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Spillover: dynamics of cross-species transmission

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Advances in genetic sequencing are now revealing the tremendous diversity of the microbial communities that inhabit or infect living organisms. However, it is difficult to identify the microbes that have the potential to spill over from other species to infect and cause disease in humans. We outline the ecological, epidemiological, and behavioral determinants of pathogen exposure and the within-host biological factors that shape susceptibility to spillover infections. By integrating the insights generated in currently isolated fields, we can quantify barriers to spillover, assess the risk of known pathogens to human health, and identify points of intervention and control.

The ecology of parasite transmission in wildlife populations: Lessons from lizards

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Models of the dynamics of parasite – host interactions often assume random encounters between infected and susceptible hosts. In wildlife populations that is rarely the case. Different animal “personalities” and social structures can have a major influence on interactions, on exposure and risk of infection, and on the spread of parasites and pathogens around populations. Our research program has explored social networks within largely solitary Australian skink species and the role of those networks in determining the infection patterns of the lizards. We ask whether the edges that link network nodes act as pathways for parasite transmission. We have found evidence supporting this transmission pathway in that more highly connected individuals are more likely to be infected, from closer genetic relatedness among parasites infecting hosts that are more closely linked on the network, and from the experimental release of genetically unique parasite strains. We also find that different transmission pathways drive infection patterns in different parasite species, and in the same species in different years. Our data come from a range of endemic parasites including bacteria, nematodes and ectoparasitic ticks, none of which has a major impact on individual host fitness at natural infection levels. We argue that understanding these transmission pathways will be vital background information for intervention management in the case where some new more virulent pathogen enters a population and threatens its persistence.

Host population density driving, and driven by, parasites

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The association between hosts and parasites results in reciprocal selective pressures over coevolutionary time. Over shorter ecological time scales, this is manifested by the negative impact of fitness-reducing parasites on host populations, and the positive effect of host density on parasite transmission and abundance. We use a large database on the densities and biomasses of all free-living and parasitic metazoans from multiple New Zealand lake ecosystems to address these reciprocal effects between hosts and parasites. First, we show that the bottom-up coupling of resource and consumer abundance predicted by ecological and epidemiological theory is evident, as seen by the positive relationship between parasite densities and those of their hosts across localities, independently of host and parasite taxa or modes of infection. From an energetic perspective, host populations appear undersaturated with parasites: parasite biomass scales with host biomass with a lower slope than predicted by theory. Second, we observed that across host populations, the variance in host density across a set of samples scales positively with mean density, following Taylor's power law. However, the slope of this relationship differs between free-living taxa that are not parasitized and those that are parasitized. This suggests that parasitism exerts measurable effects on host population dynamics. Finally, the extent to which different parasite species achieve their highest abundance in the same localities is strongly dependent on similarities in life cycles: the more intermediate and definitive hosts are shared between two parasites, the stronger the spatial covariance in their local abundances. Thus, some host populations are more likely than others to suffer additive or synergistic effects from multiple parasites. The strength of the population regulation and selective pressure exerted by parasites on their hosts is therefore measurable across a broad spectrum of taxa, and structured spatially with multiple parasite species sharing hotspots of infection.

Devil facial tumour disease (DFTD) immunisation trial on Tasmanian devils to be released in initial phase of rewilding programme

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The Tasmanian devil is threatened with extinction in the wild by devil facial tumour disease (DFTD). Measures to counter extinction include the maintenance of a genetically diverse captive insurance population, and the development of a protective vaccine against DFTD. The success of the captive insurance population has allowed progress to be made on the long-term goal of rewilding and this coincides with promising results obtained from previous immunization trials on captive devils. Consequently the 17 devils ear-marked for release in September 2015 are being used in a more substantial immunization trial.

The immunization trial comprises monthly subcutaneous injections of non-viable modified DFTD cells plus adjuvants. Blood samples are collected fortnightly to assess humoral and cell mediated immune responses.

The previous immunization trials resulted in antibody responses from all 4 captive devils as demonstrated via flow cytometry and ELISA. The cell-mediated immune responses were more difficult to demonstrate but there was evidence of killing of DFTD cells by peripheral blood lymphocytes in some of the cytotoxicity assays. Supernatants of the assays were collected and frozen with the expectation that an interferon gamma detection ELISA will be developed.

The same methods are being used to assess the responses of the 17 devils due for release. This trial started on 09/02/15 and preliminary results will be available in May 2015. After release, the devils will be monitored regularly to assess their adaptation to the wild, their on-going immune responses to DFTD, and if/ when they succumb to DFTD.

The primary purpose of immunizing these devils is to provide a large enough sample size for the immunization trial to enable a robust assessment of responses detected. It is hoped the immunizations will also provide some protection against any natural DFTD challenge these devils might face once released and thus mitigate this threat.

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Predict: Preventing emerging infectious diseases at wildlife-human-domestic animal interfaces

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The appearance and spread of diseases, such as Ebola, have had profound global health impacts and have heightened awareness regarding the interconnectedness of wildlife, domestic animal, and human health. The impacts have illustrated our vulnerability to infectious diseases affecting all species and the disappearing boundaries between the less developed and more developed parts of the world. Further, population growth and environmental change are facilitating contact with wildlife in unprecedented ways and increasing frequency, yet many nations lack the resources and infrastructure necessary to detect and respond. Management of zoonotic diseases therefore warrants vigilant attention but also provides a challenge for wildlife managers to protect species. USAID initiated the Emerging Pandemic Threats program in 2009 with the goal of strengthening capacities in developing countries to prevent, detect, and control infectious diseases. PREDICT, a wildlife surveillance and virus discovery component of the program, focused on building capacity and applying a One Health approach to this challenge. Through a consortium of partners, PREDICT's efforts focused on early detection and response to potentially high-consequence viruses in regional "hotspots" for infectious pathogen spillover. Implemented in over 20 countries, we improved early detection and response to disease threats by: 1) strengthening viral surveillance in wildlife; 2) improving virus detection and discovery capacities; 3) characterizing high-risk wildlife-human interfaces, behaviors, and drivers of pathogen spillover; 4) optimizing predictive models for disease emergence and spread; and 5) deploying cutting-edge information management and communication. In just five years, the consortium humanely sampled more than 56,000 wild animals, while training 2,500 wildlife professional in optimal field and laboratory practices, and detected more than 815 novel viruses in addition to 169 known ones. PREDICT has played a key role in investigating the cause of human and wildlife diseases and is identifying high-risk human behaviors for the protection of wildlife and global health.

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Mapping the risk and modeling the costs of emerging zoonoses

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Emerging zoonoses are a major threat to public health, causing morbidity and mortality on a global scale, and costing billions of dollars annually. Yet our understanding of how diseases emerge, and our capacity to predict and prevent their emergence, is rudimentary at best. As part of the USAID-EPT-PREDICT program, we have assembled a modeling and analytics team to analyze the causes of disease emergence and spread. Our goals are to identify the regions, sites and human-animal interfaces of highest risk for disease emergence and maximize surveillance and control programs for known and previously unknown zoonoses. In this talk I highlight some of our recent work, including a completely revised EID hotspots risk map that identifies land use change as a significant driver of EID events from wildlife. To estimate the potential for future zoonotic disease emergence, we have analyzed viral discovery curves following repeated sampling of individual species. This gives us the first ever estimate of the likely number of unknown viral pathogens of zoonotic potential in mammals – the 'zoonotic pool'. To identify which wildlife

are most likely to harbor potential zoonoses, we conducted the first ever analysis of all viral-host relationships in mammals, corrected for reporting bias. This work also sheds light on whether bats are more 'special' as a zoonotic virus reservoir than other species. To generate local scale risk maps, we have conducted human risk behavior analysis, wildlife sampling and viral discovery in three Tropical forest systems. This "DEEP FOREST" project provides a strategy to understand where disease emergence is most likely to occur in a landscape and where funds would be best spent on surveillance and control. Finally, we analyzed the cost of pandemics, and their control, and show that in the long term, prevention of disease emergence through programs like PREDICT is more cost effective than controlling diseases after they have emerged. This is borne out by our analysis of the economics of the West African Ebola outbreak, and what this signifies for future pandemic prevention programs.

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Determining the role of fruit bat population dynamics in the emergence of Hendra virus in Australia

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Hendra virus (HeV) is a bat-borne RNA virus that has recently emerged as a human public health concern in Australia. HeV circulates in the large frugivorous and nectarivorous bats of the genus *Pteropus* (known colloquially as fruit bats or flying foxes). Periodically, spillover into an intermediate host (horses) occurs which amplifies viral populations before infecting humans. Anthropogenic influence and landscape change have been implicated as catalysts in the emergence of HeV, as well as analogous bat-borne diseases, such as Ebola and Nipah virus. Therefore, a quantitative understanding of the mechanisms that drive host population dynamics and pathogen epidemiology within the context of landscape change is an important and elusive requisite to predicting the behavior of a bat-borne disease system.

Here, I present spatiotemporal models of both food resource distribution and foraging behavior of fruit bats, the combination of which enables a functional model of bat population dynamics at the landscape scale. My methods employ novel algorithms that analyze patterns in census counts at roosts of fruit bats across southeastern Queensland over the past decade, and mathematical models of social foraging behavior that characterize spatiotemporal flux of fruit bat populations over time. Some initial results indicate that large aggregations of bats are correlated with remotely sensed measures of eucalypt phenology, and the fission-fusion structure of bat populations appears to be driven by hyper-variable patterns of flowering and nectar production across the landscape.

Robust prediction of the mechanistic interaction between food resource variability and bat population distribution facilitates parameterization of epidemiological models of viral transmission that are not vulnerable to typical confounders such as spatial population heterogeneity. And more broadly, it allows construction of scenarios that demonstrate how landscape change quantitatively influences bat population dynamics and ultimately drives spillover and emergence of bat-borne pathogens.

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Routes of Hendra virus excretion in naturally-infected flying-foxes; implications for viral transmission and risk of spillover to horses

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Flying-foxes (genus *Pteropus*) are the natural host of Hendra virus (HeV) which sporadically causes fatal disease in horses and humans in Australia. While there is strong evidence that urine is an important infectious medium that likely drives bat to bat transmission, and in all likelihood bat to horse transmission, we are less certain about the relative importance of alternative routes of excretion. Differentiating between alternative modes of transmission is critical in determining transmission rates in host-pathogen models. Determining the main routes of HeV excretion in flying-foxes is also important when assessing the relative risk of spillover to horses at the bat-horse interface. The main aim of this study was to determine the primary routes of HeV excretion in three of the four Australian flying-fox species, namely *P. alecto*, *P. poliocephalus* and *P. scapulatus*. A total of 2840 flying-foxes were captured and sampled between 2012 and 2014. A range of biological samples (urine; serum; urogenital, nasal, oral and rectal swabs) were tested for HeV using RT-qPCR. Forty-two *P. alecto* had HeV genome detected in at least one sample and were classified as "HeV-positive". The 42 HeV-positive *P. alecto* returned a total of 78 positive samples, at an overall detection rate of 1.76% across all samples tested in this species (78/4436). Urine was the most sensitive sample for detecting HeV genome, with fewer detections in serum, nasal, oral and rectal swabs. There were no detections in *P. poliocephalus* (n = 1168 animals; n = 2699 samples) or *P. scapulatus* (n = 262 animals; n = 985 samples). While it has been widely assumed that all four species play an important role in HeV infection dynamics, recent evidence suggests that Black and Spectacled flying-foxes may be the main reservoir hosts; the results of this study are consistent with this hypothesis.

Hendra virus infection of Black flying-foxes (*Pteropus alecto*): Assessment of nutritional and physiological drivers

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Hendra virus has caused periodic fatal disease in 90 horses and 7 humans in Australia since 1994. Epidemiological studies suggest that flying-foxes, in particular the Black flying-fox (*Pteropus alecto*) and the closely related Spectacled flying-fox (*P. conspicillatus*) are the natural reservoir hosts. This study investigates the hypothesised causal relationship between sub-optimal nutrition and physiological stress with Hendra virus infection in the Black flying-fox. During a twelve month study of flying-foxes in South East Queensland, samples were collected from captured wild Black flying-foxes for hematologic, biochemical and urine analysis, establishing normal reference ranges. The population demonstrated no significant deviation from normal hematologic or biochemical ranges across the study, but did demonstrate statistically significant temporal changes consistent with life cycle events. Measurements from Hendra virus positive animals were within the established normal ranges, but demonstrated statistically significant differences compared with Hendra virus negative animals, notably, increased lymphocyte %, decreased neutrophil %, decreased plasma triglyceride and increased plasma alkaline phosphatase levels and increased urinary protein levels. In order to investigate physiological stress, population urinary cortisol and Hendra virus urinary excretion prevalence was measured from mixed species roosts of flying-foxes from 2 geographically distinct sites (inland subtropical SE Queensland and coastal tropical Far North Queensland). This study demonstrated no significant seasonal fluctuations in Hendra virus excretion prevalence, nor urinary cortisol for the Far North Queensland population, but significant seasonal fluctuations in both Hendra virus excretion prevalence and urinary cortisol for the South East Queensland population. As population measurements are difficult to interpret due to the presence of multiple species and cohorts, a novel urinary collection and molecular analysis method was employed over a 1 year study to collect individual animal urines from the South East Queensland roost site to identify sex and species cohorts, and assess cortisol levels and Hendra virus excretion status.

Avian bornavirus in free-ranging waterfowl in North America and Europe

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The first avian bornavirus (ABV) was identified in 2008 by researchers investigating the cause of proventricular dilation disease in psittacine birds^{3,4}. A distinctly separate genotype (ABV-CG) was discovered in 2009 in association with neurological disease in free-ranging Canada geese (*Branta canadensis*) and trumpeter swans (*Cygnus buccinator*) in Ontario, Canada¹. Since then this genotype, now identified as ABBV-1, has been identified from a variety of wild avian species⁵, predominantly waterfowl, in North America at prevalences ranging from 10 to 50%, and in 2014 an additional genotype was identified in mallard ducks (*Anas platyrhynchos*)². In order to determine whether avian bornavirus was present in European waterfowl, the brains of 333 hunter killed geese in Denmark were examined by real time RT-PCR for the presence of avian bornavirus; seven birds (2.1%) were positive. Sequences were 98.18-99.83 % identical to each other, and 97.38-98.06 % identical to a reference sequence of ABBV-1 from North America. This is the first finding of ABV in wild waterfowl in Europe, and extends the range of waterfowl species in which the virus has been identified to include the pink-footed goose (*Anser brachyrhynchus*), greylag goose (*Anser anser*), and barnacle goose (*Branta leucopsis*). Given the migration paths of these species, avian bornavirus is likely to have a much wider geographic range than has previously been suspected.

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Avian migrants facilitate invasions of Neotropical ticks and tick-borne pathogens into the United States

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Migratory birds may play an under-recognized role in facilitating the invasion of ticks and tick-borne pathogens, leading to new human and animal health threats. Every spring, billions of birds migrate north across the Gulf of Mexico in route to breeding grounds across eastern North America. Because ticks typically stay attached to their hosts for up to a week, this annual migratory movement provides an opportunity for birds to transport ticks from South and Central America to the US. Furthermore, the stress of migration may be associated with increased circulation of pathogens within bird blood. Over two spring seasons (2013 and 2014), we captured birds on the Northern coast of the Gulf of Mexico in Texas, where high densities of migrants aggregate in the coastal marsh habitats. In the field, we searched each bird and collected ticks, obtained a blood sample, and attached a leg band prior to release. In the laboratory, we identified ticks to life stage and species using both morphologic and molecular features, and screened ticks and blood for infection with pathogens in the genera *Rickettsia*, *Borrelia*, and *Ehrlichia* using PCR and DNA sequencing. Based on examination of 3,844 birds of 85 species, we found 2.9% of birds of 36 species were infested by larval or nymphal ticks. Migratory birds harbored eight different tick species, including seven in the genus *Amblyomma* and one in the genus *Ixodes*, only two of which are known to be established in the US. Among the human pathogens detected within ticks and birds included *Borrelia miyamotoi* (a relapsing fever group spirochete), *Rickettsia parkeri* (a spotted fever group *Rickettsia*) and at least three other *Rickettsia* species implicated in human disease in Central and South America. Avian migrations may provide a mechanism for the geographic expansion of neotropical tick-borne disease risk.

Lesions associated with natural Wellfleet Bay virus infection in the common eider (*Somateria mollissima*) from Cape Cod, Massachusetts and available diagnostic tools

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Mortality events have been reported in common eiders (*Somateria mollissima*) from Cape Cod, Massachusetts dating as far back as the mid-twentieth century. In 2006, a novel enveloped RNA virus was isolated from the tissues of birds from some mortality events that were investigated. Full-length genome sequencing demonstrated that this virus was an orthomyxovirus in the genus *Quaranjavirus*, and the name Wellfleet Bay virus (WFBv) has been proposed. Between 2009 and 2014, diagnosticians with the Southeastern Cooperative Wildlife Disease Study in Athens, GA, USA, examined seventy-nine common eider carcasses from mortality events in Massachusetts. The gross and histologic lesions seen in naturally infected common eiders will be discussed. Diagnostic testing, including a combination of immunohistochemistry (IHC), virus isolation (VI), serology, and reverse transcription nested polymerase chain reaction (RT-nPCR), confirmed 24 birds as positive for WFBv, 26 as suspect, 25 as negative, and 4 as previously exposed to WFBv without evidence of active infection. Gross findings associated with detection of WFBv were multifocal to coalescing, flat, tan foci disseminated on the surface of the liver and splenomegaly. Histologically, multifocal to coalescing foci of coagulative necrosis throughout the liver were associated with detection of WFBv. Liver, esophagus, and spleen were most consistently positive by VI. Molecular testing using RT-nPCR was more sensitive than other ancillary tests. Non-specific staining with IHC made interpretation of results challenging, but this test can be useful with concurrent ancillary testing and the presence of characteristic lesions. Further investigation of diagnostic techniques and characteristic lesions, will facilitate understanding of morbidity and mortality factors affecting common eider, as well as any potential population-level impacts of this recently discovered virus.

Psittacine beak and feather disease in forest red-tailed black cockatoos (*Calyptorhynchus banksii naso*) in Western Australia

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Psittacine beak and feather disease (PBFD) is a highly contagious viral disease recognised as a key threatening process to endangered psittacine birds in Australia. Management of this threat is problematic because PBFD is endemic in many populations of abundant native psittacines, resulting in a continual risk of spillover into threatened populations. The recent emergence of PBFD in forest red-tailed black cockatoos (*Calyptorhynchus banksii naso*), a threatened species endemic to south-west Western Australia, is a conservation concern because its effect at a population level is uncertain.

In 2013, PBFD was confirmed by PCR, haemagglutination (HA) and haemagglutination inhibition (HI) assays in two juvenile forest red-tailed black cockatoos with clinical signs of chronic PBFD. The affected birds originated in the wild, but had been captive for eight months at separate rehabilitation centres prior to diagnosis. Subsequent screening of all in-contact birds, including 21 forest red-tailed black cockatoos and 6 Carnaby's cockatoos (*Calyptorhynchus latirostris*), found no evidence of active infection or seroconversion, despite prolonged close contact with the infected birds.

A research project investigating PBFD prevalence in wild forest red-tailed black cockatoos commenced in July 2013. Cockatoos entering the rehabilitation program due to illness or injury were selected as the study population, and screened for PBFD at a commercial laboratory by PCR, HA and HI assays. Preliminary results of this research found a prevalence of 0% (n=112, 95% CI:0-3.3%).

While forest red-tailed black cockatoos are susceptible to PBFD, these results indicate that the disease currently occurs at a very low prevalence in the wild population. Of note is the absence of infection in cockatoos exposed to high viral loads for an extended period, which suggests that forest red-tailed black cockatoos and Carnaby's cockatoos are relatively resistant to infection with PBFD virus.

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Bettongs and blood parasites: what's driving declines in the woylie (*Bettongia penicillata*)?

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Woylie (also known as brush-tailed bettongs, *Bettongia penicillata*) populations have undergone substantial declines over the past decade, with the cause of the declines attributed partly, but not entirely, to predators. Woylie declines displayed density-dependence, and a spatiotemporal pattern of decline, similar to declines patterns observed in other disease-driven declines. Three *Trypanosoma* spp. have been identified from woylies so far, and one of these – *T. copemani* – has shown pathogenic potential. In this study, we asked if patterns of *Trypanosoma* infection were associated with patterns of decline throughout the decline region. If trypanosomes were associated with woylie declines, we expected that infection prevalence would be highest at the peak of the decline, and prevalence would decline as the declines progressed through the region. Species-specific PCRs were run for each of the three *Trypanosoma* species from woylie blood samples collected between 2006 – 2012 from the Upper Warren region (south-west Western Australia). We found a strong association between spatio-temporal patterns of woylie declines and the prevalence of *T. copemani*, but not the other two *Trypanosoma* spp. We consider this further support for the hypothesis that *T. copemani* is indirectly involved in the woylie declines, probably through enhancing the predation risk of infected individuals.

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The effects of intensive conservation management on host-parasite interactions in threatened New Zealand wildlife

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The usual suspects threaten New Zealand's biodiversity including: a suite of introduced mammalian predators; habitat fragmentation, degradation and loss; and introduced animals and plants that compete directly and indirectly with native species. The intensive conservation management of many native species is required to prevent extinction. These management strategies often have unforeseen consequences on host-parasite interactions in wildlife. I will present recent cases that demonstrate spill-over of introduced parasites into native wildlife, exposure of native wildlife to novel parasites; increased exposure, infection and disease of wildlife from native parasites; and finally the over-zealous treatment of parasites in conservation management that results in loss of native parasite diversity, perhaps with accompanying detrimental effects on the host. The knowledge gaps that hinder our ability to understand and effectively moderate host-parasite relationships in intensively managed wildlife will be reviewed and future research directions discussed.

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Baseline health parameter changes in eastern bettongs (*Bettongia gaimardi*) following reintroduction

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The eastern bettong (*Bettongia gaimardi*) is a small, nocturnal, predominantly mycophagous, potoroid marsupial that has been extinct on the Australian mainland for approximately 100 years. Recently 60 eastern bettongs were translocated from the island of Tasmania and re-introduced in to two predator-free fenced reserves, Tidbinbilla Nature Reserve and Mulligan's Flats Woodland Sanctuary, within their former range in the Australian Capital Territory. A subset of 30 (12 male, 18 female) bettongs from this translocated population underwent detailed health assessment and screening for selected infectious diseases and parasites at the point of translocation and again at 12-24 months after reintroduction.

Significant differences were evident between multiple haematological and biochemical variables pre- and post-reintroduction. Elevations in haematocrit, haemoglobin, red cell count, mean corpuscular hemoglobin, globulin, urea, creatinine and triglycerides were suggestive of an improved plane of nutrition for bettongs post-reintroduction. Limited haematological and biochemical variable differences were observed between populations at the two reintroduction sites. Ectoparasite assemblages differed pre- and post-reintroduction with five of 13 ectoparasites present initially failing to persist post-reintroduction. Four additional species of ectoparasite were present on bettongs post-reintroduction; presumably acquired from sympatric marsupials at the reintroduction sites.

All bettongs were seronegative for antibodies to *Toxoplasma gondii* at both time points. One bettong with detectable antibodies to the alphaherpesviruses macropodid herpesvirus 1 and macropodid herpesvirus 2 at the point of translocation was seronegative at repeat sampling. The novel gammaherpesvirus, Potoroid herpesvirus 1, was detected, via PCR, from one bettong at the point of translocation but not at repeat sampling following reintroduction.

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Challenges in wildlife health management: a case study of avian malaria in kiwi (*Apteryx* spp)

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This study highlights and discusses some of the issues around managing wildlife health risks when using ex-situ breeding as a conservation management tool. In 2011, an outbreak of avian malaria (*Plasmodium (Huffia) elongatum*) occurred in a captive management facility which releases kiwi over a wide range of the North Island of New Zealand. At the time, wildlife health experts made a series of recommendations to determine the significance of the event and manage potential risks it presented for wild populations. This case study is a four year follow-up on the event to review the level to which the recommendations were implemented. Of the five key recommendations, there had been good implementation where wildlife health experts were directly involved (recommendations 1-4). However, implementation was poor when the kiwi were released and wildlife health experts were no longer directly involved (recommendation 5). There could be a number of reasons for this difference in compliance including communication issues, cost, conflicting advice, lack of interest and differing perceptions of risk. All of these were evident both at the time of the recommendations and also in the follow up discussions. The implications of these findings will be discussed in more detail. This case study highlights the disease risks associated with use of captive management for species recovery, and especially the greater risk when there is congregation of animals from distinct geographic areas, and their dispersal of the offspring. It also emphasises the need for better communication of health risks and associated planning, improved understanding of wildlife health by conservation managers and scientists, and greater involvement of wildlife health experts in the decision making and follow up for disease outbreaks.

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Genomic solutions for major biodiversity diseases chytridiomycosis and white nose syndrome threatening conservation of species

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The recent global spread of chytridiomycosis across different environments and into multiple species causing mass amphibian decline provides a rare opportunity to look at host pathogen evolution writ large (Skerratt et al 2007, Martel et al 2013). We aimed to identify whether manipulating this evolutionary relationship using the simplest genomic tools such as assisted selection for disease resistance could be used as a management strategy. We found that resistance to chytridiomycosis is evolving. This is good news for the conservation of amphibians still threatened by the disease. We found greater survival in the offspring of long exposed frogs compared with those from naïve frogs as measured in laboratory transmission experiments (Bataille et al 2015). However, selection for greater survival was context specific and variable among populations. We identified MHC resistance markers which can be used to readily identify amphibian populations most suited for assisted selection. Promoting innate immunity artificially may be useful in overcoming the lack of opportunities for natural selection in the wild. In addition,

determining whether natural selection is occurring for other mechanisms enabling population persistence such as particular life history traits or behaviours is important. A welcome boost to this approach to managing the major biodiversity diseases chytridiomycosis and white nose syndrome threatening conservation of wildlife is the latest developments in synthetic biology and genetic engineering for human biomedicine.

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Immunization against *Chlamydia pecorum* in wild-caught koalas (*Phascolarctos cinereus*) is associated with a reduced chlamydial burden and less progression to pathology

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Chlamydia pecorum is a significant cause of decline in koala (*Phascolarctos cinereus*) populations in Queensland (QLD) and New South Wales (NSW), Australia (1). Current treatments have limited efficacy, and cannot restore fertility in sterile female koalas (2). Newly developed vaccines have been tested in captive animals under controlled conditions with promising results (e.g. 3), so the vaccination of free-ranging koalas was an obvious and important next step. Here, we recruited 60 free-ranging koalas, with varying levels of chlamydial infections but no clinical signs of disease, in one south-east QLD population in Australia. Thirty koalas were immunized against *Chlamydia pecorum* with adjuvanted chlamydial major outer membrane protein (MOMP), while 30 acted as the non-immunized control group. All koalas received a comprehensive veterinary check, and whole blood and swabs (urogenital and ocular) were collected before they were radio-collared and re-released. Koalas were then recaptured at 6 and 12 months post-immunization for veterinary checks and sampling. Results from the first vaccination study in a wild koala population show that immunization can significantly reduce chlamydial burden up to 12 months post-immunization in animals already infected compared to a non-immunized control group. Incidence of new infections was low and did not differ greatly between the immunized and non-immunized group, however, progression to disease was higher in non-immunized animals than in immunized animals. Lastly, the reproductive success and fitness of immunized female koalas and their offspring was greater than that of their non-immunized counterparts. This study provides the first evidence that a MOMP based vaccine against *Chlamydia pecorum* can reduce *Chlamydia* shedding and progression to disease in free-ranging koalas.

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Conservation implications of coccidian and vector-borne parasites in the endangered whooping crane (*Grus americana*)

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While the population of endangered whooping cranes (*Grus americana*) has grown from 15 individuals in 1941 to an estimated 304 birds today, the population growth is not sufficient to support a down listing of the species to threatened status. The degree to which disease may be limiting the population growth of whooping cranes is unknown. This conservation medicine project described selected parasites of potential concern infecting the only self-sustaining wild population of whooping cranes. Using a non-invasive sampling approach, we assessed the prevalence and phenology of *Eimeria* oocysts in whooping crane fecal samples collected across two winter seasons (November 2012 – April 2014) at the Aransas National Wildlife Refuge along the Texas Gulf coast, USA. Across both years, 26.5% (n=328) of fecal samples were positive for *Eimeria* based on microscopy, and 16.4% (n=262) of samples were positive by PCR; phylogenetic analyses confirmed the infection with *Eimeria gruis* and *E. reichenowi*, two species known to produce disseminated visceral coccidiosis and high juvenile mortality in captive cranes. In

addition, we obtained blood samples from these cranes to screen for several vector-borne hemoparasites via PCR. We documented *Plasmodium*, or *Haemoproteus* infections in 62% (n=61) of blood samples, and *Leucocytozoon* infections in 7%. The phylogenetic analysis revealed that most sequences for each *Plasmodium* lineage were identical to each other and formed a unique clade distinct from previously published sequences. Taken together, these results suggest that birds are being exposed to these pathogens prior to arriving on the wintering grounds in Texas. Understanding the epidemiology of coccidiosis and vector-borne pathogens, which are capable of regulating host populations, are important for management efforts to increase population growth of the endangered whooping crane.

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Declining populations of small mammals in Australia's north: is disease the smoking gun?

