desert bighorn populations.

Results indicated lower than normal rainfall and removal of domestic livestock had no influence on exposure to leptospiral or viral diseases, and disease was not a factor limiting bighorn population growth and production. However, seroprevalence of antibodies against *Chlamydia* sp. increased during a year of exceptionally low rainfall, and incidence of pneumophilic bacteria in nasal swabs declined after livestock removal. We suggest our findings are consistent with enzootic stability and levels of immunity corresponding with absence of clinical disease in the Mazatzal Mountains desert bighorn sheep population. Continued monitoring of disease exposure and population trends in relation to variables such as presence or absence of domestic livestock, densities of bighorn and sympatric ungulates, rainfall levels, and translocations are key to understanding effects and etiology of desert bighorn sheep diseases.

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OVER THE FENCE AND THROUGH THE WEEDS - THE SPREAD OF *BRUCELLA ABORTUS* STRAIN 2308 FROM ELK (*Cervus elaphus*) TO ELK AND BISON IN A CAPTIVE FACILITY

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Abstract

Brucellosis in Greater Yellowstone Area (GYA) bison and elk has been a source of controversy and wildlife management for many years. Research on brucellosis has been conducted in numerous facilities that house captive wildlife to generate data on the disease in elk, bison, and reindeer.

From 1999 to 2002, approximately 100 elk were held captive at the Idaho Department of Fish and Game Wildlife Health Laboratory in Caldwell, Idaho to evaluate the efficacy of single-dose, calfhood vaccination using *Brucella abortus* strain 19 (S19). These elk were challenged with 1 x 10^7 CFU of pathogenic *Brucella abortus* strain 2308 (S2308) by bilateral intraconjunctival sac instillation on February 28, 2002. Abortions occurred

between March and June 2002, and live births occurred in May and June 2002. All elk in the vaccine study were euthanized by late June 2002.

All resident animals at the Wildlife Health Laboratory undergo annual health checks including serological testing for brucellosis. None have been found to be seropositive to brucellosis until summer and fall of 2002. In July 2002, a 2-year old bull bison was found to be serologically positive to brucellosis at slaughter. On follow-up testing, 2 additional bison cows were found to be seropositive. In August 2002, three adult bull elk were found to be seropositive to brucellosis. One bull was culture positive for S2308 on a semen sample collected in December 2002 and again at slaughter in February 2003. In late summer and fall 2002, 3 adult elk cows were found to be seropositive to brucellosis. In addition, a group of 6 seronegative adult female elk were joined with a seropositive bull elk in fall of 2002. To date, 3 of these elk have seroconverted.

An extensive epidemiological investigation was undertaken to try to determine the source and strain of the brucellosis that appears to have crossed at least 2 fencelines uphill from the elk challenged with S19. No common use area, random contamination or biosecurity break was identified that could explain the movement of the S2308 among these animals and through the various pens.

As of March 2004, all seropositive animals on site have been euthanized and tissue samples submitted for culture. Summaries of serial serological and culture results and epidemiological data will be presented. The conclusions reached may have implications for future work with brucellosis in captive wildlife facilities.