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Over the past 20 years, there has been an alarming decline in a wide range of small native mammal species in northern Australia. The declines have occurred in remote areas that were previously considered ecologically sound. The causes of the declines are under investigation; changed fire regimes, grazing and introduced predators (including feral cats) are implicated. There is still much to learn about health and disease of small mammalian wildlife species in Australia and the paucity of knowledge has made assessment of the impact of disease in these declines challenging.

Over a two year period, research was undertaken by the Conservation Medicine Program, Murdoch University, in collaboration with Northern Territory (NT) Dept. of Land Resource Management to assess health and disease in native mammals in the tropical savannahs of the NT. Study sites were located in urban (Darwin) and remote regions (Bathurst Island, Cobourg Peninsula, Kakadu National Park and Groote Eylandt), and included both mainland and island sites. Investigation focused on four target species across four different families (brush-tail possum, northern brown bandicoot, northern quoll and brush-tailed rabbit rat). Black rats and feral cats, as potential pathogen reservoirs, were also examined and sampled.

Animals were anaesthetised and investigations included assessments of health and specific testing for a range of prioritised pathogens of concern, including toxoplasmosis, herpesvirus and blood borne pathogens. *De novo* molecular testing was undertaken to investigate the presence and potential impact of novel pathogens. Sampling and testing was undertaken on almost 200 individuals. This is one of the most comprehensive and wide ranging disease investigations undertaken on multi-species declines in Australia.

The implications of our findings will be discussed in the context of wide scale population declines in the Top End of the NT and will include recommendations for ongoing work in this area.

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Mass mortality of Eastern Grey Kangaroos (*Macropus giganteus*) associated with hepatogenous photosensitisation

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The impact of introduced toxic plant species, to which evolutionarily naïve native mammals have little tolerance, is poorly documented. Sudden blindness, icterus and subsequent mass mortalities of Eastern Grey Kangaroos (*Macropus giganteus*) occurred in the Wagga Wagga region of New South Wales in April 2014. Preliminary differential diagnoses of Wallal/Warrego virus, macropod herpesvirus or facial dermatitis were ruled out. The animals had various severities and phases of hepatopathy with lesions ranging from acute damage to chronic regeneration. Animals presented with marked corneal oedema with a ventral to dorsal progression in animals showing progressive stages of disease. This syndrome was suspected to be a plant toxicosis resulting in hepatogenous photosensitisation.

Clinical pathology investigation of the cases provides evidence for a difference in the sensitivity of enzymes used to assess cholestasis in macropods. Histopathology of the livers of the affected animals demonstrated intrabiliary acicular clefts, indicating possible saponin crystal formation, resulting in structural damage to the bile ducts and hepatocytes.

An analysis of pasture proportion and species indicated the areas where kangaroos were feeding in the evenings and early night contained a high proportion of the suspect toxic plant species *Panicum gilvum* when compared to a control location. *P. gilvum* likely contains steroidal saponins (a secondary plant metabolite) and has been associated with previous case reports of photosensitivity in lambs and other species. There are preliminary indications in metabolomics data that compounds with similarities to diosgenin are found in the leaves, stems and inflorescence of *P. gilvum* and trace metabolites of diosgenin were found in the livers of sampled kangaroos.

This disease has recurred in 2015 following climatic conditions favouring the growth of *Panicum* species in the Riverina. This study highlights the potential of introduced weeds to affect and damage vulnerable populations of native mammals.

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Chytridiomycosis-induced shift in amphibian life-history

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The emergence of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis in amphibians, is implicated in the severe decline of up to 200 amphibian species globally, 113 of which may be extinct. In remnant populations of declined species, *Bd* persists and chytridiomycosis can cause substantial ongoing mortality. The emergence of novel pathogens is predicted to drive changes in host life-history, yet the effects of *Bd* on amphibian life-history have not been examined. Here, we show that *Bd* is associated with life-history changes in amphibians that can make them more vulnerable to climatic extremes. We focus on the endangered alpine tree frog, a species that is restricted to a small number of remnant populations in sub-alpine regions of south-eastern Australia. In diseased populations, very few adults survive between years. However, *Bd* prevalence is low in early life-history stages, resulting in high recruitment and population persistence. We compare longevity, and age and size at maturity between diseased and disease-free populations, and also with museum specimens collected prior to *Bd* emergence. We demonstrate that populations with *Bd* have a severely truncated age structure driven by very high rates of adult mortality. This truncated age structure means that in chytridiomycosis-challenged populations, individuals breed once, compared with non-diseased populations where individuals likely breed multiple times. Using a combination of empirical evidence and population models, we also show that chytridiomycosis is associated with earlier sexual maturity. We use population simulations to illustrate that these shifts in life-history greatly increase the risk of decline when populations are subject to variation in recruitment success caused by drought. Our results show that chytridiomycosis can drive major life-history shifts in its amphibian hosts and underline the potential utility of minimizing other sources of mortality to mitigate the threat posed by this deadly pathogen.

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Culture-independent genome sequencing provides new insight into the relationships between *Chlamydia pecorum* infections of koalas and Australian livestock

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Chlamydia pecorum is an important global pathogen of livestock and is a burden to primary producers in a number of agriculturally productive regions in Australia. The same pathogen is also recognized as the major pathogen of the koala, causing debilitating ocular and reproductive tract diseases and threatening the long-term survival of Australia's koala populations. Molecular typing studies by our group have previously suggested a link between *C. pecorum* strains infecting sheep, cattle and koalas, however, further genetic inference has been limited by the requirement to culture strains *in vitro* for the purpose of whole genome sequencing.

This study employed a culture-independent DNA capture approach to sequence *C. pecorum* genomes directly from clinical swabs samples collected from koalas with chlamydial disease as well as from sheep with arthritis and conjunctivitis. Investigations into single nucleotide polymorphisms within each of the swab samples revealed that a portion of the reads in each sample belonged to separate *C. pecorum* strains, suggesting that all of the clinical samples analyzed contained mixed populations of genetically distinct *C. pecorum* isolates. This observation was independent of the anatomical site sampled and the host species. Using the genomes of strains identified in each of these samples, whole genome phylogenetic analysis revealed that a clade containing a bovine and a koala isolate is distinct from other clades comprised of livestock or koala *C. pecorum* strains. Providing additional evidence to support exposure of koalas to Australian livestock strains, two 'minor' strains assembled from the koala swab samples clustered with livestock strains rather than koala strains.

Culture-independent probe-based genome capture and sequencing of clinical samples provides the strongest evidence yet to suggest that sheep and cattle *C. pecorum* strains can be transmitted to koalas and raises further questions about the evolutionary origin of this pathogen in native Australian animals.

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Status of preparedness efforts for vaccination of Hawaiian monk seals (*Monachus schauinslandi*) against morbillivirus

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Morbilliviruses affect many mammalian taxa and vaccination is a routine prevention strategy for domestic animals and has been used successfully in wildlife. Canine distemper virus (CDV) and phocine distemper virus (PDV) are morbilliviruses that have caused dieoffs of tens of thousands of phocids. Exposure to CDV or PDV has not yet been detected in Hawaiian monk seals (HMS). Risk factors for HMS include out-of-habitat pinnipeds that carry morbillivirus into Hawaiian waters and interactions between HMS and infected feral or domestic dogs, which are increasingly common as human and seal populations in the main Hawaiian Islands increase. The 1,100 remaining HMS are

distributed widely across the Hawaiian archipelago, complicating detection and outbreak response. Hence, introduction of morbillivirus into this immunologically naive population would threaten the survival of this critically endangered species. Immunization trials of captive seals with a commercial canine distemper recombinant vaccine have documented production of CDV antibody without adverse effects. An implementation strategy has been developed for emergency vaccination of wild HMS in the event that exposure or disease is detected. Efforts to model the timeline and trajectory of an outbreak and the effect of vaccination schemes on disease spread are underway and will inform the strategic approach to an emergency vaccination response. Prophylactic vaccination of free-ranging HMS during directed efforts, during routine handling (e.g., tagging), combined with emergency outbreak response preparedness, is the most effective approach to protecting this critically endangered species. While designed with morbillivirus outbreak prevention in mind, this overall strategy can be easily adapted to prevention of other disease threats, including West Nile Virus.

Skin sloughing and susceptibility: the role of skin processes in the susceptibility of amphibians to chytridiomycosis

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The zoospore fungus *Batrachochytrium dendrobatidis* (Bd), which is the causative agent of the amphibian disease chytridiomycosis, has resulted in the declines and extinctions of amphibians worldwide. This generalist pathogen infects a wide range of amphibian hosts, and a combination of ecological, physiological and environmental variables likely influence differences in susceptibility within and between species. Given Bd infects the keratinised skin of amphibians, variation in susceptibility to Bd may also be influenced by aspects of skin functioning, such as the rate of routine skin sloughing or shedding. Previous work has demonstrated that sloughing may be involved in immune defence by regulating the growth of skin-associated microbes, and thus could play an important role in the pathogenesis of chytridiomycosis. To examine the relationship between skin sloughing and disease progression, we exposed adult Australian green tree frogs (*Litoria caerulea*) to Bd, and monitored the change in sloughing rate and infection load over time using infrared cameras and quantitative PCR. Sloughing rate increased with Bd infection load in infected frogs, however the act of sloughing did not reduce Bd load on the ventral skin surface. In *L. caerulea*, a susceptible species, sloughing does not appear to limit the progression of disease. Rather, increased sloughing may actually exacerbate the loss of physiological homeostasis seen in terminally ill frogs by further inhibiting water and electrolyte transport across the skin. By measuring sloughing rates directly for the first time, our results provide insight into how variation in sloughing may influence susceptibility to chytridiomycosis. We are currently examining the efficacy of skin sloughing in reducing Bd loads on the skin of amphibians with low susceptibility to chytridiomycosis. Understanding the factors driving variation in susceptibility can improve our predictions of amphibian responses to Bd in wild populations, allowing for better species conservation planning.

When feces matter: a synthesis of a multiannual investigation of *Echinococcus multilocularis* transmission ecology in a North American urban setting

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Echinococcus multilocularis is a zoonotic, tropically-transmitted cestode typically maintained within a sylvatic cycle involving wild canid definitive hosts and small mammal intermediate hosts. Herein we provide a synthesis of multiannual research conducted in Calgary, Alberta, in which the coyote (*Canis latrans*) is the main wild definitive host. The study was conducted in five different city parks, collecting coyote feces along fixed routes and sampling small mammals to detect infections in intermediate hosts. Coyote feces were analyzed with a ZnCl₂ centrifugation and sedimentation protocol to isolate Taeniidae eggs, which were subsequently identified through species-specific PCR. Feces were also used to quantify consumption of small mammal preys and to identify individual coyotes through non-invasive genotyping conducted at 4-6 microsatellite loci. Coyote genotypes and fecal parasitology were combined to assess true parasite prevalence and individual infection patterns. This study provided the first evidence of an urban sylvatic cycle of *E. multilocularis* in North America, and showed an overall prevalence in coyote feces of 25% with significant temporal and spatial variation across seasons (10.5-43.5%) and city parks (5.3-61.5%). These variations were in accordance with availability and consumption of three intermediate host species and their levels of parasite infection (0.7-1.4%). Genotyping coyote feces allowed correcting for repeated fecal sampling of the same individuals. More importantly, it allowed us to follow individual patterns of infection and observe temporal variations in parasite transmission that could not be detected when examining feces of unknown identity. Winter was a crucial period for *E. multilocularis* transmission, as the encounter rate of coyotes with the parasite was estimated to be higher than any other season (95% CI: 1.0-22.4 infected hosts ingested). Voles (*Microtus pennsylvanicus* and *Myodes gapperi*) were preyed upon proportionally more than their availability, and likely played a key role for the maintenance of the sylvatic life-cycle of *E. multilocularis* in this urban landscape.

Elephant Endotheliotropic Herpesvirus: emerging infectious disease in SE Asia or newly described endemic?

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Elephant endotheliotropic virus (EEHV) was first recorded to cause haemorrhagic disease in captive young Asian elephants in 1999³. Since then EEHV, a highly contagious disease, has been responsible for almost 60% of all deaths in young captive Asian elephants in North America and Europe¹. Whilst the virus was considered a newly emerging disease in Asian elephants, causing fatal disease in juveniles, it was considered to be non-pathogenic in African elephants³. Despite disease associated with EEHV infection being reported widely in zoos in Western countries, only limited research has been undertaken in home-range countries of Asian elephants. This study mainly focused on EEHV infection in elephants from captive facilities in Thailand, using molecular examination of archived tissue samples collected from 17 young Thai elephants that died between 2006 and 2014². Three blood samples from suspected clinical cases were also analysed. The results indicated 14/20 individuals were positive for EEHV type 1. Further sub-typing of these cases revealed 12 aligned with EEHV1A, with five different distinct strains, and two cases aligned with EEHV1B, with only one distinct strain. Positive cases ranged in age (1-9 years), sex and geographical location. The results revealed large difference in genetic variation with no epidemiological relatedness in each case, supporting the new theory that EEHV1A and EEHV1B are more likely to be ancient endemic pathogens in Asian elephant, rather than newly emerging virus⁴. This was further supported by the fact that the affected elephants in this study had never been in contact with African elephants. Therefore, it is recommended that active surveillance and routine monitoring for EEHV in Asian elephants in their home-range countries should be undertaken in order to better understand the epidemiology of this disease and manage risk factors associated with disease transmission.

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A multi-disciplinary approach to investigation of the emerging fungal pathogen *Paranannizziosis australasiensis* in Tuatara (*Sphenodon punctatus*)

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Captive tuatara (*Sphenodon punctatus*) in the North Island of New Zealand have recently been discovered to be affected by the emerging primary fungal pathogen *Paranannizziosis australasiensis* (PA), the cause of an ulcerative dermatitis often fatal in other reptile species. The affected tuatara were part of a national breeding program, and the presence of this emerging disease has prevented their planned release. Our objectives are to develop a safe, effective treatment protocol for PA in tuatara, and to improve diagnostic and surveillance capabilities for detecting PA in both captive and wild populations. A multi-disciplinary, collaborative approach is being taken to investigate this emerging disease. Methods include an *in vitro* and *in vivo* pharmacokinetics study of two anti-fungal drugs (itraconazole and voriconazole) previously used to treat mycoses in reptiles but for which pharmacological studies in these animals is extremely limited. Simultaneously the focus of collaborators has been on the development of a PCR

to enable surveillance of potential environmental reservoirs and identification of asymptomatic carriers. Results to date show significant differences in antifungal pharmacokinetics at high and low ends of the tuatara's optimal temperature zone, with both antifungals exceeding target MICs for PA. A PCR has been developed which has shown that PA DNA can be successfully amplified when spiked into soil DNA extracts, and work is ongoing to detect naturally occurring PA infections from soil samples. The pharmacokinetic study will enable the development of an evidence-based treatment regime. This, in combination with the further use of PA-specific PCR testing, will allow us to better understand the risks of this disease to captive and free-living tuatara, and develop appropriate treatment and risk mitigation protocols.

Disease ecology during the breeding season in the endangered *Litoria verreauxii alpina* and management implications

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Amphibians are currently experiencing the greatest biodiversity decline of all vertebrate taxa, and one of the major drivers of this loss is the fungal disease chytridiomycosis. Some amphibian species, like the endangered *Litoria verreauxii alpina*, have drastically declined in parts of their range since the introduction of chytridiomycosis, yet persist in a small number of seemingly stable remnant populations. We studied chytridiomycosis dynamics during the breeding season to improve our understanding of how remnant populations persist with endemic infection, and to inform disease management. We monitored two populations during their ten-week breeding season through capture-mark-recapture analysis. We found that infection prevalence and intensity increased throughout the breeding season in both sites, but infection prevalence and intensity was higher at the site that had higher population density, suggesting that *Bd* transmission is density dependant. There was low recovery from infection in *L. v. alpina*, which was lower when the animals were heavily infected with *Bd*.

With high infection intensity at the end of the breeding season and low recovery from infection, population survival of this species is dependant on high recruitment. We further explored how disease may affect reproductive output by investigating spermatogenesis in a laboratory based infection experiment. We found that spermatogenesis increases when animals are infected with *Bd*. Infected animals had more overall activity, and a larger proportion of spermatozoa bundles in the seminiferous tubules. If infected animals are increasing reproductive efforts and producing more offspring before succumbing to disease, it is possible that population level selection for disease resistance or tolerance is minimized. In the absence of population level disease resistance or tolerance, conservation and management efforts for endangered species should focus on habitat management to support recruitment.

A pharmacokinetic investigation of florfenicol as a possible treatment of chlamydial disease in koalas (*Phascolarctos cinereus*)

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Background

Florfenicol is being investigated as an alternate treatment of chlamydiosis in koalas. Systemic chlamydiosis is the most important infectious disease of koalas, causing significant morbidity, mortality and infertility(1). Chloramphenicol base suspension is reportedly the most effective treatment, (2) but this formulation was unavailable at the commencement of these trials.

Hypotheses

1. Florfenicol will be effectively absorbed and provide theoretically therapeutic plasma concentrations in koalas following subcutaneous administration.
2. Florfenicol will represent a convenient and effective alternative to chloramphenicol for the treatment of chlamydial disease in koalas.

Materials and Methods

A new sample handling method has been developed to remove interfering endogenous compounds in koala plasma to produce a florfenicol assay utilizing High Performance Liquid Chromatography.

Florfenicol (Nuflor, MSD) has been administered to koalas with naturally occurring chlamydiosis. Blood samples were taken at serial time points following subcutaneous (SC) and intravenous (IV) administration for pharmacokinetic analysis.

Results

Poor absorption was noted following a single subcutaneous injection. The mean maximum concentration (C_{max}) of florfenicol in plasma of 3 koalas given 20 mg/kg SC was 1.2 µg/ml, attained 4 hours after administration. Following intravenous administration at 10mg/kg, the mean (n=3) florfenicol concentration at 24 hours post treatment was 25µg/mL.

Conclusions

A therapeutic target of 1-2µg/mL in plasma has been established based on the minimum drug concentration necessary to inhibit *Chlamydia pecorum* growth in vitro.(3).

Florfenicol at a dose of 20mg/kg SC is unlikely to be useful in treating koalas with chlamydiosis. Field data suggests that koalas will not tolerate higher SC doses. Intravenous administration of florfenicol may well prove efficacious, however caution must be taken with administering florfenicol via this route as an anaphylactoid reaction has been observed in one koala.

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Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*)

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Conservation and management of rhinoceros species relies heavily on the ability to safely immobilize these large pachyderms. However, white rhinoceros immobilized with potent opioids experience adverse physiological changes including severe hypoxaemia, hypercapnia and acidosis. We evaluated the efficacy of butorphanol and oxygen in alleviating opioid-induced respiratory depression in boma-managed rhinoceros. Eight sub-adult male white rhinoceros were captured in Kruger National Park and housed in bomas for the duration of the experiment. Each rhinoceros was immobilized on four occasions at two-week intervals such that all animals received the same treatments in a randomized order. The treatments comprised intravenous butorphanol, oxygen insufflation, butorphanol combined with oxygen and a control. Non-invasive cardiorespiratory measurements and arterial blood gas samples were taken at 5 min intervals during the immobilization. Chemical immobilization with etorphine, azaperone and hyaluronidase, as per standard procedure for the white rhinoceros, caused severe hypoxaemia (P_aO₂ = 27 ± 7 mmHg), hypercapnia (P_aCO₂ = 82 ± 6 mmHg) and acidosis (pH = 7.26 ± 0.02) 5 min after initial recumbency. Compared to pre-intervention values, butorphanol administration (without oxygen) improved the P_aO₂ (60 ± 3 mmHg, *p* < 0.001), P_aCO₂ (67 ± 4 mmHg, *p* < 0.001) and pH (7.31 ± 0.06, *p* < 0.001), while oxygen insufflation alone exacerbated the hypercapnia (123 ± 20 mmHg, *p* < 0.001) and acidosis (7.12 ± 0.07, *p* < 0.001). Surprisingly, butorphanol combined with oxygen fully corrected the opioid-induced hypoxaemia (P_aO₂ = 155 ± 53 mmHg) and reduced the hypercapnia compared to the control trial. Oxygen insufflation combined with an intravenous dose of butorphanol was the only treatment that significantly improved the immobilization quality of boma-managed white rhinoceros by fully correcting the opioid-induced hypoxaemia. This supportive treatment (butorphanol+oxygen) should therefore be used during all white rhinoceros immobilizations to reduce the risk of capture-related morbidity and mortality.

Hookworm disease in South American fur seal (*Arctocephalus australis*) pups at Guafo Island, Chilean Patagonia

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Hookworms are highly pathogenic nematodes that parasitize a wide range of mammals, including several species of pinnipeds. At Guafo Island, in the Chilean Patagonia, this parasite reaches almost 100% of prevalence and causes mortality in South American fur seal pups. The objective of this study was to evaluate the effects of hookworm

(*Uncinaria* sp.) infection on the host and the population by experimental deworming and periodical health assessments. During the 2013, 2014 and 2015 austral summers, we treated a randomly selected subset of hookworm-infected pups (n=120) with Ivermectin, and compared this treated group to pups with different intensity of infection based on fecal analysis (n=220). Additionally, we performed necropsies on 38 hookworm infected and 20 hookworm free (control) animals. Pups with severe hookworm infection (n=90) had marked declines in growth rate, erythrocytes, packed cell volume, hemoglobin, and total protein over the first two months of the infection (ANOVA, all p values < 0.025). In the 2015 season, pups with higher number of hookworms (n=42) had moderate leukocytosis during the first two weeks of infection but they were leukopenic two weeks later. Ten of these pups died due to hookworm enteritis and bacteremia during the study period. At necropsy these pups had over 600 hookworms in the intestine with numerous parasites deeply embedded in the mucosa and sometimes free in the peritoneal cavity. At histopathology these pups had decreased numbers of T-lymphocytes and MHCII positive leukocytes in the small intestine when compared to controls (Student T test, p ≤ 0.002). Hookworm infection is a major disease in this population significantly affecting the growth and health status of up to a third of the population. The failure of severely infected pups to sustain an inflammatory response apparently facilitates the presentation of hookworm peritoneal penetration and sepsis, leading to pup death.

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Industry, fluoride and marsupials: comparative pathology, spatial epidemiology and bioindicators

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Publish consent withheld

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Variations in plasma lead concentrations in urban North Island Kaka (*Nestor meridionalis septentrionalis*) chicks suggests multiple pathways of exposure

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Lead toxicity adversely affects health and reproduction in birds and is a recognised concern to wildlife globally. Lead is naturally present in the environment, however its extensive use in society increases the risks of toxicity to wildlife, in particular wildlife in close proximity to urban areas. In recent years there has been an increase in the prevalence of lead toxicity in North Island Kaka (*Nestor meridionalis septentrionalis*), an endemic New Zealand parrot. The inquisitive nature of these birds, an expanding urban population and increased availability of food in the urban environment are the primary factors implicated in their dispersal into urban areas where there is increased exposure to anthropogenic sources of lead. We studied two populations of North Island Kaka, urban and rural, and investigated the prevalence and biological effects of lead exposure in both chicks and adults. We examined concentrations of lead in blood samples from live birds and tissue concentrations of lead in deceased birds. Lead levels in egg shells and yolk samples were investigated to identify the pathways of exposure in chicks. Preliminary results indicate a high prevalence of lead exposure amongst nestling kaka in an urban environment, that showed no detectable neurological deficits associated with early lead exposure. Early exposure of a proportion of chicks suggests a maternal transfer of lead, although the exact pathway is yet to be investigated. The pattern of lead exposure in other chicks is indicative of direct parental feeding of lead. This study presents evidence that suggests multiple pathways of lead exposure in kaka nestlings and examines the prevalence and biological effects of lead toxicity in kaka. We have detected significant concentrations of lead in both urban and rural populations of kaka in New Zealand that has the potential to contribute significantly to the morbidity and mortality of this species.

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Toxicant concentrations in Australian sea lion, *Neophoca cinerea*, pups as a proxy for maternal concentrations

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The Australian sea lion, *Neophoca cinerea*, occupies an upper trophic level within the marine ecosystem and as such, are a useful indicator species of trace element and heavy metal (toxicant) concentrations. Anthropogenic sources of toxicants when at high concentrations can be associated with increased susceptibility to disease and mortality and reduced reproductive success. Several toxicants, including Hg, Se, As, and Pb, are known to transfer from mother to foetus through placental transfer, and via lactation. As such, toxicant concentrations in pups can be used as a proxy for maternal levels. *N. cinerea* is listed as endangered (IUCN Red List, 2008) and a specific objective of the Recovery Plan for the species is to investigate and mitigate potential threats, including pollutant

exposure. As such, monitoring of toxicant concentrations in pups is important for the conservation and management of *N. cinerea*.

Inductively coupled plasma mass spectroscopy was used to determine the concentration of 13 elements, Hg, Pb, As, Se, Cd, Mg, Al, Co, Ni, Cu, Zn, Cr, and Fe, in liver and hair samples collected from *N. cinerea* pups sampled at Seal Bay and Dangerous Reef, South Australia from 2006 to 2013. Whilst the mean concentration of the majority of elements analysed were within expected reference values for pinnipeds, the concentration of mercury (Hg) in both liver (mean = 28.9 µg/g drywt; n=39) and hair (mean =5.4 µg/g drywt; n = 199) are amongst some of the highest reported in pinnipeds.

In addition to presenting reference intervals for 13 elements in pup tissues, we report the effect of colony site, live versus dead pup status, year of sampling, and pup sex on these concentrations. The suitability of hair as a non-invasive sample for monitoring trace elements and heavy metals in the endangered Australian sea lion will also be discussed.

Seroprevalence and risk factors for zoonotic transmission of *Baylisascaris procyonis* (raccoon roundworm) in wildlife rehabilitators from the United States and Canada.

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Baylisascaris procyonis, the raccoon roundworm, is a zoonotic parasite and a cause of severe neurologic disease in >130 wildlife species. Nearly all diagnosed human baylisascariasis cases have been fatal or resulted in severe, permanent neurologic complications. Most of these infections were in children who likely ingested large numbers of infectious eggs. We hypothesized that healthy adult at-risk individuals may develop asymptomatic or sub-clinical infections resulting from accidental ingestion of low numbers of eggs, and that wildlife rehabilitators may be an at-risk population due to frequent contact with raccoons and/or their feces. We collected serum samples from 273 wildlife rehabilitators from 33 states and 3 Canadian provinces and administered a questionnaire to assess potential risk factors. Samples were tested for *B. procyonis*-specific antibodies using a recombinant RAG-1 antigen immunoblot. Thus far, 19 participants (7%) were positive for antibodies to *B. procyonis*, of which 13 (68%) had actively rehabilitated raccoons in the past year. All 19 positive individuals conducted rehabilitation in areas where *B. procyonis* is present or suspected to be present in raccoons (i.e., 12 U.S. states and one Canadian province), and all reported at least some history of raccoon contact. Use of PPE was variable, but most participants reported frequent use of gloves and hand-washing, and the majority (81%) reported regular anthelmintic treatment of raccoons. In summary, antibodies to *B. procyonis* were detected in healthy adult wildlife rehabilitators indicating the occurrence of sub-clinical baylisascariasis. Currently, we are administering a questionnaire to wildlife rehabilitators to assess knowledge of *B. procyonis* and use of PPE to better understand the educational needs of this community.

Evidence for the transmission of *Streptococcus pneumoniae* from humans to wild habituated great apes

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Wild great apes habituated to human presence for research or tourism are susceptible to human respiratory viruses (Köndgen et al. 2008&2010, Palacios et al. 2011). While viral infections pave the way for secondary bacterial infections, the bacterial infection rather than the virus itself determines disease severity and outcome (Chi et al. 2007). In all reported cases of great apes dying from respiratory disease with the involvement of a human respiratory virus, *Streptococcus pneumoniae* was also present in lung tissues. With secondary infections potentially playing a pivotal role in the progression of disease, it remains unclear whether the relevant bacteria occur naturally in wild great ape populations or also stem from humans entering great ape habitats. Previously found *S. pneumoniae* strains were shown to be distinct from known human strains (Chi et al. 2007), yet it remains possible that simultaneous transmission of viruses and bacteria occur. The objective of this study was to characterize *S. pneumoniae* found in the lungs of deceased wild great apes that died from respiratory disease and compare them to known human strains to determine whether transmission occurs.

Lung tissue of three wild great apes undergoing habituation was tested for common human respiratory pathogens. Respiratory syncytial virus (RSV) and *S. pneumoniae* were detected; phylogenetic analysis revealed that RSV clustered firmly within known human strains. *S. pneumoniae* was characterized by multi locus sequence typing (MLST) using virtual clones. The allelic profile identified was identical with known human strains. Our results suggest that not only human respiratory viruses but also bacteria are being transmitted from humans to wild habituated great apes. This provides important information for the improvement of measures aimed at preventing disease transmission during habituation to protect these endangered animals.

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Prevalence of the zoonotic pathogenic bacteria, *Salmonella* and *Campylobacter*, in translocated populations of an endemic New Zealand reptile, the tuatara (*Sphenodon punctatus*)

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Tuatara (*Sphenodon punctatus*) are New Zealand endemic, cold-adapted reptiles and the sole extant representatives of the order Rhynchocephalia. Once widespread throughout New Zealand, the introduction of mammalian predators resulted in their extirpation from the mainland and restriction to predator-free offshore islands. Translocations to reintroduce and extend the range of tuatara have been essential to their conservation. In October 2012, several unprecedented large-scale translocations moved 220 adult tuatara from Stephen's Island in New Zealand's Cook Strait to four North Island sanctuaries. It is unknown how movement outside of their ecological region and associated changes in climate might affect these animals and their susceptibility to potentially harmful pathogens. In the face of rising global temperatures understanding the impact of a shift in climate on the health of a cold-adapted reptile is a high conservation priority.

Salmonella and *Campylobacter* are enteric, zoonotic bacteria that can cause illness in reptiles. Despite presence in other fauna and the environment, previous attempts to detect *Salmonella* in tuatara have been negative, suggesting that they may be innately resistant or that their low body temperatures may not support bacterial proliferation. Similarly, *Campylobacter* prevalence was equivocal. To investigate prevalence, cloacal swabs were taken from tuatara at study sites. Analyses involved selective culturing, serotyping and PCR. As a result, this research has identified *Salmonella saintpaul* in a translocated tuatara, indicating that this species is able to carry this bacteria. Preliminary *Campylobacter* results suggest that prevalence may range between 57% and 100% of sampled individuals, suggesting that *Campylobacter* could be considered commensal in tuatara. A final set of samples is currently being obtained for analysis in Autumn 2015. This work provides critical information to inform the conservation of a species of evolutionary and cultural significance, as well as testing for shifts in a reptilian bacterial community under increased environmental temperature.

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A single haplotype of *Haemoproteus* is widespread in white ibis (*Eudocimus albus*) from urban sites in southern Florida

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Urban development in Florida has major implications for wetland dependent birds. Habitat quality and use changes can alter the exposure of animals to pathogens. White ibis (*Eudocimus albus*) have become increasingly urbanized with many now relying heavily on urban and suburban habitats. Avian haemosporidia parasites can cause acute disease and reduced fitness. Because southern Florida is subtropical with a high diversity of vectors, we hypothesized that there will be a high prevalence and genetic diversity of haemosporidia in white ibis and differences would exist between urban and rural birds. Blood samples from white ibis from Palm Beach (n=263), Lee (n=18), and Broward (n=18) Counties in southern Florida were tested for hemoparasites by analysis of Giemsa-stained thin blood smears and PCR. In Palm Beach (n=11 sites), Lee, and Broward (natural sites in Everglades) Counties, 68%, 61%,

and 27% were positive, respectively. Sequences of 139 positives from urban and rural sites revealed a novel genetic haplotype of *Haemoproteus*. Morphologically, parasites were identified as *H. plataleae*. Parasitemias of 66 positive birds were very low (average 0.085%, range <0.001%-0.890%). No *Plasmodium* infections were detected in any white ibis despite a recent report of a *Plasmodium* sp. in a white ibis in Palm Beach and sympatric birds from Lee and Broward Counties having *Haemoproteus* and *Plasmodium* infections. Additional research is needed to determine if this *Haemoproteus* species has subclinical effects on ibis health and if additional *Haemoproteus* haplotypes or *Plasmodium* infect white ibis more commonly elsewhere in the southeastern US.

Enteropathogens in house sparrows along an urban gradient in Belgium

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Over the last decades major declines in urban house sparrow populations have been observed in north-western European cities, whereas suburban and rural house sparrow populations have remained rather stable or are recovering from previous declines. Pathogens that have the potential to cause large die-offs in passerines, such as *Salmonella enterica* subspecies *enterica* serotype Typhimurium and *Yersinia pseudotuberculosis*, have been suggested as jointly responsible for these declines. However, not much is known about the actual effect of urbanization on the prevalence of these bacteria in wild birds. From September 2013 until March 2014, 389 faecal samples were collected from apparently healthy house sparrows captured in 9 urban, 9 suburban and 18 rural populations across Flanders, Belgium. All of which were investigated for the presence of *Salmonella* Typhimurium and 342 were examined for the presence of *Y. pseudotuberculosis*. During the same period, full necropsy was performed on deceased house sparrows, received from Bird Rescue Centers, to determine the cause of death. *Salmonella* spp. were not isolated from the samples obtained during fieldwork, but 57% of the faecal samples carried *Yersinia* spp.. Four percent of these *Yersinia* spp. belonged to the pathogenic species *Y. pseudotuberculosis*. A pigeon specific *Salmonella* Typhimurium var. Copenhagen phage type DT99 and a *Salmonella* Typhimurium var. Copenhagen DT195 were isolated from granulomatous lesions present in the brains of two deceased house sparrows. These results suggest that *Salmonella* prevalence in Flemish house sparrows is very low, but if infections occur, these may result in death. On the contrary, *Y. pseudotuberculosis* is widely present in healthy house sparrows. The low prevalence of these infections renders a link with ongoing population declines in the urban regions of Belgium unlikely.

Molecular investigation and antemortem diagnosis of cardiovascular flukes (Digenea: Spirorchidae) in marine turtles

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Spirorchid flukes inhabit the cardiovascular systems of endangered marine turtles, affecting multiple organ systems and contributing to strandings and mortalities worldwide^{1,2}. Few studies have explored spirorchid species assemblages, host-parasite relationships and species-specific pathogenicity. Spirorchid eggs, rather than adults, are probably the cause of the most significant pathology^{1,2}. Morphological identification of eggs beyond genus level is generally not possible due to their morphological similarity, hence it is difficult to attribute pathogenic effects to individual species. Additionally, antemortem diagnosis using traditional methods (eg. faecal flotation) is unreliable. This study aims to expand the currently limited genetic database for spirorchids, develop a means for identifying mixed spirorchid eggs in tissues, and investigate the relative pathogenicity of individual species. This will lead to the development of a targeted antemortem diagnostic test for investigation, surveillance and control purposes. Deceased turtles from the central/south coast of Queensland, Australia were obtained from government or rehabilitation organisations and examined for spirorchid infection. Morphological and molecular methods were used to identify adult flukes collected and build a catalogue of spirorchid species present in the region. DNA sequences obtained were used to develop a multiplex PCR with genus specific primers, used in conjunction with restriction fragment length polymorphism (RFLP) and capillary electrophoresis to differentiate between species. Initial results have identified nine species in this area, with potential cryptic speciation occurring in populations from different ocean regions (ie. Pacific vs. Atlantic Oceans)³. Phylogenetic evidence also suggests that the validity of the genus *Learedius* should be reviewed³. The new genetic tools now allow investigation of the relative pathogenicity of individual spirorchid species, and development of a blood based real-time PCR for the diagnosis and surveillance of pathogenic spirorchids. With these tools, we ultimately hope to aid the rehabilitation and conservation of Queensland marine turtles through improved understanding and treatment of spirorchid infection.

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Prevalence and genotyping studies of *Chlamydia pecorum* in Victorian koalas

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Koalas in Victoria, Australia were re-established predominantly from an island colony after the mainland population collapsed in the early 1900s. The potential for a genetic bottleneck exists in these animals, potentially increasing their risk of experiencing disease. *Chlamydia pecorum* is a significant pathogen in koalas and is known to be present in Victorian koala populations. Although *C. pecorum* has been relatively well studied in vulnerable northern koala populations, research devoted to understanding this disease in Victorian populations has been limited. Recent studies utilising molecular techniques have successfully genotyped *C. pecorum* samples collected from koalas in New South Wales and Queensland, but only four samples from Victorian koalas have been genotyped, and the prevalence and clinical importance of *C. pecorum* in Victoria is poorly understood.

To address this we determined the prevalence of *C. pecorum* in koalas (n = 559) across six different regions in Victoria using qPCR targeting the 16S region. The overall prevalence of *Chlamydia* infection was 22%, although this varied between geographical regions. To facilitate genotype comparisons with northern populations, we sequenced the *ompA* gene of the *C. pecorum* samples (n = 100). The predominant *C. pecorum* in our samples was genotype B, occurring in 90% of cases in our study. This genotype has yet to be detected in northern koalas, but has been previously detected in South Australia. This data, along with clinical data, and data relating to bacterial load (determined by calculating *C. pecorum* genome copy numbers), is now being used to better understand the clinical significance of *C. pecorum* infection in Victorian koalas.

This research provides crucial information regarding prevalence and genotype of *C. pecorum* within Victorian koala populations, and will open up further avenues for comparisons between Victorian koalas and koalas in Queensland and New South Wales.

Severe transmissible eye infection in reindeer: bacterium or virus?

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Infectious keratoconjunctivitis (IKC) in semi-domesticated reindeer (*Rangifer tarandus tarandus*) is considered to be a multifactorial disease, which can affect tens of animals during an outbreak, and lead to the destruction of the eye and blindness. Serum and conjunctival swab samples from 321 healthy and diseased (IKC) Scandinavian reindeer have been analyzed by bacteriological cultivation, PCR and ELISA, revealing the presence of *Moraxella* sp., among other bacteria and Cervid herpesvirus 2 (CvHV2) and antibodies against CvHV2.

To determine the primary agent of a disease in a natural environment can be difficult due to uncontrolled factors. An experimental infection was performed in order to conclude if CvHV2 or *Moraxella bovoculi*, alone or in combination, are determining factors for the development of IKC in reindeer. Twentyone healthy reindeer (seronegative for CvHV2 and culture negative for *M. bovoculi*) were selected for the experiment. Reindeer were inoculated with *M. bovoculi* (group 1, n=5), *M. bovoculi* and CvHV2 (group 2, n=5), CvHV2 (group 3, n=5) and physiological salt water (controls, group 4, n=3).

Groups 1 and 4 showed no signs of IKC, while all animals in groups 2 and 3 developed intense lacrimation, swollen eyelids, corneal oedema and, in two cases, corneal ulcer. Specific antibodies against CvHV2 were detected by ELISA 6 days after inoculation. Few colonies of *M. bovoculi* and other microorganisms were detected along the experiment, which may suggest the possibility of secondary bacterial infections if the disease was allowed to develop beyond the defined experiment end-point.

Until further analysis of the data and samples produced by this study, it can be concluded that CvHV2 is a primary agent of IKC in reindeer, whereas *M. bovoculi* did not seem to be involved as a determinant agent, even if the bacterium has been associated with severe stages of IKC in reindeer during outbreaks.

Genomic analysis demonstrates continuing evolution, recombination and environmental sources of enteric bacteria in isolated wildlife populations

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The process of translocation of threatened wildlife may have unforeseen consequences on pathogen transmission and evolution in host populations. Our understanding of some of the epidemiological features of infectious disease in vulnerable populations can be enhanced by the use of genomic sequencing of microbes in natural ecosystems. The genomic epidemiology of a prevalent rail-associated endemic bacterium, *Campylobacter* sp. *nova* 1, and a host-generalist bacterium, *Salmonella* spp., was explored in a well-described population of a New Zealand endangered flightless bird, the takahe (*Porphyrio hochstetteri*). The distinctive population structure of translocated takahe provides a unique opportunity to investigate the influence of host isolation on enteric microbial diversity. Whole genome sequencing, ribosomal multi-locus sequence typing (rMLST) and CRISPR analysis was performed on 70 *C. sp. nova* 1 isolated from multiple takahe populations. *C. sp. nova* 1 was genomically diverse and multivariate analysis of 52 rMLST alleles revealed location-associated differentiation of *C. sp. nova* 1 sequence types. Possible explanations for the observed pattern include; the spatial expansion and isolation of hosts resulting in reduced gene flow of *Campylobacter* spp. and allopatric speciation, the presence of heterogeneous environmental attributes influencing sequence type carriage or cross-species transmission of *Campylobacter* spp. from sympatric reservoir hosts. The same methods were used to analyse *Salmonella* spp. isolated from a single island population of takahe and their environment. *Salmonella* spp. isolated from takahe, their environment and a reptile showed identical rMLST profiles suggesting a shared source or transmission within the location not present in other populations. This study suggests subtle but important differences in host-microbe relationships may occur as a consequence of conservation management which has important implications when relocating wildlife populations.

Mortality and morbidity of infant mountain gorillas (*Gorilla beringei beringei*): A 46-year retrospective analysis

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Long-term studies of morbidity and mortality in free-ranging primates are scarce, but may have important implications for the conservation of extant populations. Infants form a particularly important age group, because infant mortality can have significant effects on the demographics of slow-growing populations. Necropsies of mountain gorillas (*Gorilla beringei beringei*) have been conducted since 1968, and a comprehensive health-monitoring programme has been in place since 1986. For this study, gross post-mortem [n=70] and histopathology [n=53] reports for mountain gorillas under the age of 3.5 years (infants) in the Virunga massif were collated and reviewed, with the aim of providing the first comprehensive analysis of infant mortality and the histological evidence underlying morbidity in this species. Causes of morbidity and mortality were described, and compared by age, sex and over time. Trauma was the most common cause of death in infants (46%), followed by respiratory infections and aspiration (11%). Gastrointestinal parasitism (33%), altered lymphoid tissues (suggestive of viral infection on histology) (31%) and hepatic capillariasis (25%) were the most significant causes of morbidity. We also identified histological evidence for several currently un-described causes of morbidity in infant apes, including left atrial appendage inversion, fungal pneumonia resembling adiosporomycosis, and renal tubular dysplasia. This study identified trauma, both natural (e.g. infanticide) and anthropogenic (e.g. snare entrapment), to be the most common cause of death in infant mountain gorillas. Anthropogenic factors are also likely important in deaths due to respiratory disease. Identifying the causes of mortality and morbidity in infants of this critically endangered species will help to inform policy aimed at their protection and guide *ante-* and *post-mortem* health monitoring in the future.

Sarcoptic mange in San Joaquin kit fox: a spatial stochastic model to evaluate control strategies.

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The San Joaquin kit fox (SJKF) (*Vulpes macrotis macrotis*) is a federally endangered subspecies of kit fox, endemic to California's southern San Joaquin Valley (SJV). There are now 3 main and a dozen satellite populations remaining after vast habitat destruction. Remarkably, one until-recently stable satellite population is located in the city of Bakersfield, at the southern end of the SJV.

In March 2013 we began to detect fatal cases of sarcoptic mange (SM) in this urban population, the first report of *Sarcoptes scabiei* in this well-studied species. This ongoing outbreak is a threat for SJKF conservation, as SM morbidity and mortality is high in this naive subspecies (21 SM cases this city, including 12 fatalities and 7 rehabilitated) and SM may expand to surrounding rural areas. SM outbreaks in related naive urban foxes have resulted in over 90% population declines in Europe.

In order to evaluate management strategies to control the current SM outbreak in Bakersfield, we built a spatial stochastic model that iteratively simulated the SJKF population and its annual cycle in this city. We randomly introduced SM in this population and allowed transmission within and among family groups. Population distribution and demography, annual cycling, and disease dynamics were informed by appropriate probability distributions from previous research. By modelling control strategies, we tested the hypothesis that their efficiency depend on the spatial scale and duration of their application, but also on the season of SJKF annual cycle. The objective is to identify the most efficient effort level and time frame to apply control measures in order to reduce/eliminate SM in SJKF in Bakersfield. Results are currently under development and they will support federal and state agencies to manage this disease outbreak in Bakersfield and more broadly.

Agent-based modeling of respiratory disease transmission in chimpanzees to quantitatively evaluate the performance of syndromic surveillance

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Syndromic surveillance, or surveillance of general disease "types", to study disease trends in wildlife is novel. Accordingly, associated methodologies for outbreak detection and assessment of surveillance system performance are highly needed. Since 2004, syndromic surveillance has been employed in Gombe National Park, Tanzania, to collect data on several major disease syndromes affecting free-living chimpanzees. Our team utilized 9 years of syndromic data for a qualitative assessment of system performance and development of algorithms for respiratory disease outbreak detection. Here we describe the continuation of that effort with a quantitative assessment of surveillance sensitivity, or the probability that a respiratory disease outbreak is detected by the system in place. To do this, empirical data on community demographics, social contacts and frequencies of observation by surveillance were integrated with an agent-based, network disease model to simulate surveillance of respiratory outbreaks previously observed. Out of 2000 Monte Carlo simulations of disease introduction, 1064 outbreaks were produced, with mean duration of 5 weeks and mean cumulative incidence of 36 cases in a community of 60 individuals. Two algorithms of weekly outbreak detection were examined, one producing an outbreak signal when 2 or more cases were detected, and the other signaling when case prevalence exceeded 15.6% of those observed. Surveillance sensitivity was estimated as 66% (95% Confidence Interval: 63.1, 68.8%) and 59.5% (95% Confidence Interval: 56.5%, 62.4%) for weekly count and prevalence thresholds, respectively. In addition to differences between detection algorithms, differences were also observed in surveillance sensitivity between quarters of the year. Overall, disease model simulations revealed important temporal differences in outbreak characteristics, which are likely impacting surveillance system performance. Through this work, we were able to identify the best algorithm for respiratory outbreak detection and key strategies to improve syndromic surveillance performance in a free-living chimpanzee population.

Modelling transmission dynamics of a novel *Alphacoronavirus* in an Australian population of large-footed myotis (*Myotis macropus*)

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Severe acute respiratory syndrome (SARS) emerged in 2002 in southern China and Middle East respiratory syndrome (MERS) was detected in September 2012 in southwest Asia have underscored the potential of coronavirus to become emerging infectious diseases. Subsequently, the findings that the SARS coronavirus (SARS-CoV) and the MERS coronavirus (MERS-CoV) were originated from bats emphasized the importance of bats as reservoir hosts of emerging infectious diseases. Previously, mark-recapture data of a novel Australian bat *Alphacoronavirus* in *Myotis macropus* identified that this coronavirus was maintained in the population by persistent infected bats. We have taken one step further by utilizing more statistically intense methods of analyses in order to confirm the effects of persistent infection on the coronavirus maintenance in the population of bats.

We additionally analysed the data by using 'MARK' and 'OpenBUGS' to estimate parameters, which subsequently were used to build compartment model, in which the effects of persistent infections against transient infection was estimated to see how it functions in maintenance of coronavirus.

The mark-recapture analysis found no evidence that sex and age significantly affect the survival, recapture and transition rates between infection states. Infection was shown to make survival rate decreased slightly, and to make recapture rate increased. Modelling results suggested that the role of persistent infections is not quite dominant but certainly has an effect on the maintenance of coronavirus in the bat population.

This study identifies potential effects of persistent infections in coronavirus transmission dynamics, and adds weight to the suggestion that these mechanisms may be an important coronavirus maintenance mechanism in bat populations, and, by extension, may be applicable to other bat RNA virus ecology.

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Modeling chronic wasting disease-driven natural selection of free-ranging mule deer (*Odocoileus hemionus*) in southeastern Wyoming

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Chronic wasting disease (CWD), a transmissible spongiform encephalopathy of cervids, reached record high prevalence in southeastern Wyoming in 2011 with 57% of harvested mule deer (*Odocoileus hemionus*) CWD positive. The significance of high CWD prevalence and its impact on free-ranging cervid populations is largely unknown. Previously, CWD susceptibility in mule deer was linked to prion protein (PRNP) genotype at codon 225 with replacement of serine with phenylalanine. Our study investigated the relationship between the population decline and high CWD prevalence as well as the importance of disease-driven natural selection in shaping future population trends. We hypothesized that incorporation of genetic-specific CWD effects in an epidemiologic stochastic spreadsheet model would prevent local extinction of mule deer. Our objectives for this study were to 1) use life-history data collected from radio-collared deer to parameterize models that predicted future population trends within a 100-year period, 2) characterize the impact of CWD on free-ranging mule deer populations, and 3) quantify changes in parameter estimations and their influence on modeled population trends. Our genotype-specific model predicted a 96% reduction in the population with stabilization at a median size of <300 deer by year 100. In contrast, deer were extirpated within 50 years in the absence of genotype-specific incidence and mortality rates. This disparity was due to a 99% decrease and 34% increase in homozygous serine and phenylalanine genotype frequencies, respectively in our genotype-specific model. Our disease-free model predicted stable population growth rates throughout the modeled 100 years. Our results demonstrate the importance of incorporating genetic-specific CWD mortality rates when modeling population trends in free-ranging cervids. Though the negative impacts of CWD are clear despite disease-driven natural selection favoring less susceptible genotypes, persistence of mule deer in our models generates optimism that managers may have time, albeit limited, to find solutions to this epidemic.

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Participatory epidemiology and Indigenous knowledge about muskoxen and caribou in the Canadian Arctic: preliminary insights for wildlife health surveillance.

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Muskoxen and caribou are two important species for ecosystem health in the Arctic. They are also an essential source of food for Arctic people and central to community well-being, cultural heritage and identity. Recent evidence suggests that muskox population have declined around the community of Ikaluktutiak on Victoria Island (Nunavut, Canada); moreover, mid-summer die-offs of muskoxen have been reported on both Bank Island and Victoria Islands (Northwest Territories and Nunavut, Canada). To gather baseline information on the health status of wild muskoxen,

we designed a project in the community of Ikaluktutiak combining qualitative and quantitative research methods. Early on during the research we documented people observations and concerns also about caribou in the same area. We therefore implemented the project to gather baseline information on both wild ungulate species. Participatory methods, originally developed in pastoral communities of Africa, were adapted to our context to gather Inuit ethno-veterinary knowledge about the wildlife that the local community depends on for subsistence. During summer 2014, individuals (N=30) were interviewed in the community of Ikaluktutiak to compile baseline information on local muskox and caribou abundance, distribution, health and changes over time. These data were then used to design the small-group interviews (N=7) that were conducted in winter 2014 in the same community. We used participatory exercises and proportional piling techniques to document the perceived population declines, changes in body condition status, relative prevalence of diseases, and observations of endemic and emergent diseases within the studied wildlife populations. Here we discuss the importance of combined participatory methods in the context of wildlife health surveillance. Methodological insights are presented for implementing wildlife health surveillance systems using the best available knowledge and in communities with limited resources.

A burning question – how does fire impact wildlife behavior and parasitism?

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Fire can dramatically impact wildlife populations, including extensive mortality and morbidity.¹⁻⁵ However, secondary effects, such as those on behavior and parasitism, are far less well understood;⁶⁻⁹ furthermore, the interaction of these two – how post-fire changes in movement and behavior affect parasitism – has rarely been studied. This project uses the critically endangered woylie (*Bettongia penicillata*) to investigate the impact of fire on animal movements and interactions, while simultaneously exploring shifts in parasitism. We expect:

- 1) reduced parasitism by environmentally-transmitted parasites (e.g., ticks) due to proportionally higher use for foraging of low-parasite (burnt) areas
- 2) increased parasitism by organisms transmitted via direct contact or asynchronous nest sharing (e.g., fleas, lice), as appropriate nest sites (limited to unburnt areas) become scarcer and sharing increases.

The study site is a fenced reserve in Western Australia, where an intense fire occurred in December 2014. Since the start of 2014, animals have been trapped seasonally, fitted with GPS collars (n=40), and sampled for gastrointestinal, external, and blood-borne parasites. Pre- vs. post-fire comparisons will include: kernel home and nest ranges; habitat use; weighted social networks exploring connections between individuals that reflect different transmission modes (e.g., overlapping home ranges, nest sharing),¹⁰ and parasite prevalences with 95% Jeffrey's confidence intervals. Preliminary pre-fire results indicate variable parasite prevalences, ranging from 0% (0-4.34%) for fleas to 64.86% (48.83-78.67%) for lice. Other results are pending; changes in parasitism and movement patterns in the first five months after the fire will be detailed. Understanding how fire impacts wildlife behavior and parasitism can aid conservationists in determining appropriate fire management and response schemes; it is particularly relevant for the woylie, as parasites have been implicated in the species' dramatic decline and fire is a regular feature of their environment. This study will also demonstrate the utility of social networks in wildlife epidemiology.

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Searching for the reservoir of Reston ebolavirus

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Reston virus was first described in 1989 when it caused fatal infection in monkeys and sub-clinical infection in humans in a primate research facility in Reston, Virginia, USA. The virus 'disappeared' for nearly 20 years until 2008-9 when it was identified in pigs and pig-workers in the Philippines. In 2010, a multi-disciplinary, multi-institutional team assembled by the Philippine government and the Food and Agricultural Organisation of the United Nations sought to identify the origin of the virus in nature.

The co-evolutionary arms-race between rabbits and their viruses in Australia

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European rabbits are an introduced pest species that cause serious damage to the environment and agricultural industries in Australia. Biological control using the two rabbit pathogens myxoma virus (MYXV) and Rabbit Haemorrhagic Disease Virus (RHDV) has not only greatly reduced rabbit numbers and impacts, it has also provided a unique opportunity to study the initial spread and establishment of emerging pathogens and their co-evolution with their host.

Following the release of MYXV in 1950, wild rabbits rapidly developed genetic resistance and less virulent forms of the virus became dominant in the field, to facilitate virus transmission by biting insect vectors. These interactions between MYXV and wild rabbits in Australia have become the text book example for the trade-off hypothesis of virulence evolution.

By contrast, since the release of RHDV in 1995 a different pattern of co-evolution has been occurring. It is only in recent years that some Australian rabbit populations have begun to develop genetic resistance to RHDV, and consequently rabbit numbers are again on the rise. Furthermore, rapid attenuation of RHDV has not been observed in the 16 years since its release. Indeed recent research suggests that there may be selective pressure for RHDV to maintain high levels of virulence in the face of developing genetic resistance in the host. These findings suggest that, similar to MYXV, RHDV virulence may also be selected towards maximum virus transmission, with the key difference that rabbit carcasses, and not the diseased animals, are the likely source of mechanical transmission by insects.

This is an important contribution towards understanding what drives host-pathogen co-evolution in rabbits, a prerequisite for ongoing effective rabbit control in Australia. It also highlights the unique opportunity that Australia's rabbits and their viruses provide as a model system for the study of the evolution of emerging diseases in general, and in particular of mechanisms promoting high levels of virulence.

The Grim Reaper Can Teach us Valuable Lessons About Tropical Marine Ecosystem Health

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Just how does one assess ecosystem health? This has been a contentious topic with much debate in part because there are disagreements on the definitions of ecosystem and health. However, the question is particular topical for coral reefs. Coral reefs are known as the rainforests of the sea. They are among the most diverse ecosystems on earth providing habitat for fish and other invertebrates and income and storm surge protection for coastal communities. Unfortunately, coral reefs face many threats including coastal pollution, bleaching events associated with elevated ocean temperatures, ocean acidification that is impairing the ability of many marine organisms to deposit calcium, and overfishing that is altering trophic structures. Importantly, disease is playing an increasingly important role in decline of coral reefs. Over the last 40 years, we have done a good job documenting decline of corals, but we have few clues as to why these animals are dying or causes of diseases. This is unfortunate because knowing causes of disease allows one to formulate management option to mitigate their effects. Unlike terrestrial animals, investigations of disease and disease pathogenesis in tropical marine ecosystems pose their own particular challenges. A lot of the existing literature is not inspiring and so gives one little to go on for foundational knowledge. One approach to understanding ecosystem health is to look at the health of ecologically functional groups that span trophic levels. Examples will be shown here on the weird and wonderful stuff one finds when adopting this approach using organisms ranging from corals to sea turtles and a few critters in between. In some cases, this information has important management ramifications.

Strategic early warning as a tool to overcome challenges in Canadian coastal ecosystem health

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Canada has the largest coastline of any nation. This coastline, and the species dependent on it, are facing a series of unprecedented changes ranging from regime shifts, acidification, warming, urbanization and industrial development. Legislation and literature leave us with inadequate tools to detect and respond to these complex and interacting threats over the vast uninhabited distances of the Canadian coastline. New methods are required to expand the geographic coverage to produce timely observations of situations and conditions that could be indicative of the need for action to protect coastal wildlife and the services they provide society. This presentation will first discuss the theoretical possibilities of a health intelligence approach to coastal health to overcome some of these challenges and better prepare us to identify and mitigate risks to marine biodiversity in a timely and effective manner. Examples of Canadian Wildlife Health Cooperative initiatives to develop and apply these methods will illustrate the possibility of health intelligence to support an ecosystemic and cumulative effects approach to wildlife health.

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Marine mammals, sea turtles and the deepwater horizon oil spill: An overview of rehabilitation and impact assessment efforts.

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On April 20, 2010, the world watched in horror as the Deepwater Horizon rig exploded in the Gulf of Mexico. While large spills have occurred previously, this response significantly differed from other disasters in many important aspects that complicated the response, putting tens of thousands of birds, 29 protected marine mammal species, and five threatened or endangered sea turtle species at risk.

Oiled wildlife professionals were quickly mobilized to capture and treat recovered animals, with NOAA-NMFS and UC Davis' Oiled Wildlife Care Network helping to lead the effort to treat oiled marine mammals and sea turtles. From the beginning, there were significant challenges associated with planning for and responding to the crisis: responding to, in essence, a new 50-60,000-barrel spill every day; developing operational activities on site due to cetaceans and turtles never being recovered in large numbers during previous spills; and simply the sheer size of the response.

This presentation will provide an overview of the activities of the Marine Mammal/Sea Turtle Group under the Wildlife Branch for the Deepwater Horizon oil spill, including development of primary and secondary care centers for the five state region, completion and revision of animal care protocols, a review of key activities of the Group (including issues related to controlled burns, translocation of sea turtle nests, and at-sea collection of live turtles) and a synopsis of the intake, release and necropsy data. Lastly, recent information related to the potential effects of the spill on these populations will be presented

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Evolution of resistance to chytridiomycosis is associated with a robust early immune response in a wild amphibian

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The fungus, *Batrachochytrium dendrobatidis* (Bd), aetiological agent of the devastating amphibian skin disease chytridiomycosis, is now considered endemic in most climatically suitable regions around the world. The evolution of or assisted selection for host immune resistance to chytridiomycosis may be a promising avenue for ensuring sustainable long-term persistence of Bd-threatened wild amphibians. For such a strategy to succeed it is essential to understand the mechanisms by which such resistance manifests. Here we examined transcriptomic responses of alpine tree frogs (*Litoria verreauxii alpina*) to subclinical Bd infection, comparing long-exposed populations to a naïve population. We performed a blinded, randomized and controlled exposure experiment, collecting skin, liver and spleen tissues at 4, 8 and 14 days post-exposure from 51 frogs for transcriptome assembly and differential gene expression analyses. We analysed our results in conjunction with data on infection intensities and the results of a large clinical survival experiment run concurrently in the same species. We identified a large number of significantly dysregulated transcripts (liver 1043, skin 8165, spleen 1665) in the tissues from subclinically infected individuals versus unexposed negative control frog tissue, including the predominant up-regulation of numerous transcripts associated with the host immune response (liver 132, skin 645, spleen 216). Our comparison between populations highlighted variations in response to subclinical infection associated with long-term population Bd exposure history

and clinical evidence of survival. Individuals from the longest-surviving population demonstrated a larger complement of differentially expressed immune-associated genes in the skin at 4 days post exposure than frogs from the two more susceptible populations, consistent with a robust early innate and adaptive immune response. Our results support the concept of selecting for the evolution of resistance against chytridiomycosis, and suggest that an insufficient early immune response to infection may contribute to the susceptibility of this non-model species to chytridiomycosis.

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The evolution of *Trichomonas* and the pigeons and doves: a unique story in Australasia

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Trichomonosis is amongst the earliest recorded diseases of birds, and *Trichomonas gallinae* is regarded as a significant pathogen in birds of prey and columbids. In recent years the protist has expanded its host range and caused population declines of passerines in Europe and North America.

Australasia is home to more than 40% (134 species) of the world's pigeons and doves (family Columbidae). These have a deep evolutionary history in the region, having evolved in Gondwana more than 65 Mya, and exhibit marked phenotypic and ecological diversity. Of particular ecological significance are the fruit-doves, which are the most effective seed-dispersers in the region's rainforests. In order to examine the potential impact of *T. gallinae* on Australia's columbid fauna, we sampled more than 500 individuals of 25 species of pigeons and doves for *Trichomonas* in Australia and Papua New Guinea.

We found *T. gallinae* in healthy individuals of two introduced species of pigeons, as well as in four wild birds of prey with trichomonosis. By contrast, almost all wild columbid species were infected with novel lineages of *Trichomonas* and were asymptomatic. We discovered six new lineages of *Trichomonas*, more than doubling its previously described genetic diversity. Our findings suggest that *Trichomonas* is deeply co-evolved with columbids in Australasia, but that this organism is adept at host-switching. This may be a salient feature in the recent emergence of trichomonosis in other birds and in a likely spillover from columbids to humans giving rise to *T. vaginalis* (the most common sexually transmitted infection of people) at some point in the past, an event which is suggested by our data.

Potential evolutionary trade-offs for infection with *Trichomonas* in columbids will be discussed, as well as the risk to native columbids and birds of prey from the introduced pathogen *T. gallinae*.

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Have pathogens influenced the evolution of non-carotenoid colouration in dragon lizards?

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Pathogens can drive wildlife evolution in many ways. For example, pathogens can cause severe population bottlenecks, and they have played key roles in speciation events and the evolution of sociality. Pathogens can also drive the evolution of colouration through sexual selection. This phenomenon has been widely studied in carotenoid-based colouration systems but less is known about the roles of the other pigment types present in vertebrates: pteridines and melanins. These pigments differ in how they are acquired and produced; carotenoids are acquired from the diet and pteridines and melanins are endogenously produced from purines and tyrosine, respectively. The link between pathogens and carotenoid-based colouration is well understood, with carotenoid intake determined by foraging ability, which in turn is influenced by pathogen load. In contrast, much less is known about the relationship between pteridine- and melanin-based colouration and pathogens. Conflicting results have been reported with regards to associations between non-carotenoid colouration and pathogens, and the suggestion of a link between pteridine- and melanin-based pigments and the immune system is controversial. We propose to examine whether pathogen load is associated with secondary sexual colouration in the tawny dragon (*Ctenophorus decresii*). The tawny dragon is a small (<30g) agamid that inhabits rocky outcrops and ranges within South Australia and uses pteridine- and melanin-based colour patches in male-male confrontation and mate acquisition displays. We will quantify colouration variation independently of the human visual system using photograph-derived data. Pathogens will be identified, and pathogen load will be determined, using morphological and molecular methods. Little is known about the pathogens infecting tawny dragons. However, preliminary results show individual variation in the abundance of ticks, mites and haemogregarine blood parasites. This research will aid in understanding the role that pathogens play in the evolution of secondary sexual colouration in an agamid pteridine- and melanin-based colour system.

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Cockatoo Hindlimb Paralysis Syndrome (CHiPS): journey towards a diagnosis

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Since 2012, we have been documenting and characterising a syndrome colloquially known as “CHIPS”: Cockatoo Hindlimb Paralysis Syndrome. Affected birds are unable to stand or walk, and rapidly lose condition due to their inability to feed. The syndrome only affects Carnaby’s Cockatoo, which migrates to metropolitan Perth in the summer from breeding grounds in the north. With intensive supportive care, many cases will improve over 7-10 days to full use of their legs. Data collection to characterise this syndrome has become increasingly refined, allowing many common differential diagnoses for paresis and paralysis in birds to be ruled out. However full characterisation of the index case continues to be elusive due to the non-specific nature of the presentation, high incidence of secondary trauma and lack of histopathological changes at post mortem.

We are currently investigating the hypothesis that CHIPS is a result of exposure to organophosphate (OP) pesticides which are widely used on crops in northern breeding areas. Although tissue testing for pesticide residues has been consistently negative, some birds have demonstrated depression of acetylcholinesterase in plasma and brain, indicative of exposure to anti-cholinesterase compounds. The clinical presentation also has intriguing similarities to ‘OP-induced delayed neuropathy’ (OPIDN), a rare human condition in which OP molecules bind to neuropathy target esterase, initiating degeneration of distal axons and resulting in a peripheral, often reversible, neuropathy.

We have recently developed reference ranges for brain acetylcholinesterase and plasma total cholinesterase in Carnaby’s cockatoos, which may hold the key to reliable identification of CHIPS cases if OP pesticides are implicated in the condition.

Key factors in the progress of this investigation are:

- Buildup of a longitudinal database
- Revisiting differential diagnoses in the light of additional data and analysis
- Systematic testing of likely hypotheses
- Cooperative data collection and disease screening involving a number of stakeholders

Experimental inoculation of Egyptian rousette bats (*Rousettus aegyptiacus*) with five different ebolaviruses, and comparison with Marburg virus

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Ebolaviruses and marburgviruses (*Filoviridae*) cause sporadic outbreaks of hemorrhagic fever in humans and non-human primates, with case fatality rates up to 90%. The Egyptian rousette bat (*Rousettus aegyptiacus*) has been identified as a natural reservoir for marburgviruses and a source of virus spillover to humans. Cumulative evidence suggests various fruit bat species also play a role in the transmission cycle of ebolaviruses. Through a two-part experimental infection study, we investigated the susceptibility of Egyptian rousettes to viruses representing each of the five ebolavirus species (Sudan, Ebola, Bundibugyo, Taï Forest, and Reston viruses), and compared findings with Marburg virus. In a 10-day pilot study, groups of four juvenile, captive-bred bats were inoculated with a low-passage stock of one of the ebolaviruses, or with Marburg virus. There were no mortalities and no significant hematologic or histopathologic abnormalities. In ebolavirus groups, viral RNA distribution in tissues was limited, and no bat became viremic. In the Sudan virus group, viral RNA was more widespread, and liver and spleen were PCR-positive at day 5 post-inoculation. In contrast, Marburg virus RNA was widely disseminated, with evidence of viremia, oral and rectal viral shedding, and antigen in spleen and liver. In a second, 15-day serial euthanasia study, 15 bats were inoculated with Sudan virus. Viral RNA was found in multiple tissues, especially at early time points, but tissue viral loads were low, with no detected viremia or viral shedding. This is the first reported experimental infection study comparing tissue tropism, potential for viral shedding, and clinical and pathologic effects of six different filoviruses in the Egyptian rousette, a known marburgvirus reservoir. Our results suggest that Egyptian rousettes are unlikely sources for ebolaviruses in nature, and lend support to a possible single filovirus – single bat host relationship, analogous to that of hantaviruses in rodent reservoirs.

Ticks, birds and tick-borne diseases: the terrestrial cycle

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The importance of birds as reservoir hosts for tick-borne diseases has been recognised for many decades. Due to the difficulties inherent in studying birds in natural conditions, the appreciation of their role as reservoirs has lagged behind. We are currently studying the ecological processes involved in the interactions between ixodid ticks and European songbirds. Epidemiological parameters were obtained from experiments and long-term observational data (15 years) of the sheep tick (*Ixodes ricinus*), the tree-hole tick (*Ixodes arboricola*) and *Ixodes frontalis*, parasitizing some of Europe’s commonest songbirds, the great tit (*Parus major*) and blackbird (*Turdus merula*). Both songbirds are recognised as a reservoir host for *Borrelia burgdorferi* s.l.. Immature developmental stages of *Ixodes ricinus*, the most important vector of tick-borne zoonotic diseases in Europe, are frequently found on songbirds. *Ixodes arboricola* is a wide-spread nidicolous tick, adapted to a lifestyle inside tree-holes where it infests roosting and breeding birds (e.g. great tits), while *Ixodes frontalis* is an ornithophilic tick that also infests open-nesting birds (e.g.

blackbirds). In addition, these ticks are considered as competent carriers of *Borrelia burgdorferi* s.l. and Tick-borne encephalitis virus, though their ability to transmit those pathogens and therefore maintain it in birds has not been determined yet. We will report on the main factors that explain spatio-temporal infestation risks, defined by ecological risk models making use of detailed vegetation maps in conjunction with micro-climatological variables and the birds' life-history parameters. Furthermore, we will present experimentally obtained data on the virulence of ticks, the birds' resistance mechanisms, as well as the host-mediated transmission mechanisms. Additionally, the possibility of closed enzootic cycles between birds and ornithophilic ticks, with *Ixodes ricinus* as a bridge vector, will be discussed based on experimental data.

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Someone's been sleeping in my bed....the external parasites found in nest boxes provided for brushtail possums in the Strathbogie Ranges, Victoria

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Denning sites provide an important opportunity for the direct and indirect transmission of parasites between host animals. Nonetheless, we know little about the species and numbers of external parasites (ectoparasites) found in the dens of Australian marsupials. This study monitored ectoparasite prevalence and abundance in nest boxes mounted for the use of *Trichosurus* spp. at Boho South, south-eastern Australia over two years. The content of nest boxes was checked for ectoparasites every 5 weeks for the first 12 months, and then every 3 months for the subsequent 12 months, and the identity of mammal species having used the nest box was determined using hair samples. A wide range of ectoparasitic mites, including *Trichosuroaelaps* spp., *Haemolaelaps* spp. and *Ornithonyssus* spp., and ticks, including *Ixodes tasmani* and *I. trichosuri* was detected. The prevalence and abundance of mite species differed from that usually found when recovering them directly from *Trichosurus* spp. This study highlights the importance of including the host animal's environment in assessments of ectoparasite exposure.

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A one health problem: Dissemination of antibiotic resistance determinants to wildlife populations

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Antibiotic resistance has been identified as one of the world's most pressing issues. The spread of antibiotic resistance is commonly attributed to overuse of antibiotics in both human health and animal production. However, we are seeing increasing reports of antibiotic resistance in diverse wildlife species, and in the absence of antibiotic therapy. Antibiotic resistance in wildlife has been reported for both captive and wild populations, and proximity to humans is a significant factor associated with presence of resistance. Knowledge of the genetic basis of antibiotic resistance has shed light on the rapidity of emergence and role of antibiotic use as a selective agent. Class 1 Integrons are naturally occurring mobile genetic elements which facilitate the spread of antibiotic resistance within and between bacterial species. The class 1 integron is primarily associated with human clinical resistance in Gram negative bacteria. By determining the presence of class 1 integrons, and their associated resistance profiles, in wildlife species we are able to investigate penetration of antibiotic resistance into wildlife populations. We have been investigating threatened wildlife hosts of terrestrial and marine origin to determine carriage of antibiotic resistance determinants. In terrestrial environments we have identified class 1 integrons in captive bred rock wallabies that form the primary population of supplementation of wild populations. Similarly, we have found class 1 integrons in the Tasmanian devil insurance population. In marine vertebrates, class 1 integrons have been identified in little penguins inhabiting Sydney Harbour and in endangered Australian sea lions. Our data shows that antibiotic resistance elements are being transferred from terrestrial to marine environments. In captivity, the presence of antibiotic resistance potentially impacts efficacy of treatments for bacterial infections. In the wild, these elements provide an indicator of movement and colonization of bacterial species from human and domestic animal sources.

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Methods to mitigate *Trypanosoma cruzi* transmission while encouraging wildlife presence

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Trypanosoma cruzi is a protozoan parasite that infects more than 100 mammalian species, causes fatal cardiomyopathy in humans and dogs (Chagas disease), and is maintained by numerous wildlife reservoir species. Although humans are most frequently infected in Latin American countries, Chagas disease has become a global concern, with an estimated 300,000 chronically infected persons living in the US, 80,000 in Europe, and 1,500 in Australia. In general, heightened zoonotic disease awareness is often accompanied by fear and may lead to needless killing or removal of wildlife species, threatening biodiversity and disrupting food chains and ecosystem processes. It is therefore important to understand local Chagas disease transmission cycles, including *T. cruzi* presence in wildlife reservoirs and vectors. Our study took place in northern California (CA), where we evaluated reservoir, vector, and environmental components of the local *T. cruzi* cycle. First, we performed mark-

recapture of woodrats (*Neotoma* species) on several private rural residential properties. Blood samples screened via polymerase chain reaction (PCR) assays yielded an overall *T. cruzi* prevalence of 14%, suggesting that woodrats likely serve as *T. cruzi* reservoirs in a previously unstudied northern CA region. Second, we collected *Triatoma protracta* (a *T. cruzi* vector found in the western US and northern Mexico) to assess *T. cruzi* prevalence and identify subtypes known as discrete typing units (DTUs). Only DTU Tc1 was represented, with approximately 55% of the vector specimens testing PCR-positive, highlighting the potential risk for *T. cruzi* transmission to people and domestic animals. Finally, via hierarchical logistic regression we evaluated woodrat microhabitat use with respect to vegetation parameters. Our habitat analyses, in combination with woodrat movement data, provide guidance for habitat modifications to decrease the risk of human exposure to *T. cruzi*, while still allowing for the presence of woodrats and the ecosystem benefits that they offer.

Evidence for inter-epidemic infections of Rift Valley fever virus in wildlife and livestock in the Serengeti ecosystem, Tanzania

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Rift Valley fever (RVF) is a mosquito-borne zoonotic disease which presents in epizootic form over large areas of a country following heavy rains and flooding, and is characterized by high rates of abortion and neonatal mortality, primarily in sheep, goats and cattle; and fatal haemorrhagic syndrome in humans. In Southern and Eastern Africa, RVF occurs at intervals of between 5-12 years and the last outbreak was in 2006/2007. Little is known on where the virus is maintained during the inter-epidemic period; therefore this study was conducted to investigate sero-prevalence of RVF in wild and domestic ruminants, and small mammals during the inter-epidemic period. Serum samples from buffaloes, gazelles, cattle and rodents were tested for specific RVF IgG antibodies using the indirect enzyme-linked immunosorbent assay (I-ELISA) based on the recombinant nucleocapsid protein (rNp) of Rift Valley Fever Virus (RVFV). The results revealed that 31.4% of buffaloes (n=102), 23.1% of Grant's gazelle (n=13), 14.5% of Thomson's gazelle (n=69), 19% of Impala (n=21), 20% of hartebeest (n=20), 6.5% of topi (n=31), 2.3% of wildebeest (n=88), 11.6% of cattle (n=593), 4.5% of goats (n=110) and 3.8% of sheep (n=53) were sero-positive for RVF. It was interesting to note that some sero-positive animals including 7 buffaloes, 20 cattle, 4 goats, 1 sheep, 2 topi, 2 hartebeests, 1 Grant's gazelle and 1 impala were born 1 to 4 years after the end of the last outbreak, indicating occurrence of new infections in the absence of overt clinical signs during the inter-epidemic period. These results indicate presence and circulation of RVFV within the Serengeti ecosystem five years after the last disease outbreak. The implications of the results in relation to the role of wildlife in the ecology of RVFV, surveillance, preparedness and response to RVF are discussed.

Australia's national wildlife disease surveillance system

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Wildlife are increasingly recognised as the source of emerging diseases with potential impact on biodiversity, livestock health/trade and human health. The key to early detection of emerging diseases is surveillance, and an integrated system for reporting and data capture.

Australia has a national system for wildlife disease surveillance co-ordinated by Wildlife Health Australia (WHA), the peak body for wildlife health nationally. The system consists of WHA Coordinators based in government agencies in each State/Territory, surveillance programs based around zoo and sentinel wildlife hospitals, and focus groups (Universities, Zoo Animal Health Reference Group, Bat Health Focus Group). Targeted surveillance programs are in place for significant diseases such as avian influenza in wild birds and Australian bat lyssavirus.

National collation of wildlife disease data occurs in the electronic Wildlife Health Information System (eWHIS), a central web-enabled database. Information is used to identify significant disease events in wildlife, to inform decisions during disease events and outbreaks, for national and international reporting, and to assist decision-making by policy-makers.

WHA is currently scoping the expansion of the national wildlife disease surveillance program to formally integrate contributions from Australian universities. WHA has coordinated a Universities Focus Group since 2008, facilitating coordination and communication within and between universities and government. As a means to continue developments in the area, WHA has recently progressed investigations to quantify the potential wildlife disease surveillance contributions from Australian universities. The initial priority is to assess wildlife disease information generated by university veterinary clinics and/or pathology departments. It is envisaged that this will facilitate WHA's development of a tailored surveillance program to complement and enhance the existing national wildlife disease surveillance capacity.

Other sources of information for future consideration for integration into the national wildlife disease surveillance system include private veterinary practices and wildlife rehabilitation groups.

Australia's wildlife health system: Recent developments

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Wildlife Health Australia Incorporated (Wildlife Health Australia; WHA) is an initiative of the Australian government comprising a network of stakeholders across Australia with an interest in wildlife health. WHA evolved from the Australian Wildlife Health Network (AWHN) as the next step in the development of Australia's wildlife health system.

In 2012, after ten years of operation it seemed timely to review the AWHN in preparation for its next ten years of activity. After consultation with key stakeholders and funding bodies a decision was made by the AWHN Management Group to re-brand and incorporate AWHN as a not-for-profit, separate legal entity under the New South Wales Incorporated Associations Act 2009. The association would be named WHA and would become the peak body for wildlife health in Australia. It would continue managing and growing programs already developed by AWHN, but its objectives and activities would be reviewed and better targeted to suit the needs of the country for its next ten years of operations. The core business activity would remain coordination of wildlife health surveillance information that can be used to improve decision making, management and policy development to protect Australia's trade, human health, livestock health, biodiversity and tourism.

Funding is primarily from the Australian Government Department of Agriculture (DoA) through the Caring for Our Country (CFoC) program, with the understanding that, with other funding, WHA could also become more involved with biodiversity, human health and environmental issues.

This paper notifies the international wildlife community of the formation of WHA and presents WHA's future priorities.

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Geoscape: A new technique to track potential zoonotic disease sources in wild birds

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The small size of birds has made it difficult to track their movements, particularly the smaller passerine groups. This has prevented wildlife disease workers from being able to pinpoint source locations from where diseased birds have originated. When birds produce their feathers, a permanent signature of elements that exist at that location is incorporated into the feather. That signature will be carried in the feather until molted by the bird. By understanding molt patterns, researchers can utilize stable hydrogen and heavy metal isotopes in feathers to more accurately determine locations of feather production. Information from Geoscape can be used in the future to allow wildlife disease workers to more accurately track sources of avian zoonotic disease outbreaks. This information can also provide a benefit of substantiating where concentrated metal deposits occur over the earth's surface. Proof of concept for the "Geoscape" technique will be provided from passerine bird studies conducted across western North America.

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The globalization of parrot viruses as the result of the trade in wild caught parrots and its potential conservation implications

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The global trade in wild caught parrots, although eventually curtailed in many countries by the Convention on the International Trade in Endangered Species (CITES) and legislation in importing countries, resulted in over 4,500,000 wild-caught birds being imported into North America and the European Union (EU) from 1975 to 2011. Unknown numbers of captive bred parrots were also traded during this time and continue to be traded. Quarantine surveillance focussed on poultry pathogens whilst other diseases of parrots were largely ignored. By means of literature review, we identified a minimum of 15 viruses that had been introduced to North America, EU, Japan or Australia through this trade, 12 of which pose potential threats to aviculture, the poultry industry, and/or parrot conservation. We also found that anthropogenic factors, such as mixing of species, housing animals in close contact, and a disregard for basic biosecurity measures played a major role in the disease dissemination. Key features of the viruses that did establish themselves in aviculture were their ability to subclinically and persistently infect many species of parrots. Pressure continues from aviculturalists to allow limited trade in parrots to resume. One argument for allowing the resumption of the international trade of parrots is that diagnostic tests now exist that would allow the selection of pathogen-free parrots. A review of current molecular based diagnostic technology showed that satisfactory tests do not exist for all of these viruses and due to this and on-going emergence and discovery of previously unrecognized viruses, we conclude that a resumption of the global parrot trade would be premature.

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Pandemic (H1N1) 2009 influenza A virus infection associated with respiratory signs in sloth bears (*Melursus ursinus*)

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An 8-week-old hand-reared sloth bear at Smithsonian's National Zoo in Washington DC presented with respiratory signs in February 2014. Nasal swabs were tested for influenza A virus (IAV) by rRT-PCR and virus isolation. IAV viral RNA was detected and subtype H1N1 was isolated. Sequence analysis confirmed pandemic (H1N1) 2009 lineage which has zoonotic potential.

Within one month prior to the onset of respiratory signs in the cub, several of its caretakers developed influenza-like illness and subsequently the juvenile and adult sloth bears housed separately in the same building developed signs of respiratory disease. Human-to-bear transmission of the H1N1 virus, concurrently circulating in the human population, was strongly suspected. Bear-to-bear or bear-to-human transmission could not be confirmed. The cub was treated with supportive care and respiratory signs resolved within two weeks.

Additional nasal swabs and serum from the cub and banked sera from six other collection sloth bears dating back to 2002 were tested. Serologic evidence revealed introduction of H3N2 IAV (also a predominant subtype in humans) into the sloth bear collection prior to this 2014 introduction of H1N1.

The influenza A pandemic (H1N1) 2009 virus has demonstrated infectivity in a broad array of domesticated species as well as black-footed ferrets, skunks, cheetah, giant anteater, badger, binturong and giant pandas. In many of these exotic species, infection was suspected to have been transmitted from human caretakers, suggesting that revised biosecurity measures be considered to protect human and animal health and to minimize opportunities for development of new reservoir species and subsequent emergence of novel strains. This is the first report of infection in sloth bears, listed in Appendix I of CITES and as vulnerable and decreasing by the IUCN primarily due to habitat loss and poaching.

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Wild bird avian influenza in Australia: What have we learnt since 2006?

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Avian influenza viruses (AIVs) are found worldwide in domestic and wild birds, sometimes causing disease in gallinaceous poultry and other species. Surveillance of wild bird reservoirs provides an opportunity to better understand the epidemiology of AIVs. Given Australia's isolation both geographically and ecologically, the epidemiology of AIV is expected to be different from other geographic regions.

In 2006, Australia established the National Avian Influenza Wild Bird Surveillance Program as a collaborative project involving a range of government and non-government organisations. The program allows national coordination of surveillance activities and collation of data to further the understanding of AIV epidemiology in Australia. Surveillance is conducted Australia-wide, and includes i) pathogen-specific, risk-based surveillance via convenience sampling of apparently healthy, live and hunter-killed wild birds, and ii) enhanced passive surveillance via investigation of significant, unexplained morbidity and mortality events in wild birds.

Between July 2005 and June 2014, over 80,000 samples were collected from healthy live or hunter-killed wild birds and over 1700 wild bird morbidity and mortality events were reported.

No highly pathogenic avian influenza (HPAI) were identified, but a wide range of low pathogenic avian influenza (LPAI) subtypes were found in Australian wild birds, including H5 and H7 subtypes. LPAI H5 subtypes were found to be more common and widespread than H7 subtypes, despite all previous HPAI outbreaks in Australia poultry being caused by HPAI H7 subtypes. Phylogenetic analysis has shown Australian AIVs typically form separate sub-clades of the Eurasian avian influenza lineage, with infrequent introductions of North America lineages. The dynamics of Australian wild bird AIVs are complicated and associated more with large scale rainfall patterns than simple seasonal patterns.

The NAIWB surveillance program continues to maintain AIV sampling and diagnostic capability and capacity in Australia and inform risk assessment and decision making that may benefit the Australia poultry industry.

Australian bat coronaviruses

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Coronaviruses were responsible for the global outbreaks of severe acute respiratory syndrome (SARS) in 2003 and 2004, and Middle East respiratory syndrome (MERS) in 2012. Bats have since been identified as the natural hosts for a number of novel coronaviruses, including the likely ancestors to SARS and MERS coronaviruses. It is essential for Australia's biosecurity preparedness, and for the broader understanding of this previously unknown group of viruses, that coronaviruses in bats in our region are identified, characterised and their ecology understood. 2,195 bats collected from Australia and neighbouring countries between 1997 and 2009 were tested for evidence of coronavirus infection. The study identified coronaviruses belonging to two genera (*Alpha-* and *Betacoronavirus*) in Australian bats, and serological evidence of infection in other bats from East Timor, Indonesia, Malaysia and Papua New Guinea. It also identified the interspecies transmission of a variant of the alphacoronavirus *Miniopterus bat coronavirus HKU8* from *Miniopterus spp* to bats of the genus *Rhinolophus*, supporting the hypothesis that bats from this genus are more likely to foster host shifts and pose a risk for the emergence of other bat coronaviruses. A two year longitudinal study of *Miniopterus spp* between 2006 and 2008 was used to collect data and develop a model hypothesis of the infection dynamics of an *Alphacoronavirus*. The findings suggested that bats have an anamnestic (immunological) memory which limits secondary coronavirus infections with a stronger and more rapid production of antibodies, compared to a primary infection. Finally, a modified mark/recapture study on a maternal population of *Myotis macropus* identified that individual bats were infected with a novel unclassified putative *Alphacoronavirus* for up to 11 weeks. The observed pattern of infection supports not only a hypothesis of persistent coronavirus infection in bats, but also suggests that acute infection, and intermittent viral is possible.

Polymerase chain reaction as a diagnostic tool for identification and speciation of avian mycobacteriosis – replacing standard culture with molecular diagnostics

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Avian mycobacteriosis is a significant cause of avian morbidity and mortality, affecting commercial, aviary/pet, zoological and wild birds.¹⁻⁷ Over a period of 7 years, Taronga Zoo had a 9.4% incidence of avian mycobacteriosis in birds based on histopathological and/or microbiological examinations. Although *Mycobacterium avium* was successfully cultured in 30% of histopathology positive cases, 70% were unspciated and suspected cases couldn't be confirmed using culture. This study aimed to identify mycobacterial species in unspciated and suspect cases using molecular diagnostics. It also explored fecal testing as a potential method of ante-mortem detection and examined epidemiological features of avian mycobacteriosis within the collection, including species predilection, environmental risk factors and application of molecular diagnostics for use in wild birds.

Conventional pan-mycobacterial nested-PCR and sequencing identified mycobacterial species in archived tissue samples from cases between 2007-2013. From 80 birds, mycobacterial DNA was identified in 104 samples as *M. genavense* (66), *M. avium* (48) or *M. terrae* (2); 12 samples had dual infection. PCR increased the rate of post-mortem mycobacteriosis detection by 30% and increased the rate of *Mycobacteria* speciation by 331%. Ante-mortem fecal testing using real-time PCR didn't detect *M. genavense* in clinically affected birds, including those later confirmed to have *M. genavense* on tissue PCR. Two clinically healthy birds had *M. genavense* in fecal samples.

In total, 38 bird species were represented, with affected birds displaying a wide range of clinical signs and gross findings. The highest incidence of mycobacteriosis was in Columbiformes (16.1%) suggesting greater susceptibility. Psittaciformes had a low incidence (3.4%), contrasting previous studies suggesting high susceptibility.¹

This study highlighted the need for molecular diagnostics on tissues for avian mycobacteriosis diagnosis and confirmed *M. genavense* as the leading cause of mycobacteriosis in the collection. Further research includes relevance to wild populations and further development of reliable ante-mortem diagnostics.

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Unexpected outcomes following the experimental infection of a phylogroup 2 rabies virus into its natural reservoir host

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Lagos bat virus (LBV) is a phylogroup 2 lyssavirus which causes rabies when it infects the brain of terrestrial mammals¹. Vaccination against classical rabies virus (phylogroup 1) does not protect against LBV as there is little or no serological cross protection across these lyssavirus phylogroups². To understand risk factors for public and domestic animal health, an understanding of viral infection dynamics in reservoir hosts is required³. The straw-coloured fruit bat, *Eidolon helvum*, is a natural reservoir host of LBV¹. Three LBV isolates obtained from wild *E. helvum* were available, including the type virus isolated from a bat from Lagos Island, Nigeria in 1956. Prior to investigating viral infection dynamics in this host, we conducted a pilot study to determine if each of these isolates would cause disease should it reach the brain of *E. helvum*. Each of three groups of captive-bred *E. helvum* known to have never been exposed to phylogroup 1 or 2 lyssaviruses (or any viruses that serologically cross react with them) were experimentally inoculated intracerebrally with one of the LBV isolates. Equal titres of virus were inoculated across all isolates using a standard site and rate of inoculation. A fourth group was mock-infected using virus-free tissue culture fluid. While mock-infected animals remained healthy throughout, each LBV isolate produced a distinct disease phenotype with a clinical duration of less than 24 hours. The incubation period and the pattern of viral spread to the salivary glands also appeared to be isolate-specific.

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Disease and mortality factors in American Crows in Ontario, Canada 2000-2008

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From 2000-2008, over 9500 corvids from Ontario, Canada, including 6897 American Crows (*Corvus brachyrhynchos*), were tested during surveillance for West Nile virus (WNV). A subset of 589 crows, 189 from 2000, prior to the establishment of WNV in 2001, and 400 testing negative for WNV over the period 2001-2008, were examined for gross and microscopic lesions, and tested retrospectively for agents that might explain mortality. Findings were evaluated in relation to age, sex, year, time of year and body condition, and statistical relationships between various pathologic syndromes, including severe lymphoid depletion, and viral, bacterial, mycotic, parasitic and toxic agents were assessed. Integrating history, gross and microscopic findings, and results of ancillary diagnostic tests, final diagnoses (mean = 4.4/bird) were established, and cause of death was inferred, where possible. Of the 125 final diagnostic terms, 106 were considered potentially to have a negative impact on health, or to be of epidemiological or other scientific relevance. A cause of death was identified in 72% of birds. Twenty causes of death other than euthanasia (9%) were recognized, in descending order of frequency: trauma (total 45%), caused by blunt force, electrocution, gunshot, predation; infectious (total 11%), involving unknown or identified bacterial, fungal, and viral agents, alone or in various combinations; toxicity (total 3%), involving Avitrol[®], anticoagulants, cholinesterase inhibitors and botulism; starvation (3%); dehydration (<1%); and heart failure (<1%). Gastrointestinal parasitism (24%), microfilariaemia (19%), and *Leucocytozoon* infection (12%) were common, but not associated with mortality. Compared with the overall prevalence of WNV (23.9% of 5728 crows tested 2002-2008), other infections were uncommon in the study group. Avian pox (2.7%), aspergillosis (2.4%) and sepsis/polyserositis associated with Gram positive and Gram negative bacteria and *Mycoplasma* (4.8%) were most prevalent. An avian reovirus-like agent was associated with fatal necrohemorrhagic enteritis in <1% of crows.

A tale of two colonies: Marburgvirus resurgence in bats in a Ugandan mine following extermination attempts

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Marburg virus (MARV) and Ravn virus (RAVV), collectively called marburgviruses, cause a rapidly progressive, often fatal hemorrhagic fever in humans. Marburgviruses were first isolated from *Rousettus aegyptiacus* bats inhabiting Kitaka mine near Ibanda, Uganda, following a Marburg hemorrhagic fever (MHF) outbreak there in 2007. Following this outbreak, the colony was exterminated by local miners in an attempt to regain access to the mine after it was closed due to the presence of the virus. In 2012, MHF reappeared in Ibanda town in the largest outbreak in Uganda to date, resulting in 15 laboratory-confirmed MHF cases. An ecological investigation revealed Kitaka mine had been re-opened and subsequently repopulated by *R. aegyptiacus* bats. Analysis of bat tissue samples by quantitative reverse transcriptase PCR (qRT-PCR) revealed elevated levels of MARV infections in bats inhabiting the mine after repopulation had occurred. Our findings elucidate that the destruction of a colony of *R. aegyptiacus* is not an effective control strategy. Permanent non-lethal exclusion practices may prove more effective.

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Macacine Herpesvirus 1 (B virus) in Wild-caught Long-Tailed Macaques (*Macaca fascicularis*) Following Capture and Transport in Malaysia

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Macacine herpesvirus 1 (MaHV1; B virus) is a zoonotic virus that naturally infects macaques (*Macaca spp.*) and can cause fatal encephalitis in humans. In Peninsular Malaysia, wild macaques are abundant and translocation is used to mitigate human-macaque conflict. Most adult macaques are infected with MaHV1, though the risk of transmission to wildlife personnel who handle them during capture and translocation is unknown. We investigated MaHV1 shedding in 392 long-tailed macaques (*Macaca fascicularis*) following capture and translocation by the Department of Wildlife and National Parks (DWNP), Peninsular Malaysia. PCR was performed on urogenital and oropharyngeal samples to detect MaHV1 DNA. Overall, 39% of macaques were shedding MaHV1 DNA, with no difference in detection rates between sample types. Males were more likely to be shedding at the time of sampling than females, with 44.1% ± 6.6% (n = 220) and 33.1% ± 7.1% (n = 169) prevalence respectively (Z-Statistic = 2.1925, df = 1, p = 0.0001). Additionally, males were significantly more likely to be shedding in saliva than females, with 26.4% ± 5.8% (n = 220) and 16.0% ± 5.5% (n = 169) prevalence respectively (Z-Statistic = 2.4575, df = 1, p = 0.007). There was no significant difference in shedding status among age groups: 37.6% ± 6.3% (n = 221) in adults, 38.8% ± 10.6% (n = 80) in sub-adults and 43.8% ± 10.3% (n = 89) in juveniles. This study demonstrates that MaHV1 was shed by a significant proportion of macaques following capture and transport and suggests that personnel handling macaques under these circumstances may be at risk of MaHV1 exposure.

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Emergence of avian influenza A (H10N7) in harbour seals (*Phoca vitulina*) in Sweden associated with a mass mortality event

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Cross-species transmission of avian influenza A viruses (AIV) to marine mammals sporadically occurs. However, confirmed AIV-associated mortality has been restricted to harbour seals (*Phoca vitulina*) in the United States. In March, 2014, increased mortality of harbour seals was observed on the Swedish west coast and by December, 444 dead seals had been reported. On April 16, 2014, a 12 year old male harbour seal with respiratory distress was euthanized and examined by necropsy. It suffered from severe bronchopneumonia and AIV and *Escherichia coli* were detected in the lungs. AIV was further characterized by PCR and identified as H10N7. Phylogenetic analyses showed it was most closely related to the Eurasian lineage of AIVs circulating in wild and domestic birds,

suggesting initial transmission from an avian source. Investigation into the extent of this outbreak using tissue, swab and/or serum samples from harbour seals found dead ($n = 20$) and from the general population ($n = 144$) is on-going. To date, AIV (H10N7) has been detected in lungs of nine individuals collected from April 16 - Sept 7, 2014. Preliminary results suggest that a significantly larger proportion of the seal population was exposed to the virus than the proportion that died during the mortality event and secondary complications (e.g. bacterial pneumonia) may have contributed to a fatal outcome. To our knowledge, this is the first report of AIV in seals in Europe, the first time it has been associated with mass mortality in seals outside of the United States, and the first time that H10 has been identified in seals. Inter-species transmission of AIV to mammals has potential public health significance and monitoring of AIV circulation in marine mammals coupled with further investigations into the pathology, epidemiology and zoonotic potential of H10N7, as well as adaptation of H10N7 to seals are warranted.

Taxonomy and genetic population structure of hookworms in the endangered Australian sea lion

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Hookworm infection causes significant clinical disease and mortality in Australian sea lion pups (*Neophoca cinerea*). Pups are infected via the transmammmary route during the immediate post-partum period with infection causing anaemia, enteritis, and reduced pup growth. All *N. cinerea* pups are infected and hookworm disease is estimated to contribute towards approximately 40% of all pup mortality, which oscillates between ~20–40%. The duration of patent hookworm infection is 2–3 months. As female *N. cinerea* demonstrate extreme natal site fidelity, and male pinnipeds are considered to be dead-end hosts for hookworm infection, we hypothesised that *N. cinerea* hookworm populations would be highly genetically structured. To better define the epidemiology of hookworm infection, we investigated the taxonomic identity, phylogenetic relationships, and diversity of hookworms from pups found dead ($n=117$) at three allopatric colonies in South Australia.

Morphological examination and molecular analyses of nuclear ribosomal loci (ITS1, ITS2, and 28s) demonstrated that a single, novel hookworm species, *Uncinaria sanguinis*, infects *N. cinerea* pups. Contrary to expectations, analysis of the mitochondrial cytochrome oxidase I gene (COI) revealed high hookworm diversity ($h=0.986$; $\pi=0.013$), unrelated to geographical location, with a large number of haplotypes ($n=45$).

The findings of this study challenge assumptions about the ecology of *N. cinerea* and the epidemiology of hookworm infection; in particular, the method/s of transmission and dispersal of hookworm between colonies. The results of further investigations to address the possibilities of patent adult infection, the New Zealand fur seal (*Arctocephalus forsteri*) as a means of hookworm dispersal, and paratenic hosts are presented and discussed. The characterisation of hookworm infection is vital to addressing key knowledge gaps relating to a significant disease threat; this study contributes towards our understanding of the *N. cinerea*–hookworm relationship, informing conservation of this endangered pinniped (IUCN Red List, 2008) and its parasitic fauna.

Systemic coccidiosis in green turtles (*Chelonia mydas*) in Australia: An emerging infectious disease

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Outbreaks of infectious disease are increasingly recognised in marine turtles and, in some cases, may be indicative of a broader underlying ecosystem disruption. The coccidial parasite *Caryospora cheloniae* was first described in captive green turtle (*Chelonia mydas*) hatchlings experiencing severe inflammatory disease of the hindgut in the Cayman Islands in 1973.¹ The first epizootic of coccidiosis in wild green turtles occurred in Moreton Bay, Australia in 1991. On this occasion, turtles had lesions throughout the intestine, sparing only the duodenum. Many also had extra-intestinal lesions of which the brain was a common target organ. Based on the morphology of the oocysts which was similar but not identical to those described previously, this organism was thought to be *Caryospora cheloniae*.² Subsequently, epizootics of these *Caryospora*-like organisms in green turtles have occurred repeatedly along the east coast of Australia. We have collected all of the available temporal, spatial, clinical and necropsy data

on known outbreaks occurring between 1991 and 2014. Using this data we will provide the most comprehensive picture to date of the number and timing of disease outbreaks in green turtles caused by this Caryospora-like organism, the age class of affected turtles, clinical signs, and distribution of lesions. We will also show whether or not these epizootics are linked with specific climate conditions. The data generated by this study will help to better understand the epizootiology of this disease and hopefully provide a means to mitigate its impact.

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Human development and land cover predict *Toxoplasma gondii* exposure in California sea otters

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Protozoal meningoencephalitis, caused by *Toxoplasma gondii* and *Sarcocystis neurona*, represents a significant cause of mortality for reproductive-aged sea otters. Individual and behavioral risk factors for *T. gondii* exposure include male sex, and diet preferences including a high percentage of kelp snails. The only known definitive hosts shedding infectious oocysts are terrestrial felids, but despite this, little is known about features of the terrestrial environment that determine risk of pathogen transfer into the marine environment. In this study we present a novel method for evaluating the relationship between landscape-based features and disease exposure risk. We conducted a spatially-weighted regression using a weighting index calculated from discharge of each river within the study area and distances between capture location and river outflows. Distances were calculated based on real-world coastal topography to reflect the distance an otter must swim between two points. This index was used to weight exposure of each study animal to indices of development and land-cover, calculated for coastal watersheds adjacent to the entire sea otter range in California as well as comparison sites in Washington and Alaska. While a simple comparison of the features of the study sites with seroprevalence indicated a positive association with some land-cover categories (cropping - spearman's $\rho = 0.9$, $p = 0.0001$; pasture - $\rho = 0.72$, $p = 0.01$), weighted regression analysis found these factors to be less accurate predictors of serum antibody status than human housing unit density. A 2-fold increase in housing density in adjacent watersheds was associated with a 1.3-fold increase in disease exposure odds among sea otters ($p < 0.0001$). We conclude that specific features of areas of denser human habitation are conducive to greater oocyst loading or mobilization into freshwater. This effect may be driven by increased definitive host abundance in areas of human habitation or altered watershed permeability.

Toxoplasma in the New Zealand marine environment

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Water-borne toxoplasmosis is increasingly recognised as a problem world-wide, and studies in California suggest that infectious oocysts reach the sea in freshwater run-off contaminated by cat faeces¹. Novel *Toxoplasma* genotypes arise by recombination in the cat host², and oocysts can be concentrated in filter-feeding invertebrates¹. We have established that an atypical type II genotype causes fatal disseminated toxoplasmosis in Hector's dolphins³ and in several native bird species in New Zealand⁴. We are currently conducting studies to characterise the distribution of this genotype in cat populations throughout New Zealand, and to assess levels of oocyst contamination in the coastal marine environment using mollusc species as bioindicators. Faecal samples collected from wild-trapped feral cats were examined by faecal float and by direct DNA extraction from faeces, followed by PCR targeting the *Toxoplasma*-specific dhps gene. Presence of *T. gondii* was confirmed by sequencing. Haemolymph was collected from commercially sourced and environmentally sourced greenlip mussels, and subjected to DNA extraction followed by *Toxoplasma*-specific PCR and sequencing. New Zealand appears to have an unusually high prevalence of infection in coastal mussels, with 12/56 (21%) commercially sourced mussels and 31/154 (20%) wild mussels testing positive on PCR. 4/133 (3%) feral cats were oocyst-positive on faecal float, and 40/97 (41%) whole-faeces DNA extractions processed to date were positive. We hypothesise that the higher prevalence on whole-faeces preparations reflects the presence of sexual phases of *T. gondii* within sloughed intestinal epithelial cells. Genotype analysis of 5 genetic markers revealed a possible atypical type #13 from two cat oocyst samples. Further genotyping is in progress for the remaining cat oocysts and the mussel samples. An extended study is underway on mussels at key coastal sites to detect changes in seasonal prevalence which may accompany factors such as increased surface-water input due to seasonal rainfall and snow thaw.

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Diphtheritic stomatitis in yellow-eyed penguins: an epidemiological analysis of 15 years of field data on an idiopathic disease in endangered New Zealand penguins

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The yellow-eyed penguin (hōiho, *Megadyptes antipodes*) is one of the world's rarest penguin species, with a population c. 4000 and a range limited to southeastern New Zealand and its subantarctic islands. Threats to the species include habitat loss, introduced predators such as mustelids, and emerging infectious diseases. The New Zealand Department of Conservation and community groups have monitored this species since the 1980s. During the last decade several significant and distinct disease entities have emerged within yellow-eyed penguin chicks. A major entity is diphtheritic stomatitis, an idiopathic disease marked by ulceration and proliferative stomatitis of the bill commissures and oropharynx. Diphtheritic stomatitis was first identified in two chicks during the 1999/2000 breeding season, and has since spread to affect all known breeding sites on the Otago Peninsula and in North Otago. The disease spread in conjunction with histopathological features suggest an infectious aetiology. In an effort to better understand the disease, we analyse 15 years of nest-monitoring data to describe the basic epidemiology of the disease and identify risk factors. Our goal with this study is to increase the body of knowledge about diphtheritic stomatitis with a view to improving disease management in the field.

Conservation - a decisive Public Health campaign of the 21st century?

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'Conservation is good for human health' was a central message at the IUCN World Parks Congress in Sydney, November 2014. This builds on the advice of the Millennium Ecosystem Assessment and the Convention of Biodiversity Aichi Targets. Healthy wildlife - both as a consequence and a driver of healthy ecosystems and of healthy people was an awkward poor cousin in the overall discussion of Health and Well Being, and of the 'Healthy Parks-Healthy People' campaign at the Congress. Rates of type 2 diabetes, obesity, some cancers, depression - all rising global epidemics in an increasingly urban human population- can be reduced with increased physical exercise and contact with nature. However these outcomes do not require a healthy park or well functioning ecosystems to be realised. Pursuit of these goals, alone, could mismanage ecosystems, not only causing ecological degradation but in some cases facilitating infectious disease transmission. Engaging the conservation-health debate in the thick of human activity (i.e. beyond protected areas) is essential to avoid distancing issues and sidelining public concern.

In an era when action to address the global biodiversity crisis is critical and conservation is appealing to human health for support, wildlife disease researchers need to be able to contribute digestible complexity to this debate. The co-benefits of wildlife health to human and ecosystem health need to be better researched and articulated and research must be accessible to the agents of change. In this presentation we will use examples from Australasia to look at the type of evidence we have, the policy framework in which it can be considered and the mechanisms of translation. We will also frame conservation as a decisive 21st century public health campaign and consider the current progress and hurdles against those of previous major public health campaigns.

Young citizen scientists monitor a deadly disease threatening regional amphibian communities in Humboldt County, CA

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We address regional chytridiomycosis through an effective citizen-science program. Ecoclub youth (4-16 years) and families assessed prevalence and distribution of *Batrachochytrium dendrobatidis* among amphibians of Humboldt Bay National Wildlife Refuge (HBNWR) and Redwood National and State Parks (RNSP), Humboldt County, California (USA). Following required educational workshops with professional biologists, 25 youth and 24 supervising adults sampled 154 metamorphosed amphibians by skin swabs and 15 tadpoles by buccal swabs, May 2013 through December 2014. Using real-time polymerase chain reaction tests, 30/169 (18%) samples were positive

for *B. dendrobatidis*, including 8/68 Pacific chorus frogs (*Pseudacris regilla*), 3/22 Western toads (*Anaxyrus (Bufo) boreas*), 3/25 northern red-legged frogs (*Rana aurora*), 16/26 foothill yellow-legged frogs (*Rana boylei*), 0/3 ensatina (*Ensatina eschscholtzii*), 0/2 California slender salamanders (*Batrachoseps attenuatus*), 0/5 coastal giant salamanders (*Dicamptodon tenebrosus*), 0/3 rough-skinned newts (*Taricha tenebrosus*), and 0/15 tadpoles. At HBNWR, fewer [6/73 (8.2%)] metamorphosed amphibians were positive, compared to RNSP [20/69 (29%)], and the workshop training wetland [4/12] ($\chi^2=10.36$; $p=0.0056$). Median chytrid zoospore intensity among positive samples was 1.7 (range: 1-33). Fifteen of 75 (20%) amphibians sampled by youth <12 years old were positive; similarly, 15/94 (16%) amphibians of participants >12 years of age were positive. Except for *Rana aurora*, all are first-time species reports for Humboldt County. These findings were comparable to other regional chytrid studies, supporting the potential value of involving children and families in citizen-science projects. The project incorporates traditionally underserved communities: 10 of 25 participating students have Latino heritage, Native American heritage, and/or African-American heritage. Socioeconomically, about half of all families in the school district fall below 130% of the federal poverty guidelines. Besides addressing a pressing wildlife health issue, direct participation of Ecoclub youth and families in this research contributes to science education among a multigenerational public and encourages broader stewardship.

Colonization dynamics of a new endogenous retrovirus in mule deer from chronic wasting disease- free and endemic regions

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Endogenous retroviruses [ERVs] are relicts of infectious retroviruses that have infected and integrated the viral genome into a germ cell, essentially creating a new host gene. The history of retrovirus epizootics in a population is therefore reflected by ERVs. The genomes of all mammalian species contain ERVs but most of these derive from retroviral colonization events that occurred in the ancient history of a contemporary species. We identified a new family of ERV in mule deer, CrERV, which has repeatedly colonized the host genome over the last 200,000 years up to present time and retains transcriptional activity in all animals. While ERVs clearly have a fundamental role in host genome evolution and contemporary phenotypes, they also have an enigmatic association with disease. It is particularly intriguing that an active ERV is present in mule deer because the prion disease - chronic wasting disease [CWD]- occurs naturally in this species; elevated retroviral transcripts have been detected in experimental CWD infection in several species. The aim of our research is to determine if the CrERV colonization histories differ in mule deer from states with endemic (Wyoming) and absent (Montana and Oregon) CWD because differences in the distribution of full length retroviruses among deer genomes can affect genome structure and function. We have developed new empirical, computational, and statistical tools and a draft of the mule deer genome to comprehensively assess the genomic location and estimate the population frequency and evolutionary history of each CrERV in all sampled animals. Our results demonstrate that mule deer in Montana and Oregon share a similar CrERV colonization history that is very different from that experienced in Wyoming deer. These data suggest that the genomic landscape shaped in part by an endogenous retrovirus may contribute to the natural susceptibility or resistance of mule deer to CWD.

Anaemia in neonatal pinnipeds: Distinguishing between correlation and causation by haematophagous parasites of the Australian sea lion

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Neonatal anaemia is a phenomenon observed commonly in many species. For pinniped pups, this anaemia has generally been attributed to physiological responses to host-environment changes such as the increased oxygen availability post-partum, compared to the *in utero* environment, and the expansion of plasma volume with growth. However, despite the widespread host distribution of haematophagous hookworm and lice species, few studies have considered parasitosis as a cause of anaemia in pinniped pups. In this study, we investigated the impact of hookworm (*Uncinaria sanguinis*) and lice (*Antarctophthirus microchir*) infections on the health status of free-ranging neonatal Australian sea lion pups (*Neophoca cinerea*; $n=295$) by estimating their effects on haematological parameters. Additionally, in order to determine whether these parasites are simply correlated with the occurrence of neonatal anaemia or play a causative role in its development, we experimentally manipulated naturally-occurring infections and compared changes in the haematological values between ivermectin-treated pups ($n=31$) and saline-treated pups ($n=24$).

Key findings of this study include the identification of *U. sanguinis* as an important agent of disease for neonatal *N. cinerea* pups, with infection causing significant anaemia, hypoproteinaemia, and a predominantly lymphocytic-eosinophilic systemic inflammatory response. Conversely, *A. microchir* has a lesser impact on *N. cinerea* pup health with infestation causing mild anaemia and hyperproteinaemia; high intensities of lice are likely secondary to hookworm infection or other causes of disease. Critically, by classifying the erythroid response to anaemia as regenerative, we demonstrated that anaemia is not solely a benign physiological response to host-environment changes, but largely reflects a significant pathological process in this species. These findings inform the conservation management of free-ranging *N. cinerea* and improve our understanding of the impact of parasitic infections on the health and development of haematological parameters in pinniped pups.

An outbreak of colonic amoebiasis in wild cane toads (*Rhinella marina*) in northern Australia

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Cane toads were introduced into Queensland in 1936 in an attempt to control the sugar cane beetle. Since then, they have dispersed across a million square kilometers, arriving in Darwin, Northern Territory, in 2005. Despite intense interest in the diseases of this invasive amphibian species, no infectious or parasitic diseases that produce significant mortality have been identified in wild cane toads. In August 2014 ("dry" season), toad ecologists noticed numerous dead, or emaciated and weak wild cane toads in the vicinity of their research station near Darwin. Although wild toads are usually thin during the dry season, the degree of emaciation and behavior of the toads was unusual. Euthanasia, necropsy and histopathology of a wide range of organs was performed on several emaciated weak toads and several relatively normal toads from the local population. In addition, several toads were sampled from a neighboring population, 30 km away. All toads from the local population had some degree of colitis, ranging from severe extensive ulcerative pyogranulomatous colitis in emaciated weak toads to less severe, more proliferative, and lymphoplasmacytic colitis in relatively normal toads. In all toads in which there was colonic mucosal epithelium remaining, there were amoebae intermingled with the epithelial cells. Histologically appreciable amoebic colitis was not evident in the toads from the neighboring population. During December ("wet" season), when toads are generally in better body condition, several toads in excellent body condition sampled from the local population did not have histological evidence of amoebic colitis, while relatively thin toads did. Efforts are underway to identify the pathogenic amoebae, and develop a means to diagnose the infection in live toads to enable investigation of the ecology of the host-parasite relationship.

Rats about town: Parasites and pathogens carried by rats in Vancouver, Canada

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Rats are the source of numerous pathogens responsible for significant human morbidity and mortality in cities globally. Despite evidence that rats are thriving in cities, there is little contemporary research on urban rats and rat-associated health risks in Canada. To address this gap, the Vancouver Rat Project captured 725 rats over the course of one year from Vancouver's Downtown Eastside to study the ecology of rat-associated pathogens. During Phase I of the project, rats underwent a full necropsy and ectoparasites were removed from the fur. Rat tissues were tested for zoonotic organisms using various techniques, including PCR, ribotyping, and whole-genome sequencing. We isolated several pathogens including *Bartonella* spp., *Clostridium difficile*, *Leptospira interrogans*, and methicillin-resistant *Staphylococcus aureus*. However, certain pathogens thought to be endemic in rats worldwide (e.g., Seoul hantavirus and *Rickettsia typhi*) were conspicuously absent. Additionally, rats were infested with fleas, lice, and mites; this is important because fleas are known vectors for certain zoonotic bacteria. Prevalence of rat pathogens and ectoparasites varied by block, with some blocks having many infected/infested rats and others having none. This uneven distribution may be a result of local rat population structure, and suggests that the likelihood of contacting a rat and its pathogens is site-specific. To investigate this hypothesis, in Phase II of the project we are performing a genetic analysis of rats to infer population structure and movement patterns of rats, and relating these to environmental features of the capture site. Ongoing research also includes sequencing of flea-associated bacteria to determine whether rat fleas carry known or novel zoonotic pathogens. By integrating data on rat ecology, parasites, and pathogens we aim to better understand rat-associated public health risks – information that can be used in turn to inform programs that monitor and mitigate impacts of rats in the urban environment.

Investigations into Pathogens of Potential Public Health and Biosecurity Significance in Australian Wild Birds

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The Australian Wildlife Health Centre (AWHC) at Zoos Victoria's Healesville Sanctuary provides veterinary care for approximately 1300-1500 wildlife patients each year. Roughly 60% of these patients are birds, with common species including Crimson rosellas, Tawny frogmouths, Sulphur-crested cockatoos, Rainbow lorikeets, Eastern rosellas, Australian king parrots and Galahs. These patients may be brought in by wildlife carers and community members, or by referral from other veterinary clinics, for treatment of sickness and injury.

Wild birds may carry pathogens of potential public health significance, such as *Chlamydia*, Influenza, *Salmonella*, *Campylobacter* and *Yersinia*. These and other pathogens may also be of potential importance

to zoo biosecurity and threatened species programs. Understanding the prevalence and significance of these pathogens is important for managing infectious risks, particularly for the veterinarians, veterinary nurses, keepers, wildlife carers and members of the public who handle sick and injured birds. Two of Healesville Sanctuary's priority native threatened species are birds (the Orange-bellied parrot and Helmeted honeyeater), and as such these captive breeding programs are particularly at risk from pathogens carried into the sanctuary by wild birds.

For this investigation samples including choanal/tracheal and cloacal/intestinal swabs are being collected from wild birds presenting to the AWHC for veterinary care. No zoonotic bacterial enteric pathogens (*Salmonella*, *Campylobacter* or *Yersinia*) were isolated by microbiological culture techniques, although coliforms were cultured from 60 of the 102 birds sampled thus far. Polymerase Chain Reactions to detect *Chlamydia*, Avian Influenza, Avian Paramyxovirus, Avian Coronaviruses, *Salmonella* and *Campylobacter* are in progress.

This investigation hopes to improve our understanding of significant pathogens of Australian wild birds for public health, zoo biosecurity and risk to threatened species programs, and then target detected pathogens in developing guidelines to help reduce infectious risks for people handling wild birds, as well as to reduce risk of transmission to captive animals.

Disease risk analysis and disease risk management for avian reintroduction projects

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Marked declines in biodiversity are leading to ambitious interventions for wildlife conservation, such as reintroductions. Reintroductions have had relatively low success rates to date, and further research into their limitations, methodology and management is needed in order to improve conservation outcomes. Disease hazards are one of the constraints on successful reintroduction. Guidelines for disease risk assessment (DRA), and health management, for reintroduction of specific taxonomic groups would be beneficial, to build on existing, overarching guidelines. This PhD project (2014–2020) is using two UK reintroduction projects – for the Eurasian crane (*Grus grus*) and corncrake (*Crex crex*) – as case studies by which to review, and further develop, DRA and health management strategies for avian reintroduction.

The project comprises:

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

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

Enemy release and novel weapons: the role of avian malaria parasites in the spread of the invasive common myna (*Acridotheres tristis*) in Australia

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Invasive species can have competitive advantages in non-native ranges through decreased pathogen pressure or through the spread of invasive pathogens that spillover to native species. The common myna (*Acridotheres tristis*), one of the world's most invasive species, carries a high prevalence of avian malaria (*Plasmodium* and *Haemoproteus* spp.) in its native range and is a fierce competitor in its introduced Australian range. We tested whether this competitive advantage stems from pathogen release by comparing malaria prevalence between introduced and native mynas and relating these to prevalence in native Australian birds. We also used a global database of malaria DNA sequences to identify potentially invasive malaria lineages carried by introduced mynas. Malaria prevalence did not differ between introduced and native mynas. However, compared to native birds, *Plasmodium* prevalence was significantly higher in introduced mynas while *Haemoproteus* prevalence was significantly lower. Nine *Plasmodium* lineages were shared between Australia and the myna's native range, and eight of these occurred in both introduced and native mynas. Importantly, four of these shared lineages were also found infecting Australian native birds despite strong phylogeographic evidence for their origination in the myna's native range. We propose that mynas experience a competitive advantage by avoiding *Haemoproteus* infections in Australia and by harbouring introduced *Plasmodium* lineages that occasionally spillover to native birds.

Influenza A exposure in feral swine of South Australia.

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Wild waterfowl are known to be the main reservoir for circulating avian influenza viruses (AIV) with spillover into domestic poultry occurring infrequently. In the southern hemisphere, the breeding season and habits of wild waterfowl are not as predictable as in the north, and the dynamics which perpetuate AIV in populations continues to be investigated. Other wild animals, which share their environment, are also subject to exposure to influenza viruses. In Asia and North America, pigs have been found to be a suitable mixing vessel for influenza viruses, with occasional viral spillback into waterfowl. The exposure of feral pigs to AIV has not been investigated in Australia to date, and the extensive population and distribution of feral pigs in wetland areas, sharing habitats with waterfowl creates an opportunity for exposure and infection. Blood samples for serology and nasal swabs for virus identification were obtained from the carcasses of 23 feral pigs shot as part of a State government control cull around two Ramsar wetlands in South Australia, Kingston SE in the south-east of the state, and Innamincka in the north-east corner. The serum samples were analysed using the IDEXX Influenza A Antibody Test Kit (Netherlands). Three of the 23 samples tested positive for influenza A antibodies, indicating that the feral pigs had been exposed to influenza A across both sites. We propose that feral pigs in these habitats are frequently exposed and could present an appropriate mixing vessel for new strains of AIV. With both avian and swine influenza being notifiable diseases in Australia and worldwide, surveillance methods utilising available species and focusing limited resources by cooperation between agencies could provide a viable adjunctive method of monitoring incursions of disease.

Disease investigation during a bird washing experiment

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During an experiment investigating the efficacy of washing oiled birds with saltwater compared to freshwater, eight out of forty-four mallards (*Anas platyrhynchos*) became ill within 2-9 hours after the washing process. They presented with weakness and inability to stand. A presumptive diagnosis of capture myopathy and heat stress was made based on clinical signs and a history of prolonged periods of stress during catch up and high ambient temperature (34°C). All affected birds had been washed and rinsed in seawater (~3.5% saline). Treatment for capture myopathy was started immediately and involved aggressive fluid therapy with intravenous and oral isotonic fluids as well as meloxicam and midazolam. Despite treatment, five of the sick birds died after several hours. Initial findings on gross necropsy of the five dead birds revealed pale thigh muscles, consistent with myopathy, as well as brain congestion and cardiac haemorrhages. Blood samples were taken from the remaining live birds and revealed elevated serum sodium and creatinine kinase levels. The treatment protocol was immediately changed as the treatment for hypernatremia is the opposite for capture myopathy because serum sodium levels need to be reduced slowly to prevent swelling of the brain. Drinking water was restricted, with freshwater being offered to the birds at a rate of 0.5% of body weight every 30-60 mins throughout the day until they had drunk a total of 100-120 ml (twice their normal fluid maintenance needs). As the birds' clinical signs improved they were slowly offered increasing amounts of freshwater every 30-60 mins and after 4 days of treatment all three birds had recovered. Changes made to the husbandry procedures on Day 2-4 of the experiment to prevent further illness will be discussed. The mallards used in this experiment were raised in captivity and freshwater-adapted and therefore appeared highly sensitive to saline water.

Forced Moul in Sub-Antarctic Yellow Eyed Penguins (*Megadyptes antipodes*).

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Penguins are reliant on a waterproof plumage to survive. The moult in Yellow Eyed Penguins is a seasonal process that occurs once a year, during which the animals go through fattening and fasting periods and high levels of stress to achieve the full replacement of old feathers. The plumage replacement takes three to four weeks, which penguins spend sitting ashore unable to feed. Although hormonal induction of moult has been achieved in chickens and other species, until now successful results have not been achieved in penguins. Four juvenile Yellow Eyed Penguins that were being prepared for release in a rehabilitation centre were referred to Wildbase for treatment following feather breakage and various degrees of pododermatitis. The four Yellow Eyed Penguins had a full physical examination including radiographs, bloods and the collection of faecal samples to assess their health. The pododermatitis was treated with serial debridement and bandaging under anaesthesia. The damaged feathers were plucked over several

procedures, however the results were poor. In a rehabilitation setting, it is not feasible to keep penguins in captivity until the next natural moult occurs which could be 10 to 12 months. Complete moult was induced and achieved by oral supplementation of fresh beef thyroid. The induction of the moult was complete at around fourteen days following the treatment. The Yellow Eyed Penguins replaced their entire plumage successfully and their waterproofing was adequate, allowing release back into the wild.

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Mortality of passerines and relatives submitted to a wildlife diagnostic laboratory (Southeastern Cooperative Wildlife Disease Study, USA): a 36-year retrospective analysis

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Data gathered by wildlife laboratories from submissions provides a rare opportunity to examine long-term, demographic, temporal and spatial patterns of mortality, which may have important management implications. From 1976-2012, we retrospectively examined records of avian case submissions to the Southeastern Cooperative Wildlife Disease Study (SCWDS; University of Georgia, USA) in order to determine the relative importance of mortality factors for birds over time. During this period, SCWDS received 2,583 wild bird specimens from the orders Apodiformes, Caprimulgiformes, Cuculiformes, Passeriformes, and Piciformes originating from 22 states, mostly located in the southeastern USA. Data from 2,001 of these birds were analyzed using log-linear models to explore correlations between causes of mortality, taxonomic family, demography, geographic location, and seasonality. Toxicosis was the most frequent cause of mortality, followed by trauma, bacterial infection, physiologic stress, and viral infections. Birds submitted during fall and winter had a higher prevalence of parasitic infections, trauma, and toxicoses, while those submitted during the spring and summer were more likely to die of infectious disease, physiological stress, or trauma. We noted a decrease in toxicoses concomitant with an increase in bacterial infections and trauma diagnoses after the mid-1990s. The majority of adults died from toxicoses, whereas the main cause of death in juveniles was physiologic stress, trauma and viral infections. Infectious agents were frequently diagnosed within the Cardinalidae and Fringilidae families, while non-infectious etiologies were the primary diagnoses within the families Bombycillidae, Parulidae, Sturnidae, Turdidae, and Icteridae. It is important to note that there are inherent limitations in the interpretation of data from diagnostic laboratories, as submission of cases varies in timing, frequency, location and species, and is often influenced by factors such as media coverage of high-profile events.

Genetic characterization of two bacterial species isolated from wild goose eggs on the Arctic Coastal Plain of Alaska

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In 2011 and 2012 the rate of nonviable eggs in a nesting population of greater white-fronted geese (*Anser albifrons*) on the Arctic Coastal Plain of Alaska was noted to be higher than in other monitored populations. Additionally, field biologists noted that some nonviable eggs had a foul smell. Preliminary data recovered DNA sequences from 3 potential egg pathogens. During the summer of 2013, we collected 36 addled eggs and aseptically collected egg contents. We isolated bacteria and obtained 16S rRNA gene sequences from egg contents and pure cultures for identification of bacterial species. A *Neisseria* species was isolated from 21 addled eggs and sequence data identified the same species in 2 additional eggs. *Streptococcus uberis* was isolated from 4 addled eggs. 16S rRNA sequence data and preliminary 23S rRNA sequence data suggest that this may be a new species of *Neisseria*. A multilocus sequencing approach (glucose kinase, transketolase, D-ala-D-ala kinase, thymidine kinase, carbamate kinase, triosephosphate isomerase, and acetyl CoA acetyltransferase) shows that our *S. uberis* isolates are not clonal and likely have been introduced to this population numerous times. Swab data show that *S. uberis* DNA (16S or 23S rDNA) is not present in nest contents. These are the first reports of any *Neisseria* species isolated from bird eggs, and the first report of *S. uberis* being isolated from bird eggs.

Physiological stress and parasite infection in a critically endangered marsupial, the woylie (*Bettongia penicillata*)

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Conserving healthy wildlife populations requires an understanding of the factors which affect infection patterns. Stress, particularly if chronic or severe, may impair immune function and influence disease susceptibility and severity. This is particularly concerning in endangered species which may be more susceptible to the impacts of stress and disease for various reasons including low abundance limiting the population's capacity to adapt and restricted range preventing dispersal away from stressors. In this multidisciplinary study, we used parallel stress physiology (faecal glucocorticoid metabolites), immunology (phagocytosis flow cytometry) and parasitology analyses (faecal flotation for intestinal helminths, immunofluorescence for *Giardia* and *Cryptosporidium* spp. and PCR for *Trypanosoma* spp. of haemoparasites) to investigate the relationship between stress, immunocompetence and infection in a critically endangered marsupial, the woylie or brush-tailed bettong (*Bettongia penicillata*). Woylies, once numerous and widespread across Australia, have declined by 90% since the 1990s and only two indigenous populations remain in the southwest corner of Western Australia. It has been suggested that stress, immunosuppression and infectious disease may be acting synergistically to contribute to the species' decline. We predict that woylies experiencing a higher level of physiological stress will demonstrate compromised immune function and higher prevalence and intensity of parasite infection. We explore implications for the future of the species and wildlife conservation.

A comparison of diets used during a colony translocation of New Zealand Grey-faced petrel chicks, *Pterodroma macroptera gouldi*.

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Translocations of petrel colonies is a valuable conservation tool used to conserve rare Procellariiform seabirds and restore natural habitats. The seafood diets selected to feed petrel chicks during translocations are often heavily influenced by the practical limitations of storage, cost and availability in remote locations. The same diets are fed to various seabird species, irrespective of their different foraging strategies and diverse prey items. In New Zealand a tinned sardine in soya oil based diet has been used to feed over 14 different species of petrels during translocations, including the critically endangered Taiko *Pterodroma magenta*. Feeding this sardine based diet for longer periods has resulted in deaths related to nutritional disease. This raises the concern that birds may be fledging with malnutrition which could impact on their survival at sea.

This study assess the effects of feeding different artificial diets to petrel chicks by running a dietary trial during a colony translocation of 76 grey-faced petrel chicks, *Pterodroma macroptera gouldi* from Motuhora Island (37°52'S, 176°58'E) to Cape Sanctuary (39°40'S, 177°06'E). The chicks were fed either tinned sardines in soya oil, or a powdered Mazuri fish analog® diet supplemented with fish oil. Results of the dietary trial will be presented; comparing the effects of the diet on growth parameters, fledging rates and the disease incidence during the translocation.

To compare the nutrient content of artificial diets with wild diets, proventricular samples of freshly fed wild chicks were collected from a control colony of grey-faced petrels in West Auckland (36°54'S, 174°27'E). Blood samples from wild and translocated chicks were also taken to measure the change in erythrocyte phospholipid ratios that occurred while being transitioned from the wild diet to different oil based diets, over a 3 week period. The results of this study will improve the diet choices for future seabird translocations.

Humoral immune responses in koalas and its impact on vaccine development

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The number of koalas has been decline throughout the South-East Queensland (SEQ) and it's linked to several threatening process like habitat destruction, dog attacks, motor vehicle trauma and most importantly chlamydial infection. Chlamydial infection in koalas is significant and causes infertility, morbidity and mortality. Current antibiotics approach has certain limitations like low efficacy rate for chronic infection, increased persistent infection and mostly adverse effect on the gastrointestinal microflora. So, the vaccine development would be the ideal option. Recent mathematical modelling suggests that a 5 -10 years long vaccination program could be able to reverse the population declining trends. Our vaccine research group has been developed a vaccine by using recombinant major outer membrane (rMOMP) protein as antigen. Initially, we applied immune-stimulating complex as vaccine adjuvant. Recently, polyphosphazine based poly I: C and host defense peptides are successfully employed as vaccine

adjuvant. These combination adjuvants have the ability to elicit long lasting cellular and humoral immunity via a single dose vaccination. The chlamydial pathogenesis and the host immune response are the key interactive features for the efficient vaccine production. T cell through the activation of interferon gamma mediated immune response proceeded to the infection procedure. Besides, antibodies has subordinate role in the infection process but it could modulate the cellular response in several animal models. In this article, we analysed the humoral immunity both in koala that has natural live infection and/ or has received vaccine and its impact on vaccine development process. We observed current infection in koalas elicit very low level of *C. pecorum* specific neutralizing antibodies *in vitro*. Subsequently, these animals were affected by chlamydial diseases or unable to clear the infective stage. Alongside, we identified the current vaccine were able to boosted the antibody response via introducing *C. pecorum* specific neutralizing antibodies.

“As long as we’ve had bats, we’ve had Hendra” – Horse owners’ knowledge and risk perception of flying foxes in regards to Hendra virus

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Hendra virus (HeV) is a zoonotic paramyxovirus causing neurological and respiratory disease with high mortality rates in horses (75%) and humans (57%). Discovered in Queensland, Australia in 1994, it sporadically spills over from flying foxes (*Pteropus* spp.) to horses and from horses to humans. While outbreaks significantly impact the equine industry, they additionally propagate fear and misinformation amongst horse owners about flying foxes, leading to calls for more radical flying fox management approaches, including dispersal and culling. This study investigates the knowledge, risk perception and attitudes of horse owners towards flying foxes as reservoirs of HeV to identify knowledge gaps and misconceptions, while informing effective communication strategies and policy development.

Data presented here are part of the ‘Horse owners and Hendra virus: a longitudinal cohort study to evaluate risk’ (HHALTER) project. Five online surveys at six-monthly intervals were administered to horse owners between November 2012 and December 2014 to assess changes in their knowledge, perceptions and attitudes on a range of HeV related topics, including flying foxes and their management. Additionally, the study monitored changes in risk mitigation practices, including uptake of horse HeV vaccination.

Overall, 1,449 horse owners participated in at least one survey. Over half (57%) of the respondents of the fifth survey (N= 580) reported seeing flying foxes nearby or on their horse property, but only 30% of these perceived them as a current health threat to their horses. Furthermore, the majority of respondents (73%) agreed that flying foxes play an important role in the environment. Further ongoing descriptive and regression analysis will be presented that explore relationships between these perceptions, demographic information and the subsequent uptake of biosecurity practices and vaccination.

The findings of this study will be used to advocate sustainable flying fox camp management strategies and to ensure protection of these endangered species.

Seroprevalence of *Toxoplasma gondii* in mainland and sub-Antarctic New Zealand sea lion populations

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Toxoplasma gondii is an emerging risk to marine mammals globally and is known to be present in New Zealand waters. Archived Sera of New Zealand sea lions (*Phocarcos hookeri*) from two recolonizing mainland populations at the Otago Peninsula and Stewart Island as well as a declining population at Enderby Island in the New Zealand sub-Antarctic were tested for antibodies to *T.gondii*. Sera were screened using commercially available ELISA and latex agglutination tests (LAT) and then confirmed with western blot analysis. Antibodies were found in 13.6% (3/22) of mainland samples but not sub-Antarctic samples. The positive LAT titres in three adult females were strong (1:2048-4096) and in two of these animals persistent for up to two years, but there was no evidence of associated clinical signs or reproductive failure. Continuing surveillance is pertinent to assess subclinical and clinical impacts of *T.gondii* on these threatened populations.

Investigating avian malaria transmission dynamics: the New Zealand situation

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Avian malaria, caused by protozoan blood parasites of the genus *Plasmodium*, is a concern for native New Zealand birds; this mosquito-borne disease has impacted both captive populations and wild individuals in the country. However, whether or not it is a cause of concern to native, wild populations is still unclear. In Hawai'i, avian malaria has been a major factor in the population declines of native forest bird species and limits the elevational distribution of many remaining species. At our study site (Nelson Lakes National Park, South Island, New Zealand), declines in native forest bird abundance have been reported over the past 30 years. Avian malaria is one possible cause of these declines, since they generally occurred at lower altitudes where more potential reservoir birds and mosquito vectors may be present. In addition to the invasive mosquito vector *Culex quinquefasciatus*, also found in Hawai'i, New Zealand's native *C. pervigilans* is also suspected of playing a role in malaria transmission, complicating our understanding of local transmission dynamics.

Blood samples were taken from native and non-native forest birds along an elevational gradient (650m to 1400m) during three summers (2012-13 to 2014-15), complimented with mosquito sampling. Here we will present our findings to date, describing the avian malaria community detected (including data on host prevalence, vector surveys, and *Plasmodium* spp. sequencing results). We will also discuss our work in adapting a malaria-forest bird epidemiological model (originally developed in Hawai'i) to fit the New Zealand situation, incorporating both *C. quinquefasciatus* and *C. pervigilans*. This work has potential for informing on management beyond avian malaria as well, as other mosquito-borne diseases (e.g. West Nile, Ross River, and dengue) could eventually make their way into New Zealand.

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To treat or not to treat? Investigating the impact of polyparasitism in translocated woylies (*Bettongia penicillata*), and the effect of anti-parasite treatment on host fitness and survivability

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Polyparasitism, in which a host is co-infected with various parasite species or intraspecific strains, is common in wild animal populations. Whilst parasites have been implicated in a number of species declines, the role of polyparasitism as a potential factor contributing towards translocation failures has never been investigated. This project is currently evaluating how fauna translocations impact the transmission of parasites in woylies (*Bettongia penicillata*), and what consequences this has for translocated hosts and other cohabiting species. We are testing the hypothesis that fauna translocations lead to a higher diversity of parasites within the resultant host-parasite community, and thus a higher incidence of polyparasitism; which in conjunction with the disruption of established host-parasite associations, may exacerbate the negative impacts of parasites on their hosts to the detriment of translocation success. Secondly, as the effects of anti-parasite treatment in translocated hosts are relatively unknown; we are also assessing the effect of parasite removal in translocated hosts. We are testing the hypothesis that anti-parasite treatment reduces the incidence of polyparasitism, thereby improving host fitness and survivability. In June 2014, 182 woylies were translocated from Perup Sanctuary to two unfenced sites within Western Australia. Pre- and post-translocation, woylies from both the source and destination sites were measured and weighed, and pouch activity was recorded for females. Blood, ectoparasite and faecal samples were also collected for parasitological examination. In each destination site, cohabiting species were sampled to quantify parasite transmission between species post-translocation. In order to evaluate the effect of anti-parasite treatment, we treated half the woylies with Ivermectin prior to translocation and repeated our sampling at four and 12 weeks post-treatment. We have observed changes to the predominant species of *Trypanosoma* woylies pre- and post-translocation, and that anti-parasite treatment has had an effect on both target and non-target parasites of the translocated hosts.

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Spatial and temporal variation in trace element and heavy metal concentrations in the Australian fur seal (*Arctocephalus pusillus doriferous*)

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Toxicants, including the heavy metals mercury, cadmium, lead and arsenic can have deleterious impacts on marine mammal health and survival. The vulnerability of pinnipeds to toxicant bioaccumulation means they have a role as sentinels of ecosystem health; establishing baseline toxicant concentrations in upper trophic species, such as the Australian fur seal (*Arctocephalus pusillus doriferous*), is essential for monitoring anthropogenic impacts in the marine ecosystem.

In this study, trace element and heavy metal concentrations will be determined in pup fur and blood serving as an indirect measure/proxy of maternal trace element and heavy metal concentrations. As the first hair coat (lanugo) grows in-utero, pup fur concentrations mirror maternal blood concentrations of these toxicants during gestation. Similarly, toxicant concentrations in pup blood are a secondary indicator of maternal concentrations in the milk.

During the 2013/14 census, blood (n=100) and fur (n=229) samples were collected from Australian fur seal pups at several breeding colonies in Victoria and Tasmania. These colonies (Lady Julia Percy Island, Seal Rocks, The Skerries, Tenth Island and Judgement Rocks) reflect varying degrees of intensity of coastal industrial enterprises across the species' range. Analysis of blood samples collected from four of these colonies in 2007-2008 (n=50 from each colony) also provides archive samples for temporal comparisons of toxicant concentrations with the 2013/14 samples. The concentration of 13 trace elements and heavy metals (Hg, Se, Zn, Fe, Al, Cu, Ni, Co, Cr, Cd, Pb, As, Mg) in fur and blood will be determined by inductively coupled plasma mass spectrometry.

In addition to presenting reference values for trace elements and heavy metals across the geographical range of the Australian fur seal, we report the effect of varying anthropogenic influence on these concentrations. Demonstration of anthropogenic impacts associated with environmental toxicants will inform mitigation strategies, aiding species and marine ecosystem conservation.

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Habitat suitability analysis of elephant (*Elephas maximus*) in Palamau Tiger Reserve, Jharkhand using geospatial technology

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Nature supports a great variety of ecosystems with diverse flora and fauna. Since the turn of this century people have speeded up the species extinction process by polluting the environment, destroying habitats, including plant and animal species. Hence, Wildlife is at the brink of destruction. Elephant, largest living land mammal, found in tropical regions of Africa and Asia. The influence elephants have over much plant and animal species means they are often referred to as keystone species that are vital to the long-term survival of the ecosystems in which they live. Now a days it has threat for survival due to anthropogenic reasons and the destruction of natural habitats.. In the present study, evaluation of elephants habitat in Palamau Tiger Reserve is carried out using remote sensing, ground and other ancillary sources and is integrated using GIS using Weighted Overlay model. Different thematic maps viz., land use/cover, railway buffer, drainage buffer and multiple ring buffers of roads have been prepared to support the objective of the study. The weighted overlay analysis model is used for identifying different potential areas of habitat for this threatened species. The most suitable area for Elephants within the Tiger Reserve is found about 70.43% of the total area which could be probably due to dense forest, least disturbance from people, away from road and railway line, presence of bamboo trees. Other reasons of elephant's occurrence in this region may be due to the presence of food and water body and being least prone to poachers. The moderate and least suitable areas come about 22.25% and 4.85%, respectively. Identification of habitat potential areas for Elephants species could be considered as one of the most important steps towards the conservation and geospatial technology could be utilized for any similar species for this purpose which is under threat.

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Avian Malaria in an avian community in Torres Strait, Australia

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An Australo-papuan avian community in Torres Strait (QLD, Australia) is being investigated for detectable effects and responses to natural exposure of a high diversity and prevalence of avian haemosporida (avian malaria). At present there is a poor understanding of the effects these parasites impose on Australo-papuan avian communities and investigations from this region currently contribute very little to the general understanding of avian haemosporida. Numerous studies elsewhere have revealed a variety of species-specific responses to these parasites ranging from undetectable to highly undesirable including population declines and species extinctions.

Studying interactions between haemosporida and Australo-papuan avifauna provides a unique perspective on the dynamics of these parasites with direct relevance to Australian avifaunal communities. Detectable effects in avian hosts are expected to vary with lineage of parasite, determined by cytochrome b gene sequencing, as well as between avian host species. Thus a broad range of effects are necessarily addressed. Birds are all caught and sampled from a single island community. For each individual morphometric data are collected and used to determine effects in conjunction with blood analyses such as blood cell counts and biochemical tests of organ function. Data collected over a period of eight years are included. The role of tolerant avian host species as reservoirs in this community is also investigated, determining the effect these host species have on parasite diversity within the community.

The uniqueness of the Austalo-papuan region and its avifauna necessitate local investigations of avian parasites. An Australo-papuan perspective adds greatly to the understanding of host-parasite dynamics and interactions in this region as well as contributes to the broader understanding of these globally present haemosporida.

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Human Tiger Conflict in Chitwan National Park, Nepal

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Human- tiger conflicts are serious issues of conflicts between local people and park authority and the conflicting situation potentially play negative role in park management. The study aimed (1) To determine the trend and nature

of human-tiger conflicts (2) To understand peoples? Perception and mitigation measures towards tiger conservation. Both primary and secondary information were used to determine human-tiger conflicts in Chitwan National Park. Systematic random sampling with 5% intensity was done to collect the perception of the villagers regarding human-tiger conflicts. The study sites were selected based on frequencies of incidences of human attacks and livestock depredation viz. Rajahar and Ayodhyapuri VDCs respectively. The trend of human casualties by tiger has increased in last five year whereas the trend of livestock has decreased. Reportedly, between 2008 and 2012, tigers killed 22 people, injured 10 and killed at least 213 livestock. Conflict was less common in the park and more intense in the sub optimal habitats of Buffer Zone. Goat was the most vulnerable livestock followed by cattle. The livestock grazing and human intrusion into tiger habitat were the causes of conflicts. Developing local stewardship and support for tiger conservation, livestock insurance, and compensation policy simplification may help reduce human-tiger conflicts.

Differential infection dynamics and susceptibility to *Baylisascaris procyonis* in *Peromyscus* species.

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Deer mice (*Peromyscus* spp.) are intermediate hosts for *Baylisascaris procyonis* (raccoon roundworm) and *P. leucopus* likely serves an important role in parasite transmission. Although infection has been reported in several *Peromyscus* species, no data are available on differential susceptibility of the various species. We compared infection dynamics of *B. procyonis* in four species (*P. leucopus*, *P. maniculatus* ssp. *bairdii*, *P. californicus*, *P. polionotus* ssp. *subgriseus*) across regions of varying habitat types and *B. procyonis* prevalence. Groups of six captive-bred mice of each species were inoculated per os with one of three doses (~10, ~50, or ~500) of embryonated *B. procyonis* eggs. Animals were monitored twice daily for clinical signs and behavioral abnormalities and were euthanized at the onset of severe CNS symptoms or at 45-48 days post infection (DPI). Larvae were enumerated in the brain via microscopic examination and in skeletal muscle and visceral organs via artificial digestion with HCl-pepsin. Mortality in the high-dose group was 83% for *P. californicus* and 100% for the other species. In the medium-dose group, mortality was 33% for *P. leucopus*, 50% for *P. californicus* and *P. polionotus*, and 85% for *P. leucopus*. No mice were euthanized in the low-dose group in *P. leucopus*, *P. californicus*, and *P. polionotus*; one *P. maniculatus* was euthanized in the low-dose group. Survival data were analyzed using a Weibull regression model. Overall, *P. leucopus* had greater survival than *P. maniculatus*, *P. californicus*, and *P. polionotus*, which did not differ significantly from each other. Interestingly, larval recovery rates were nearly identical across species and doses. These data indicate that *P. leucopus* is less likely to develop acute severe disease compared to the other species, and that even closely-related rodents may experience differential mortality. Additionally, natural mortality of rodents due to *B. procyonis* may be higher than currently recognized.

Investigation into the presence of anthroponotic *Cryptosporidium* sp. in wild and captive Australian grey-headed flying foxes (*Pteropus poliocephalus*)

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Bats are of vital ecological and economic importance, due to their role as pollinators, seed dispersers, insect predators and bioindicators. Their populations are however under increasing pressure from anthropogenic impacts, in particular habitat loss, increased urbanisation and climate change. As a result Australian grey-headed flying fox (*Pteropus poliocephalus*) populations are seeking shelter in regional and urban centres, thus increasing contact rates between humans and flying foxes. *Pteropus poliocephalus* are known vectors for a range of zoonotic pathogens, but reverse pathogen transmission (zooanthroponosis) from humans into bat species has rarely been investigated. The identification of zooanthroponosis in *P. poliocephalus* could have important implications both from a conservation and One Health perspective. To identify potential zooanthroponosis we are testing wild and captive populations of *P. poliocephalus* for the presence of human-borne *Cryptosporidium* species.

Cryptosporidium is one of the most common causes of enteric illness in humans. The zoonotic *C. parvum* is capable of infecting both humans and other vertebrates and is therefore of high clinical importance. We propose that captive *P. poliocephalus* populations may be at increased risk of human-borne *Cryptosporidium* infection as a result of feeding and handling, and reduction in immune function in cases of illness and injury.

DNA was extracted from faecal samples collected from wild (n=149, 6 locations) and captive (n=36, 1 location) populations in NSW. Detection and characterisation of *Cryptosporidium* was performed at the 18S rRNA gene. Sequencing data indicates the presence of novel genotypes of *Cryptosporidium* spp. in wild and captive *P. poliocephalus* populations in NSW. Further genetic analysis is currently being performed to confirm these novel findings.

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Do avian ticks (*Ixodes hirsti*) influence host phenotype: greater tail asymmetry in tick-infested birds

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From a zoonotic perspective, *Ixodes* ticks are of biological significance given their role in the transmission of a range of diseases across taxa, including, Lyme disease and tick typhus. Understanding the life-history impacts of ticks on their avian hosts is important to identify possible fitness costs of ticks for their hosts to inform the conservation management of endemic fauna and risks to disease transmission. Here, we test for effects of *I. hirsti* ticks on its endemic songbird host, the New Holland Honeyeater (*Phylidonyris novaehollandiae*). We mist-netted 293 adult and 45 juvenile birds from 11 sites across South Australia to test for effects of ticks on adult versus juvenile birds in relation to (i) host body condition (a measure of fitness), (ii) tail feather symmetry, and (iii) haemoglobin concentration. We predicted that juvenile and adult birds in tick-infested areas would have lower body conditions, greater feather asymmetry and lower haemoglobin concentration. The results showed different effects of ticks across age classes. Compared with tick-free birds, tick-infested adult birds had lower body condition (no significant difference in juvenile birds) and tick-infested juvenile birds had greater tail asymmetry (no significant difference in adult birds). Haemoglobin concentration did not differ significantly across age class or study site. These findings show age-specific effects of ticks on hosts and point to the role of parasites to alter host phenotype during ontogeny.

Impacts of toxicants in the marine ecosystem on the health of Australian fur seals (*Arctocephalus pusillus doriferus*)

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Persistent Organic Pollutants (POPs) cause toxicity effects including endocrine dysfunction, developmental defects, and neoplasia, in a wide range of species. Species which dominate the upper trophic level have particular vulnerability to bioaccumulative toxic effects caused by environmental pollutants. For the Bass Strait food web, the Australian fur seal (*Arctocephalus pusillus doriferus*) is an important sentinel species for ecosystem health.

An alopecia syndrome has been recognized at high prevalence (up to 50% of juvenile females) in Australian fur seals at Lady Julia Percy Island (LJP), Victoria, an important breeding site for this species. Alopecic seals are only occasionally seen at other colonies. Previous investigations suggest causality could be due to a pollutant acting as an endocrine analogue. The alopecic syndrome has significance for thermoregulation and is a likely risk factor for mortality.

In order to investigate whether POPs are associated with endocrine disruption and alopecia, we compare POP's concentrations in the fur of alopecic (n=50) and non-allopecic (n=51) juvenile seals sampled at LJP. Fur samples collected from pups at LJP and four other colonies in Victoria and Tasmania will serve as baseline colonies for comparison.

The concentration of selected POPs including Dioxin/Furans (PCDD/Fs), polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs) and Perfluoralkyl compounds was determined in fur using High Resolution Mass Spectrometry or Liquid Chromatography-Mass Spectrometry. Results to date indicate noteworthy levels of PCBs and PCDD/Fs in pup samples from Seal Rocks. Detection of POPs in these samples suggests that pinniped pups are at risk of POPs mediated toxicity in-utero, a particularly susceptible developmental stage.

We discuss the significance of POPs concentrations in relation to the causality of alopecia and the conservation management of the marine ecosystem, and assess the usefulness of fur as a non-invasive biomarker to assess POPs exposure in this sentinel species.

The first full length genome sequence analysis of a marsupial herpesvirus: macropodid herpesvirus 1.

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BACKGROUND: Herpesviruses cause significant morbidity and mortality in a range of animal species, including those of the order *Marsupialia*. There is currently no published marsupial herpesvirus genome available.

OBJECTIVE: The objective of this project was to perform the first comprehensive whole genome sequence analysis of a clinically important marsupial herpesvirus, macropodid herpesvirus 1 (MaHV1). This was undertaken in order to facilitate the development of improved diagnostic tools and to contribute a unique insight into the relationship between herpesviruses from evolutionarily diverse mammalian species. **METHODS:** Full genome sequencing, annotation and analysis were performed in conjunction with *in vitro* gene expression studies that utilised reverse transcription PCRs. **RESULTS and CONCLUSIONS:** The draft genome of MaHV1 was prepared and preliminary gene transcript analysis confirmed the *in vitro* expression of six novel genes. An absence of several conserved genes: UL3, UL4, UL56 and gJ was also observed. Interestingly, the full genome sequence results confirmed findings from earlier studies that showed MaHV1 to cluster with a number of alphaherpesviruses from primate hosts.

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Host-parasite interactions and dermal mycoflora of New Zealand reptiles

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New Zealand is home to a large number of endemic reptiles, including the Tuatara (*Sphenodon punctatus*). 46% of these species are listed as endangered or threatened. There have only been limited studies on the presence and significance of parasites and microflora. Opportunistic sampling from endemic reptiles has detected a large number of as yet unidentified parasites. Many of these parasites appear to belong to orders reported to cause significant pathology in other reptile species.

The dermal mycoflora of New Zealand reptiles is similarly poorly studied beyond several case studies describing mycotic dermatitis. Of concern to wild population health, a newly emerging fungal pathogen, *Paranannizziopsis australasiensis* has recently been diagnosed at two captive facilities in New Zealand. The origin of these infections and the prevalence of this organism in wild and captive reptile populations in New Zealand is currently unknown.

New Zealand reptiles are often exposed to varying environmental conditions, as a result of intensive conservation activities, such as captive breeding and translocations, and also due to the changing climate. This study aims to investigate the mechanisms by which changing climatic conditions can affect host-parasite relationships in captive and wild reptiles endemic to New Zealand. Three reptile groups, (tuatara, geckoes and skinks) will be sampled from 3 populations (captive, wild and translocated) to investigate the prevalence and significance of endoparasites and fungal flora. A captive population will also be kept for the purpose of examining the effects of environmental manipulation on the host-parasite relationship. As reptiles are poikilothermic animals it is hypothesised that a change in climate will have a significant effect on the host-parasite relationship and dermal mycoflora. This study will help to improve the baseline knowledge of parasites and microflora of endemic reptiles and will have implications for the future conservation management of New Zealand reptiles.

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One health infectious diseases in the Arctic: Emerging challenges for animals and humans

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The Arctic Ecosystem is especially vulnerable to climate changes with several pathogens extending their distributions northerly and infecting hereinto naïve animal populations. At the same time, human driven activities change habitats, reduce resources or introduce additional stresses to several wild species, which leads to changes in infection patterns.

In aquatic ecosystems we observe infectious salmon anaemia and salmon pancreatic disease, two viral infections of salmonids, expanding north along the Norwegian coast as a result of, among others, higher water temperatures. Brucellosis, a well-known bacterial zoonosis of terrestrial animals, has also been found and isolated from seals and whales in Norwegian waters. More recently influenza related mortality in seals along southern Scandinavia raised new questions on spread of viral diseases.

In terrestrial ecosystems vector borne diseases are perhaps the main concern, as new vectors expand to higher latitudes. Tick borne encephalitis has been reported in the Arctic and we have identified cervids positive for TBE. Deer ked, a biting fly causing alopecia in moose but often also biting humans, continues to expand north and has been found to carry the zoonotic bacteria *Bartonella* spp. In reindeer/caribou we have observed outbreaks of infectious keratoconjunctivitis connected to alphaherpesvirus infections, with outbreaks likely linked to increased stress due to food shortages, habitat reductions, and changes in husbandry.

Any bird or mammal, including marine mammals, may potentially be infected by the protozoan parasite *Toxoplasma gondii* as shown by recent studies in the Svalbard high Arctic Archipelago. Also Rabies, caused by a lyssavirus, has been reported in the Svalbard Archipelago (last in 2011) with arctic foxes and reindeer being infected.

Increased awareness/research on emerging pathogens in the Arctic is paramount to understand the underlying infection biology mechanisms in this very quickly changing ecosystem and better predict disease emergence and evaluate mitigation options.

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Do introduced nematodes pose a risk to endemic birds in New Zealand?

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Helminth parasites have not been widely studied in wild endemic birds in New Zealand and heavy infections are generally only a winter problem in juveniles undergoing nutritional or environmental stress. However, when native bird habitats are shared with introduced species, some endemic avian species may be exposed to introduced nematodes to which they are not well adapted resulting in pathogenic infections.

Ground feeding birds such as saddlebacks (*Philesturnus carunculatus*) and kiwi (*Apterynx sp*) appear to be particularly prone to these infections probably because of their consumption of invertebrates found on the forest floor which are often intermediate hosts to nematodes. More than 10 cases of intestinal perforation and an associated severe fibrinous peritonitis due to migrating *Porrocaecum* spp have been seen in North Island saddlebacks obtained from three different populations located on Tiritiri Matangi Island, Mt Bruce and Karori/Zealandia wildlife sanctuaries where they have been breeding successfully for the past 10 years. Introduced birds such as starlings (*Turdus vulgaris*) frequently flock in trees near the saddleback habitats.

Migrating ascarid larvae have been found to cause of visceral larval migrans in 12/232 (5.2%) of brown kiwi in which the liver, lung and heart were examined histopathologically. Neural larval migrans was found in 6/101 (5.9%) of brown kiwi. Infection rates in some populations could be higher than this as most kiwi mortalities are due to predation and the brain and viscera from such cases are not routinely examined histopathologically. PCR analysis was completed on some archived infected tissues using specific *Toxocara* primers but the results showed no evidence of infection with either *T. cati* or *T. canis*.

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Identification and molecular characterisation of blood-borne protozoan parasites in native mammal species from the Northern Territory – Australia.

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Little is known about the prevalence of blood-borne protozoans in native mammals from the Northern Territory in Australia and their potential contribution to the rapid and broad-scale decline of many species. The present study sought to provide baseline information on the prevalence and genetic diversity of potential pathogenic blood-borne protozoans in native mammals from the Northern Territory in Australia. A total of 221 blood samples from four target mammal species (northern brown bandicoots, common brushtail possums, northern quolls and brush-tailed rabbit-rats) were screened by PCR at the 18S rDNA locus for trypanosomes and piroplasms. Sequencing, phylogenetic analysis and microscopy were also conducted. Overall, 27.6% of the animals were positive for at least one haemoprotozoan species. The prevalence of trypanosomes was 17.6%; of these *Trypanosoma vegrandis* comprised 13.5%, while the remaining 4.1% were positive for *Trypanosoma sp.* AP-2011a isolate 64, previously reported in possums from Western Australia. This is the first report of *T. vegrandis* in northern brown bandicoots and first report of *Trypanosoma sp.* AP-2011a isolate 64 in possums from the Northern Territory. The prevalence of *Babesia sp.* and *Hepatozoon sp.* was 5% respectively and phylogenetic analysis identified a novel *Babesia* species and two novel *Hepatozoon sp.* species. Further investigation is needed to determine the potential clinical impact of these parasites upon their hosts in the Northern Territory and the role they may play in population decline events.

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What are fruit bats doing in my back yard? The disease and ecological implications of Grey-headed flying foxes in South Australia.

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Grey-headed flying foxes (*Pteropus poliocephalus*) arrived in Adelaide in 2010, outside their normal distribution range. Since then, despite pup mortality each summer due to heat stress, the population has continued to increase to ~ 3,000 bats. Within this population, Hendra virus (HeV) and Australian bat lyssa virus (ABLV), known to cause disease in horses and people, have been isolated, and a unique, as yet untyped Hendra-like virus of unknown pathogenicity has been discovered.

Why have the bats made Adelaide home? What do they feed on and where do they go? Can they survive in Adelaide and are they shedding HeV and other viruses which may pose a risk to horses and people in South Australia?

The presentation will provide an overview of Grey-headed flying fox ecology, preliminary epidemiological modelling data (using Outbreak software) and initial movement and foraging data using solar powered GPS collars.

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Haemolytic anaemia associated with theileriosis in an orphaned platypus (*Ornithorhynchus anatinus*).

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Haemoparasites are relatively common in many species of native Australian mammal; however, their potential role as disease agents and their influence on wildlife ecology is not well understood. The protozoan piroplasm *Theileria ornithorhynchi* is thought to cause little harm under normal circumstances, but in an immunosuppressed platypus may become a significant pathogen. A subclinical infection may become clinical when there is an alteration in the host-agent-environment relationship.

A tick-infested, juvenile female platypus was seen on the bank of the Murrumbidgee River near Oura during daytime following a flood and was brought into care. Blood was collected aseptically from the dorsal bill sinus and Diff Quik stained blood smears were prepared. Haematology revealed yellow plasma, a PCV of 0.17 and red cell count of $4.4 \times 10^{12}/l$ (reference ranges 0.49 - 0.51 l/l and 9.9 - $10.3 \times 10^{12}/l$ respectively) and a marked regenerative anaemia with reticulocytes, anisocytosis and nucleated erythrocytes. Large numbers of erythrocytes contained parasites morphologically consistent with *Theileria*. The life cycle of this organism is unknown, but is believed to have developmental stages within the tick. A semi-nested PCR using extracted DNA from whole blood produced an 18S rDNA gene that aligned with other *Theileria* and *Babesia* genotypes. Haematology also showed a left shift and toxic changes in the neutrophils, a monocytosis and some phagocytosis of parasitised erythrocytes. Despite tick removal and PCV improvement; the platypus' condition deteriorated, it died and was necropsied on day 5. Histopathology revealed a moderate erythroid hyperplasia of the bone marrow and spleen. Foci of hepatocellular necrosis and renal tubule deposits of bilirubin or haemosiderin were present. A pure growth of *Klebsiella pneumoniae* was isolated from liver.

The animal's death was attributed to a severe immune mediated haemolytic anaemia secondary to *Theileria ornithorhynchi* infection, accompanied by a terminal *Klebsiella pneumoniae* bacteraemia and septic hepatitis.

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Plasmodium species in bats in Kenya

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Bats are recognised reservoirs of zoonotic diseases. This project examined bats from households in Kenya for zoonotic pathogens. A concurrent cross sectional study provided information on zoonotic disease exposure in human and domestic animals from the same households. The project aimed to discover novel pathogens not previously reported in these hosts.

The study was conducted in western Kenya between April and November 2012. The 89 bats collected were comprised of *Pipistrellus* sp (15), *Chaerophon* sp (54), *Epomophorus* sp (8), *Scotoecus* sp (10) and *Taphozous* sp (2). Animals were captured from randomly selected households and anaesthetised for collection of heart blood. The animals were then euthanized for collection of fresh and fixed tissues. Blood smears were examined for haemoparasites.

Total nucleic acids (DNA/RNA) were extracted from serum, whole blood and tissue samples using the automated Roche MagnaPure LC instrument. Multiplexed libraries were prepared for both DNA and RNA and sequenced on the Roche 454 GS-FLX platform.

Malaria parasites were identified in the blood smears of four bats (*Epomophorus* sp). Individual libraries were prepared from 12 selected bat samples including all *Epomophorus* bats and a random selection of other bat species. Among the 12 selected bat samples 11 samples (92%) had *Plasmodium* species identified by sequencing.

Further work is being performed to confirm the *Plasmodium* species. Samples are also being examined by PCR for a variety of pathogens including hantavirus, lyssavirus and coronavirus. Histopathology is planned to document any pathological changes related to disease in these animals. Ongoing work will identify pathogens in the human inhabitants of these households.

Disease information from peridomestic wildlife together with information of disease exposure in humans and domestic animals will further our understanding of multi-host pathogens in this setting, allowing a "One Health" approach to understanding the epidemiology of zoonotic disease in households in western Kenya.

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Bovine TB Control in New Zealand: A real-time example of the convergence of human, animal and conservation medicine.

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New Zealand has had a long history of *Mycobacterium bovis* (TB) infection in cattle, and latterly deer, herds. This has been largely driven by wild animal infection, primarily possums, an introduced pest into New Zealand which is also responsible for extensive flora and fauna damage. New Zealand has been able to control its bovine TB problem

through the targeted control of these infected wild animal populations and at the same time achieve significant collateral benefit to flora and fauna protection. The New Zealand TB control strategy provides a working example of the convergence of human, animal and conservation medicine.

This paper will describe the history of the introduction of mammals (wild and domestic) into New Zealand which has led to the establishment of a complex ecological and epidemiological web of bovine TB in man, domestic animals and particularly wildlife species. The same introductions have led to large scale destruction of native flora and fauna. The paper will describe the various plans and strategies implemented over a seventy year period to successfully control bovine TB in New Zealand, especially the control of the disease in wildlife populations over the past 40 years.

The paper will also present a series of cases studies of the collateral beneficial impact on native flora and fauna protection resulting from the wildlife pest control strategies implemented for bovine TB control.

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Protecting Critically Endangered Sea Turtles from Invasive Predators in the Caribbean

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In the island of St. Kitts and Nevis in the Caribbean, the small Indian mongoose (*Herpestes javanicus*) predated on nests and hatchlings of critically endangered hawksbill and endangered green sea turtles. Mongooses are one of the 100 worst invasive species and they are responsible for extinctions and population declines of wildlife in the Caribbean and other locations where they were introduced. With this project we will provide an immediate benefit for critically endangered sea turtles, while obtaining baseline data for managing an invasive predator. By characterizing reproductive features of mongooses we will obtain important information for future research on non-lethal methods for fertility control. From a series of trapping of mongooses from the sea turtle nesting sites along with fitting a proportion of individuals with VHF telemetry collars, we will obtain data on population ecology that will be critical to understand population trends, habitat range and movement patterns. By testing for rabies virus antibodies and for *Leptospira* spp. we will assess the potential of mongooses as reservoirs of these important zoonotic diseases. We will investigate on the social dimensions (public attitudes, values, and knowledge) of invasive species management and wildlife conservation that will be instrumental for designing outreach and education efforts. This will also ensure public support that is a determinant concept for success and sustainability in future mongoose population control programs. Finally, the data generated of these various aspects will be critical for future research on wildlife conservation, animal welfare and invasive species management in St. Kitts and other regions in the Caribbean. This project is currently underway and we will present an update of the main findings during the conference.

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AMRRIC: A grass-roots One Health approach

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AMRRIC is a national not-for-profit charity that uses a One Health approach to coordinate veterinary and education programs in Indigenous communities. By working with remote Indigenous communities to improve the health and wellbeing of their pets, we are helping to create healthier, safer and happier communities.

Whilst our focus is on companion animal health and wellbeing, the geographic isolation of the communities with which we work, as well as the free-roaming nature of dogs and cats in these communities means that our work has direct relevance to local wildlife and its conservation. Some of the ways that our work supports wildlife populations include:

- Working with communities, building capacity and general knowledge around animal health and zoonoses;
- Working with local ranger groups and regional councils, providing educational resources about the impact of feral cats on the local environment, as well as the importance of good cat management;
- Educating community members about the reproductive and social differences between dogs and dingos, thereby helping to ensure that interactions between domestic dogs and local dingo populations are minimised;
- Supporting and training local Indigenous animal management workers to recognise and report exotic diseases such as rabies and old-world screw-worm fly.

AMRRIC has a strong academic focus and has collaborated on a wide variety of research topics. We assist ethically designed and culturally appropriate research by helping communities to determine the questions they would like solved, linking communities to researchers, advising on appropriate research design for remote Indigenous communities, and collecting and providing samples from the animals with which we work. AMRRIC aims to encourage and facilitate appropriate and culturally sensitive research programs to underpin and inform effective animal management and community health programs. We welcome enquiries from researchers in regards to research proposals.

Please visit www.amrric.org for further information.

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Caught in the act: the catastrophic collapse of the mountain chicken frog due to chytridiomycosis.

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Amphibian chytridiomycosis has caused precipitous declines of hundreds of species worldwide¹. These declines are often extremely rapid, with amphibians disappearing from sites before they are known to be infected, thus preventing the collection of real time population or infection dynamics data during the chytridiomycosis epidemic phase². By tracking two island populations of the Caribbean endemic mountain chicken frog (*Leptodactylus fallax*) before, during and after the emergence of amphibian chytridiomycosis, we were able to quantify the biological impacts of a near-extinction event due to this disease, possibly for the first time. We report one of the fastest declines of any species ever recorded, with a loss of over 85% of the population in less than 18 months on Dominica and within 12 months on Montserrat. Although collected only from the last intact population ahead of the epidemic wave on Montserrat, a conservation assurance population captured a representative sample of genetic diversity from the wild population, possibly because it was genetically homogenous across this small island. The disease led to a marked loss of genetic diversity from the Dominican population. The emergence of chytridiomycosis in the mountain chicken, and its spread from one island population to the other, was predictable, but the species decline could not be prevented. This case study highlights an urgent need to mitigate the emergence and spread of wildlife disease at both national and international scales if the CBD 2020 targets for halting biodiversity loss are to be attained.

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Malformations and disease in the Cururu Toad (*Rhinella jimi*) on the Archipelago of Fernando de Noronha, Brazil: A potential model for ecosystem health monitoring.

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The archipelago of Fernando de Noronha (FN) is located about 350km of the northeast coast of Brazil and is recognized as a UNESCO World Heritage site for the importance of the marine environment. Island environments are unique with many endemic species and compared to the continent there is greater vulnerability of species and habitats to threats. For Brazilian oceanic islands, invasive species and habitat loss are leading short-term threats to biodiversity. On FN, the current fauna of terrestrial vertebrates is mostly composed of introduced species including the Cururu Toad (*Rhinella jimi*). Previous work showed a high prevalence of toads on FN with limb and eye malformations and limited surveys demonstrated evidence of underlying disease problems (e.g. mycobacteriosis). To better define the extent of malformations and disease, we randomly collected and examined 100 adult toads and collected samples for histopathology, microbiology, parasitology and radiology. Apparent anomalies were found in 45 animals with 84.4% affected the limbs (e.g. short, missing or fused digits) and 11.1% affecting the eyes (e.g. microphthalmia or phtthis bulbi). Radiology and histopathology suggests some anomalies are not developmental, but instead were caused by trauma (e.g. fractures) or infectious diseases (e.g. osteomyelitis or panophthalmitis). Twenty-nine percent of the animals had granulomas in the liver, spleen and kidney with intralosomal acid-fast bacteria. A *Mycobacterium marinum*-type organism was isolated from most of these cases. A small number of animals had a protozoan parasite in the brain with morphologic similarities to both *Toxoplasma gondii* or *Cystodiscus* sp. (identification pending). The health alterations presented by these toads, introduced in an altered habitat, suggests that they can become useful as a model for ecosystem health monitoring for endemic wildlife species on FN.

Characterising parasites of an endangered South Australian lizard as risk assessment for a future translocation

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The Pygmy Bluetongue Lizard (PBT), *Tiliqua adelaidensis*, is endemic to the native grasslands of the Mid-North region of South Australia and exclusively inhabits burrows dug by lycosid and mygalomorph spiders. Habitat destruction by urbanisation and cropping has restricted its distribution to a small number of isolated populations across its former range. Bio-climatic modelling indicates that the PBT is further threatened by climate change. Translocation is an arguably viable conservation strategy, and an experimental translocation is planned for early 2016, allowing us to assess the ecological and also parasite/pathogen transmission risks posed by uniting PBT lizards from a donor population with PBT lizards from the recipient community.

Variation within and between PBT populations will be assessed prior to the translocation, by characterising endoparasitic biota from up to ten PBT individuals from ten isolated populations from across the species range. Parasite taxa targeted for typing will include enteric bacteria (using high-throughput metagenomic techniques), a PBT-specific nematode *Pharyngodon wandillahensis*, and if present, blood and gut protozoans (to be detected by microscope), and viruses (to be detected by serology). Inter-population variation will be assessed by parasite community composition analysis and by whether there are genetic differences between common parasitic species. Any distinct inter-population differences in parasite biota will serve as markers of host population of origin, as transmission dynamics between translocated and resident reptiles are monitored after the translocation. In the two years following the translocation, parasite biota of PBT individuals will be compared to their status as donor or resident, and to measures of fitness (body condition, reproductive success), allowing us assess the risk posed by parasite/pathogen transmission and the viability of translocation as a conservation strategy for *Tiliqua adelaidensis*, and more broadly for other endangered species.

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Developing a population health index for an apex marine Arctic predator, the narwhal (*Monodon monoceros*), using biological, medical and sociocultural measures to predict resilience in a time of climatic, economic and social change.

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The objective of this study is to create a comprehensive and integrative tool, using established and innovative measures of health (traditional knowledge, community monitoring, medical and pathological data) and stress (blubber cortisol, stress related proteins, skin microbiomes), for culturally and economically important Arctic marine mammals. By utilizing archival and current data and samples, this cross-disciplinary approach will enable a predictive assessment of adaptation to future regional ecological and climatic change, and economic development. Marine mammals are apex predators in the Arctic and sentinels for the ecosystem as a whole. Narwhal summer in areas of north Baffin Island (Pond Inlet & Arctic Bay) that are being affected significantly by climate change and resource development, yet we lack knowledge of past and current health markers which could facilitate evidence based policy decisions in this area. Proof of concept for this health index will be developed using archived narwhal tissues (1982-2013), field samples collected in collaboration with hunters (2013-2017), community focus groups, hunter and elder interviews. Data will be measurable against recorded climate parameters, and resource development activity in specific areas such as Milne Inlet. Preliminary data on hematology, serum chemistry and respiratory tract flora are presented from live whales captured for telemetry studies conducted over a 10 year period. While no food safety issues were identified, a pathology survey of harvested whales provided by the Mittimatalik Hunters and Trappers Association, documented respiratory pathology and neoplasia. Analysis of corticosteroid levels in blubber, and skin microbiomes are underway. We believe this new health assessment index will help policy makers and communities to promote economic development in an environmentally sustainable manner by providing evidence of stable or changing health status earlier than reliance on crude measures of mortality or changing biological indices.

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Flying-fox roost disturbance and Hendra virus spillover risk

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Flying-foxes (genus *Pteropus*) are the natural host of Hendra virus (HeV) which periodically causes fatal disease in horses and humans in Australia. The presence of flying-foxes in urban areas has increased in recent decades, and often provokes negative community sentiments resulting in calls for the dispersal of urban flying-fox roosts. However, it has been hypothesised that disturbance of urban roosts may result in a stress-mediated increase in virus excretion, promoting infection in flying-foxes, and increasing the risk of spillover to horses and consequently humans. We sought to examine the impact of dispersal on urinary HeV infection and cortisol dynamics in flying-foxes. The data were analysed in generalised linear mixed models using restricted maximum likelihood. The difference in mean HeV prevalence in samples collected before (4.9%), during (4.7%) and after (3.4%) roost disturbance was non-significant ($P = 0.440$). Similarly, the difference in mean urinary cortisol concentrations was non-significant (before = 22.71 ng/mL, during = 27.17, after = 18.39) ($P = 0.550$). We did find associations between cortisol concentration, season and region, while the effect of dispersal on cortisol concentration approached statistical significance for region, plausibly reflecting the nature and timing of disturbance. We also found a positive statistical association between HeV excretion status and urinary cortisol concentration, but elaborating any causal association was beyond the scope of the study. These findings usefully inform public discussion and policy development in relation to Hendra virus and flying-fox management. Qualitative assessment of behavioural distress associated with roost disturbance showed that the severity of impact reflected the nature and timing of the activity, and highlights the need for a 'best practice' approach to dispersal. While the mobility of flying-foxes provides some capacity to escape anthropogenic disturbance, their increasing urban presence may subject them to chronic roost disturbance and harassment, the consequences of which are unknown.

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Evaluation of semen characteristics of Persian fallow deer (*Dama Dama Mesopotamica*) in dasht-e -naz refuge in Iran

behrang ekrami

Persian fallow deer (*Dama dama mesopotamica*) is belonging to the family Cervidae and is only found in a few protected areas in the northwest, north, and southwest of Iran. The aims of this study were analysis of inbreeding and morphometric parameters of semen in male Persian fallow deer to investigate the cause of reduced fertility of this endangered species in Dasht-e-Naz National Refuge, Sari, Iran. The Persian fallow deer semen was collected from four adult bucks randomly during the breeding and non-breeding season from five dehorned and horned deer's BY an artificial vagina. MANY DAG defect abnormalities observed in all samples. The post-mating season collected ejaculates contained abnormal spermatozoa, debris and secretion of accessory glands in horned bucks and accessory glands secretion free of any spermatozoa in dehorned or early velvet budding bucks. Microscopic evaluation in all four bucks during the mating season showed the mean concentration of 9×10^6 spermatozoa/ml. The mean \pm SD of age, testes length and testes width was 4.60 ± 1.52 years, 3.58 ± 0.32 and 1.86 ± 0.09 cm, respectively and the DAG defects may be the cause of high rate of polymorphism because of small primary herd size of Persian fallow deer that needs more genetically evaluation

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Occurrence of the Koala Retrovirus (KoRV) and *Chlamydia pecorum* in the Mount Lofty Ranges koala population

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Two key infectious pathogens of koalas (*Phascolarctos cinereus*) are the koala retrovirus (KoRV) and *Chlamydia pecorum*. High KoRV viraemia is associated with lymphoid neoplasia and immuno-compromise in infected koalas, leading to secondary infections with opportunistic pathogens such as *Chlamydia*, which cause ocular, respiratory and urogenital tract disease.

Both pathogens are highly prevalent in eastern Australian populations, however the prevalence in the South Australian Mt Lofty Ranges koala population is unknown and thought to be low. This study identified the occurrence of KoRV and *C. pecorum* in the Mt Lofty Ranges and whether there is an association between KoRV and *Chlamydia*.

Blood samples were collected prior to euthanasia from 21 euthanised koalas submitted for necropsy. Haematology and KoRV status as determined by PCR were assessed. Dry swabs of the conjunctiva and urogenital sinus were collected for chlamydial detection using Clearview Chlamydia MF test and q-PCR. An additional 10 samples were collected from hospitalised koalas that were released.

The occurrence of KoRV-provirus was 77.4% (24/31) with 12.5% of affected koalas having lymphoma (3/24). *Chlamydia pecorum* was identified at 32.3% (10/31) with half of these koalas showing clinical disease (5/10) and the remainder subclinical carriage (5/10). Reproductive tract disease was the most common presentation of chlamydial disease. A trend towards significant association was identified between KoRV and Chlamydial infection ($P = 0.083$).

The high occurrence of KoRV and chlamydial infection in rescued koalas indicates these pathogens are significant in the Mt Lofty Ranges koala population, requiring further investigation to determine the true prevalence in this population.

Assessment of metal ingestion in female northern pintails (*Anas acuta*) wintering along the Texas coast

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Lead poisoning through shot ingestion was historically one of the largest health issues affecting waterfowl in North America. Lead shot was banned for use in waterfowl hunting in the United States in 1991, and in Canada in 1997. However, biologists need to understand how and if lead shot remaining in the environment will continue to impact waterfowl. Our goal was to estimate lead and non-toxic shot consumption by female northern pintails (*Anas acuta*) wintering along the Texas Coast. We found shot in the gizzards of 39 (17%) of 227 female northern pintails collected along the Texas Coast. Of these, lead shot was found in 7 gizzards, steel shot was found in 24 gizzards, and other non-toxic shot was found in 20 gizzards. Some females consumed multiple shot types. Overall shot (lead and non-toxic combined) ingestion rates were similar to those found prior to the lead ban in Texas (14%) and Louisiana (17%); however, lead ingestion rates were considerably lower, suggesting that lead is becoming less available over time. All northern pintails that had lead shot in their gizzards were collected from coastal habitats. Whereas, it seems that lead consumption by northern pintails has decreased, monitoring lead consumption rates from different regions will provide insight into lead's resilience and prevalence in different habitats and under various environmental conditions.

Lack of human awareness and the need for increased public education regarding the emerging zoonotic parasite, *Baylisascaris procyonis*

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Publish consent withheld

Apicomplexan protozoal infection, presumably *Theileria cervi*, associated with brain abscessation in a free-ranging, white-tailed deer from Georgia, U.S.A

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An eight month old, male free-ranging white-tailed deer was submitted to the Southeastern Cooperative Wildlife Disease Study in Athens, GA with a history of abnormal behavior. Gross post mortem findings included diffuse hepatic fibrosis, cranioventral bronchopneumonia, and a brain abscess from which *Trueperella pyogenes* was cultured. Histologically, there was extensive meningoencephalitis associated with intracellular protozoal organisms. By immunohistochemical staining, the protozoal organisms were negative for *Neospora caninum*, *Toxoplasma gondii*, and *Sarcocystis neurona*. On genetic sequencing, the organism was most closely related to *Theileria cervi*. In the brain, multiple intracytoplasmic ovoid apicomplexan organisms that measured 3940nm by 2579nm with a visible conoid apparatus were observed by transmission electron microscopy. Infected cells had positive cytoplasmic immunohistochemical staining for glial fibrillary acidic protein and negative staining for neurofilament, CD3, CD79a, and CD20, supporting the histologic appearance suggestive of astrocytic origin. *Theileria cervi* is transmitted by the tick *Amblyomma americanum*. Infection with *T. cervi* is common in free-ranging white-tailed deer within the United States with rates of infection exceeding 50% in enzootic areas. Clinical disease is rare and typically only reported in heavily infected fawns. Mycotoxins and bovine viral diarrhea were also not detected with ancillary testing. This is the first report, to the authors' knowledge, of *T. cervi* being associated with a brain abscess in a white-tailed deer.

A review of parasites causing disease in squamates: methods for their detection and implications for conservation and zoonosis

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Squamata is a large and diverse order of reptiles comprising lizards and snakes, with over 7000 described species. In a view to provide a resource for biologists, we aimed to review squamate parasites particularly those that cause a fitness effect on their wild host. We also review the detection methods available. Secondly, the review will cover the role that parasites and pathogens play in squamate conservation, and investigate incidences of squamate zoonoses. This review will make it easier for biologists to investigate pathogens in wild populations and will highlight areas requiring further study, including pathogens that present a *risk* to squamate conservation and human health.

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Pathology in the female upper genital tract of koalas (*Phascolarctos cinereus*) (Victoria, Australia)

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Abnormalities within the female reproductive tract of koalas (*Phascolarctos cinereus*) have long been recognised. During a retrospective study of koala health with Wildlife Health Surveillance Victoria, nine adult, female koalas were identified at necropsy with abnormalities within the genital tract. Gross findings included cystic and papillary structures within the upper genital tract (ovaries, periovarian tissue and oviducts), thickening of uterine horns, as well as mammary masses within the pouch. Samples were collected for histopathology and neoplastic lesions were evaluated based on WHO classification of ovarian neoplasia in human medicine. Histopathology of the ovaries revealed the presence of ovarian cysts (1/9), papillary hyperplasia (1/9) and cystadenoma (1/9), borderline tumour (3/9) and carcinoma (1/9). Carcinoma also appeared to arise within the fallopian tube (2/9). In most cases (6/9), extensive fibrosis containing distorted and cystic endometrial glands was observed within uterine horns, along with mild to moderate chronic inflammation within the uterus and/or cervix. The masses observed within the pouch were consistent with mammary adenoma and lipoma. PCR from cloacal swab revealed the presence of *Chlamydia pecorum* in one case and PCR from spleen was positive for Koala retrovirus (KoRV) in four cases. The histopathological findings of benign changes observed within the upper genital tract are similar to those described in the literature. However, our findings suggest that malignant neoplasia is part of the spectrum of pathology within the koalas' female reproductive tract. *Chlamydia* infection has been associated with chronic inflammation and fibrosis within the uterus leading to cystic changes within the upper genital tract. Failure to detect *Chlamydia* sp. in the majority of these cases could indicate intermittent shedding or resolved infection. Interestingly, all animals displaying carcinoma were positive for KoRV. Further investigations should be performed to identify possible association between the presence of these agents and the development of malignant lesions.

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Atlantic cod (*Gadus morhua*): a transmission host for *Brucella pinnipedialis*?

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High prevalences of anti-*Brucella* antibodies have been reported in hooded seals (*Cystophora cristata* - HS), suggesting that brucellosis (a bacterial infection inducing abortion in terrestrial mammals) may contribute to the dramatic decline of the Northeast Atlantic stock. However, pathological changes have never been identified in infected HS. Age-dependent serological and bacteriological patterns suggest that seals are exposed to an environmental source of *B. pinnipedialis* during their first year of life (after weaning), followed by the clearance of infection. This raises questions about the existence of a reservoir of *B. pinnipedialis* in the HS food web.

Atlantic cod (*Gadus morhua*) is part of the diet of adult HS, and was, therefore, experimentally infected with a *B. pinnipedialis* HS wildtype strain. Fish were challenged with 10^5 *B. pinnipedialis* intraperitoneally. No mortality or macroscopic pathology was recorded. Samples of blood, liver, spleen, muscle, heart, head kidney, female gonads were collected from 5 fish at day 1, 7, 14 and 28 pi to determine the bacterial load. *Brucella pinnipedialis* HS strain was retrieved from all organs investigated, except muscle. Our results show that *B. pinnipedialis* induce bacteremia in 6/8 Atlantic cod for an extended period of time (up to 28 days pi).

Anti-*Brucella* antibodies were detected in fish at day 28 pi by RBPT and ELISA.

Primary leukocytes were isolated from head kidneys of non-infected fish and challenged with *B. pinnipedialis* (reference strain) or the HS strain used in vivo. Both strains entered the leukocytes and survived intracellularly without any reduction in retrievable numbers of bacteria for at least 48 hours.

Our results indicate that *B. pinnipedialis* has a prolonged course of infection in Atlantic cod. Atlantic cod may thus act as a transmission host for *B. pinnipedialis* in the marine environment.

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Natural Hendra virus infection in flying-foxes, tissue tropism and risk factors.

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Hendra virus (HeV) is a lethal zoonotic agent that emerged in 1994 in Australia. Pteropid bats (flying-foxes) are the natural reservoir. To date, HeV has spilled over from flying-foxes to horses on 51 known occasions, and from infected horses to close-contact humans on five occasions. We undertook screening of archived bat tissues for HeV by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Tissues were tested from 310 bats including 295 Pteropodiformes and 15 Vespertilioniformes. HeV was detected in 20 (6.4%) individual flying-foxes from various tissues including spleen, kidney, liver, lung, placenta and blood components. Detection was significantly higher in *Pteropus alecto* and *P. conspicillatus*, identifying species as a risk factor for infection. Further, our findings indicate that HeV has a predilection for the spleen, suggesting this organ plays a vital role in HeV infection. The lack of detections in the foetal tissues of HeV-positive females suggests that vertical transmission is not a regular mode of transmission in naturally infected flying-foxes, and that placental and foetal tissues are not a significant source of infection for horses. A better understanding of HeV tissue tropism will strengthen management of the risk of spillover from flying-foxes to horses and ultimately humans.

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Granulomatous pneumonia associated with haemosporidial schizonts in little red flying foxes (*Pteropus scapulatus*)

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Pteropid bats harbour a diversity of haemosporidial parasites, but associated disease has not been reported previously. The best known of the pteropid haemosporidia are several species of *Hepatocystis*, which also occur in rodents and primates¹. Recently two new monospecific genera, *Sprattiella alecto* and *Johnspretentia copemani*, have been described from black flying foxes (*Pteropus alecto*) in Queensland^{2,3}. *Hepatocystis*, *Johnspretentia* and *Sprattiella* have schizonts within the liver, lung and kidney respectively, but no significant pathology has been attributed to their presence. At this laboratory small numbers of unidentified haemosporidial schizonts are sporadically seen in the lungs of both black and little red flying foxes (LRFF), *Pteropus scapulatus*, during the course of disease investigations; they are generally considered an incidental finding. However haemosporidial schizonts were associated with granulomatous, eosinophilic pneumonia observed in a series of six LRFF from southeast Queensland during March - May 2014.

In these cases, schizonts of variable morphology were present within lungs of all six bats, most frequently within blood vessels. Inflammation was angiocentric, varied from eosinophilic to granulomatous, and often spread into adjacent alveoli. Pneumonia was deemed mild in half of these bats and moderate to severe in half. Protozoal schizonts were somewhat similar to *Johnspretentia copemani*. They varied from round to oval to sinuous, and from a minimum diameter of 21µ up to 150µ or more in length. Using fresh lung from two LRFF, a 486bp portion of the cytochrome b gene was amplified by PCR and sequenced¹. 99% identity was obtained with a parasite described as "*Hepatocystis* sp." from *Pteropus vampyrus* in Malaysia. Attempts to characterise these parasites continue.

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Diversity of MHC *DQB* and *DRB* genes in the endangered Australian sea lion pups (*Neophoca cinerea*).

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Major histocompatibility complex (MHC) class II molecules have an important role in vertebrate adaptive immunity, being responsible for recognising, binding and presenting specific antigenic peptides to T lymphocytes. We have studied the MHC class II *DQB* and *DRB* exon 2 genes of the Australian sea lion (*Neophoca cinerea*), an endangered pinniped species that experiences high pup mortality. Following characterisation of *N. cinerea DQB* and *DRB* by molecular cloning, and evaluation of diversity in pups across two colonies using variant screening (n = 47), three *DQB* alleles and ten *DRB* variants (including one pseudogene allele) were identified. The higher diversity at *DRB* relative to *DQB* is consistent with other studies in marine mammals. Despite overall lower MHC class II allelic diversity relative to some other pinniped species, we observed similar levels of nucleotide diversity and selection in *N. cinerea*. In addition, we provide support for recent divergence of MHC class II alleles. The characterisation of

MHC class II diversity in the Australian sea lion establishes a baseline for further investigation of associations with disease, including endemic hookworm infection, and contributes to the conservation management of this species.

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Mycobacteriosis in New Zealand Birds

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Mycobacteriosis is an important disease of poultry, companion birds and wild (including captive) birds. Mycobacterium avium complex (MAC) and Mycobacterium genavense are the two most common isolates seen in birds. Both these isolates also have zoonotic potential. Post mortem diagnosis of avian mycobacteriosis is straight forward, but ante-mortem diagnosis is difficult as clinical signs, haematology and biochemical changes are non-specific and can mimic other disease processes. Acid-fast staining of faeces is also problematic as faecal shedding of these organisms can be intermittent and differentiation of pathogenic versus non-pathogenic species of mycobacteria is not possible. In birds routes of infection are most commonly via ingestion, but aerosol and cutaneous routes of infection are also reported. A search of out post mortem database revealed 7/6631 (0.001%) cases of mycobacteriosis in four different species of wild bird. Three birds, a Southern Giant Petrel and two Blue Penguins had severe respiratory mycobacteriosis; the two Blue Penguins were from the same captive institution. Two birds were Weka, both from the same captive institution; one bird had focal involvement of the small intestine while the other bird had generalised involvement of the intestine as well as microscopic lesions in the liver and spleen. Two birds were Australasian Harriers; one bird, from a Rehabilitation facility, had generalised mycobacteriosis, with lesions in the gastrointestinal tract, spleen, lungs, skin and bone, while the second bird, which was wild, had cutaneous lesions around the feet. Mycobacterium avium complex (MAC) was isolated from the lung of one of the Blue Penguins and culture results from one of the Weka are pending. A diagnosis of mycobacteriosis in a captive facility poses logistic difficulties in terms of what to do with the remaining population of in-contact birds, as well as management practices to reduce the chance of zoonotic spread to staff.

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Review of pathology of a population of captive Tasmanian devils (*Sarcophilus harrisii*) 2007-2015

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The Tasmanian devil (*Sarcophilus harrisii*), the world's largest carnivorous marsupial, has suffered significant wild population declines in the last two decades, primarily due to Devil Facial Tumour Disease. One strategy implemented to protect the species from extinction has been establishment of an insurance population in selected zoos and parks throughout Australia. Whilst disease processes of wild Tasmanian devils remains an area of active research, comprehensive descriptions of the pathology of captive devils are lacking.

A retrospective study was undertaken to summarise pathological conditions diagnosed ante-mortem and post-mortem in an insurance population of Tasmanian devils (*Sarcophilus harrisii*) housed at Taronga Western Plains Zoo in Dubbo, Australia between 2007 and 2015.

Complete medical records for 74 Tasmanian devils held at the zoo during the study period were reviewed. The incidence of each identified disease process was defined and associations between diagnoses examined. The influence of factors such as age, gender, origin (wild versus captive), length of time in captivity and parentage was explored for each disease process.

The most commonly identified pathologies included cutaneous mycobacteriosis and a variety of other dermatoses, disorders of the cardiovascular system and neoplastic processes. Confirmed diagnoses not previously reported in Tasmanian devils include brainstem astrocytoma, congestive heart failure manifested as second degree atrioventricular block and infundibular keratinizing acanthoma.

This study expands the understanding of the diseases of captive Tasmanian devils and provides an important base for further development of health monitoring programs for this important population of animals.

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***Avioseerpens* in North American *Aechmophorus occidentalis* (Western Grebe): A new host and geographic record for a dracunculoid nematode and implications of migration and climate change**

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Aviosempens is a dracunculoid nematode common in domestic ducks in Taiwan. Dracunculoid worms are rarely recorded in North American bird species. This is a report of *Aviosempens* sp. in *Aechmophorus occidentalis* (Western Grebe) from southern Arizona, a bird species of rare occurrence in the Sonoran Desert of the southwestern United States. The bird was submitted to the Tucson Wildlife Center (TWC) for examination and treatment of crash-landing trauma, and was subsequently found to harbor dracunculoid nematodes. The worms were collected, identified, and a treatment plan outlined prior to release. This is the first record of *Aviosempens* from a Western Grebe, and the first record of the parasite in the desert southwest of the USA. Its discovery underlines the importance of veterinary involvement in diagnostics, intervention, and parasite identification in the wildlife rehabilitation setting. The implications of climate change, alterations in migratory patterns, and the possibilities for migratory spread of unusual parasites are discussed.

Salmonella serotypes isolated from reptiles in the Kimberley, Western Australia

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There is often a high prevalence of *Salmonella* carriage and shedding in captive reptiles. It has been suggested that this may be due to contact with humans and domestic animal species, or the stress of captivity. Little is known about the ecology of *Salmonella* in truly wild reptiles. In Australia, the rates of human salmonellosis are highest in Northern Australia, with consistently high rates observed in the Kimberley region of Western Australia. The aim of this study was to investigate the prevalence and serotypes of *Salmonella* in wild reptiles in the Kimberley.

Methods:

In May 2011, 130 wild reptiles belonging to 41 different species were caught in the Kimberley region of Western Australia. Cloacal swabs were taken for the isolation of *Salmonella*. Isolates were obtained via selective enrichment, and identified via biochemical tests and serotyping.

Results:

Forty percent of reptiles sampled were positive for *Salmonella*. Nineteen different *Salmonella enterica* serotypes were identified, belonging to subspecies I, II, IIIb and IV. The most common serotype was *Salmonella* Rubislaw (prevalence = 10.8%).

Conclusion:

This study showed a high prevalence of *Salmonella* in wild reptiles in the Kimberley. These reptiles have had minimal contact with humans and domestic species, suggesting that they are natural carriers of *Salmonella*. The main serotypes identified differed from the most common isolates in cases of human disease, but many have known pathogenicity in humans. Three of the top ten human *Salmonella* serotypes identified in 2011 in Western Australia – Paratyphi B biovar Java, Chester and Muenchen – were isolated from reptiles in this study. The high prevalence of *Salmonella* in these wild reptiles and the *Salmonella* serotypes reptiles and humans have in common, suggests that reptiles are likely to be significant contributors to the natural ecology of *Salmonella* and a significant reservoir for the organism.

PREDICT - Wildlife Disease Surveillance in Viet Nam

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The PREDICT¹ project was designed to strengthen capacities in developing countries to detect, control, and prevent zoonotic viruses of pandemic potential. In Viet Nam the first stage of the extensive study identified potential high-risk interfaces for zoonotic disease spillover from wildlife to humans. Many of the high-risk interfaces identified in Viet Nam were linked to the domestic and international trade of wildlife. Samples were collected at these high-risk interfaces from wild rodents, bats, carnivores and non-human primates. Through this collaborative effort with Vietnamese research, academic and government institutions, PREDICT collected nearly 7,000 samples from wildlife and completed over 16,300 assays in Vietnamese and international laboratories to identify known and novel viruses. Viral Family/Genus conventional PCR assays were applied followed by cloning and sequencing for confirmation of results and virus identification. In Viet Nam 24 novel viruses (2 Corona, 2 Herpes, 5 Paramyxo and 15 Rhabdoviruses) and 3 known viruses (1 Corona, 1 Paramyxo and 1 Influenza) were detected. Further work will be focused on understanding the biological and behavioral factors influencing the spillover, amplification and spread of pathogens at these high-risk interfaces to mitigate the risk of pandemic disease emergence.

Risk Factors Associated with the Prevalence of Antibodies to *Leptospira interrogans* in Wild Lowland Tapirs (*Tapirus Terrestris*) in the Brazilian Pantanal

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There is an increasing need to understand epidemiology and risk factors of wildlife infectious diseases, especially for emerging zoonosis. The objective of this study was to investigate the major risk factors associated with the prevalence of *Leptospira interrogans* antibodies in wild lowland tapirs (*Tapirus terrestris*) in a seasonally inundated floodplain. The study was carried out in a private cattle ranch in the Brazilian Pantanal, Mato Grosso do Sul State (19°20'S; 55°43'W). Seventy-nine blood samples from 45 wild lowland tapirs (27♂ and 18♀) were collected between 2008 and 2014. Microscopic agglutination (MAT) was used to test the samples for 26 serovars of *Leptospira* spp. Antibodies against *L. interrogans* were observed in 65% (95% CI: 50-77%) of tapirs and 73% (95% CI: 63-82%) of tested samples. Nine *L. interrogans* serovars were found with considerably high antibody titers: Pomona 100–3200, Icterohaemorrhagiae 100–800, Bratislava 100–800, Grippotyphosa 100–400, Canicola 100, Copenhageni 100–400, Pyrogenes 800, Wolfii 200, and Hardjo 200. Age was negatively correlated with presence of *L. interrogans* (simple logistic regression; $p < 0.01$) and juveniles presented lower infection rate (Fisher-Freeman-Halton test, $p < 0.01$) when compared to sub-adults and adults. Higher titers of Pomona serovar were significantly correlated to the presence of other serovars ($p < 0.05$) and to higher numbers of these ($r = 0.40$; $p < 0.01$). There were no significant differences when comparing sex (males and females), seasons (wet and dry) or locations with higher or lower volumes of water during the seasonal floods. Further statistical analysis will investigate temporal variation of antibody titers (for individuals captured and sampled multiples times throughout the study), spatial ecology (home range overlap) and kinship relationships. Despite the high antibody titers found in lowland tapirs, no clinical signs or laboratory results indicated disease in these animals. Nevertheless, potential health issues caused by exposure to *Leptospira* spp. should not be disregarded.

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Investigation of bat-to-bat transmission of Marburg virus in the Egyptian fruit bat, *Rousettus aegyptiacus*

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The Egyptian fruit bat, *Rousettus aegyptiacus*, was recently identified as a major natural reservoir host for Marburg virus (MARV). Experimental inoculation of *R. aegyptiacus* from the laboratory-breeding colony with MARV confirmed that this bat species is indeed the natural reservoir host of the virus. However, the mechanisms of bat-to-bat transmission of MARV among *R. aegyptiacus* remain unknown. Therefore, the objective of this study was to determine whether bat-to-bat transmission of MARV can occur in a controlled laboratory environment through the direct, indirect and/or airborne routes. This study used a total of 38 juvenile bats: 12 inoculated donor (ID) bats, 24 naïve contact (NC) bats and 2 negative control (NEG CO) bats. The ID bats were inoculated with MARV, the NC bats received no inoculation and the NEG CO bats were inoculated with media. The ID and NC bats were housed in partitioned cages, separated by wire mesh or solid metal partitions. Blood, urine, and oral and rectal swabs were collected from bats daily from 0 through 25 dpi and then weekly through 56 dpi. These specimens were used to monitor viremia and virus shedding by Q-RT-PCR and virus isolation, and to monitor the MARV IgG antibody response. This study identified multiple routes of MARV shedding in the ID bats and evidence of oral exposure in NC bats. Further transmission studies will be necessary to better characterize bat-to-bat transmission of MARV in a controlled laboratory environment.

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Novel paramyxoviruses in Australian flying-fox populations support host-virus coevolution.

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Understanding the diversity of henipaviruses and related viruses is important in determining the viral ecology within flying-fox populations and assessing the potential threat posed by these agents. This study sought to identify the abundance and diversity of previously unknown paramyxoviruses in Australian flying-fox species (*Pteropus alecto*, *P. scapulatus*, *P. poliocephalus* and *P. conspicillatus*) and in the Christmas Island species *P. melanotus natalis*. Using a degenerative RT-PCR specific for the L gene of known species of *Henipavirus* and two closely related paramyxovirus genera *Respirovirus* and *Morbillivirus*, we identified an abundance and diversity of previously unknown

paramyxoviruses (UPV), with a representative 31 UPVs clustering in eight distinct groups (100 UPVs/495 samples). No new henipaviruses were identified. The findings are consistent with a hypothesis of co-evolution of paramyxoviruses and their flying-fox hosts. Quantification of the degree of co-speciation between host and virus would strengthen this hypothesis.

Anaesthetic Considerations for Free-ranging Adult Male Australian Sea Lions

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The anaesthesia of free-ranging male sea lions in a shoreline environment presents many challenges. The size and physical strength of adult male sea lions precludes induction via face mask and their tendency to haul out and remain close to the water line necessitates careful selection of individuals and thorough planning to mitigate the risk of darted animals returning to the water while becoming anaesthetised. The Department of Parks and Wildlife (DPaW), the Department of Fisheries and Perth Zoo undertook a collaborative project to anaesthetize free-ranging adult male Australian Sea Lions (*Neophoca cinerea*) at Carnac Island, Western Australia, to facilitate fitting of satellite tracking devices to determine their feeding and activity patterns. Four sea lions were anaesthetised during two field trips in October and November 2009. Body weights of sea lions were estimated by wildlife rangers from DPaW to be in the body weight range of 150-180kg. Sea lions were anaesthetised with tiletamine-zolazepam (Zoletil®) at an estimated dose rate of 1-1.1mg/kg. Three of the sea lions were intubated and maintained on isoflurane after induction; the fourth animal roused itself during supplementary mask induction with isoflurane, before it could be intubated, so the procedure was discontinued. This animal was monitored for several hours after darting, both in and out of the water. Of the three animals fitted with tracking devices, two lost their devices naturally. The third was anaesthetised again five weeks later, using the same anaesthetic protocol, for removal of the device. Careful planning, consultation with groups undertaking similar studies and engagement of personnel experienced in sea lion biology, ecology and anaesthesia were important factors in the success of the procedures.

Trichuris trichiura in African Green Monkeys: a One-Health Approach to a Zoonotic Threat for Humans.

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Trichuris trichiura is the third most common nematode infection in humans (>600 million people infected), predominately in children. Infection occurs from consumption of food, water or soil contaminated with larvated eggs. To prevent infection, focus has been placed on hygiene education, sanitation and mass deworming of children. Preliminary data indicates that African Green Monkeys (AGM; *Chlorocebus aethiops*) on St. Kitts are infected with a strain of *T. trichiura* that also infects humans. We hypothesized that infections in AGM pose a zoonotic threat to humans. Feces from trapped AGM (69 samples, n=69) were found to contain *T. trichiura* eggs. The Biomedical Science Foundation trapped AGM between 16 and 26 of January 2015 from Tabernacle, Phillips and Estridge Mountains, Monkey Hill, West Farm and Saddler's St. Kitts. All procedures were conducted under an approved IACUC protocol or SOP. Rainfall from the mountain locations was determined to feed into surface water collection areas for human consumption and/or dams for agriculture irrigation. Approximately 30% of the water for human consumption on St. Kitts is from surface water treated via sedimentation, sands filters and chlorination; however, *T. trichiura* eggs are only effectively removed via sedimentation. Dam water for irrigation is not treated. Therefore, there is the potential for monkey feces with *T. Trichiura* eggs to contaminate drinking and irrigation water. A One-Health approach is required to ensure that previous controls are not lost due to a new source of infection.

Evaluating common storage methods of wildlife corn to reduce aflatoxin production

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Populations of northern bobwhites (*Colinus virginianus*) have steadily declined in the United States, prompting wildlife managers to provide supplemental feed. Grain can contain aflatoxin, which is a harmful fungal metabolite of *Aspergillus flavus* and *Aspergillus parasiticus*. Our objective was to assess common grain storage methods of wildlife corn that can shed light on how to reduce aflatoxin production. We placed wildlife corn in open air, storage

shed, and pavilion environments, and in metal containers, aluminum containers, and plastic containers, which mimicked feeder-type structures. We determined weekly aflatoxin levels and grain moisture content. In addition, we monitored weather parameters (daily temperature, relative humidity, and dew point) inside and outside of each storage structure. Grain moisture and aflatoxin concentrations fluctuated weekly within each type of storage container ($F_{6,28} = 7.23$, $P < 0.0001$) and ranged from 10.4–97.5% and 0–1,200 ppb, respectively. Each storage type contained corn samples that exceeded recommended aflatoxin levels deemed safe for wildlife within 2 weeks. After 8 weeks of storage, aflatoxin concentrations began to increase significantly in each storage method. Condensation build-up within metal storage containers increased mold growth on corn, which subsequently resulted in greater aflatoxin concentrations of corn along the sides of such containers compared to corn sampled within the center of the same container. Black light tests, qualitative tests, and quantitative tests that use small grain samples (<10 g samples) potentially mask the aflatoxin concentration of grain. Obtaining a low aflatoxin concentration does not necessarily mean low concentrations will occur thereafter, and vice versa. A high concentration of aflatoxin could be followed by a low concentration of another sample within the same bag of grain because aflatoxin is rarely, if ever, evenly distributed throughout grain. We recommend storing grain <2 months and to thoroughly clean and dry wildlife feeders often.

Transmission dynamics of *Borrelia* bacteria in a bird tick community

Dieter Heylen, Hein Sprong, Erik Matthysen

We examined the *Borrelia burgdorferi* s.l. circulation in a tick community consisting of three species (*Ixodes ricinus*, *I. frontalis*, *I. arboricola*) with contrasting ecologies, but sharing a common host: the great tit (*Parus major*), one of the most common birds of European gardens and woodlands.

Field data show that the birds hosted *Borrelia*-infected larvae of both *I. frontalis* and *I. ricinus*, indicating the facilitation of *Borrelia* transmission. The low, but significant numbers of *Borrelia* in unfed *I. arboricola* ticks collected from bird nest boxes, provide the first evidence that it is competent in maintaining *Borrelia* over long periods of time. Aside from the known avian genospecies (*B. garinii* and *B. valaisiana*), several less dominant genospecies were observed in the three ticks, including *B. turdi* and some mammalian genospecies.

In laboratory experiments, we imitated the natural situation during the bird's post-fledging period, in which *Borrelia*-naïve juvenile birds are repeatedly exposed to infected *I. ricinus* nymphs. Birds developed systemic infections of the avian genospecies. Although birds showed a very low competence to facilitate the transmission of mammalian genospecies, a low number of birds remained permissive for *B. afzelii*. Infected birds were able to transmit *Borrelia* to naïve *I. frontalis* and *I. arboricola* individuals, however, latter tick species were not able to transmit the bacteria to a new host.

When using the great tit as a host, transmission cycles are driven by *I. ricinus*, and are not maintained by the ornithophilic ticks (*I. frontalis* and *I. arboricola*). Still, spill-over of the bacteria from *I. ricinus* to the ornithophilic tick species often occurs in the wild. The use of bird species, other than the great tits (e.g. thrushes and finches), may result in different transmission outcomes than reported here.

Acute phase proteins and maternally and actively acquired IgG in Australian sea lion (*Neophoca cinerea*) pups with hookworm (*Uncinaria sanguinis*) infection

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Hookworm (*Uncinaria sanguinis*) causes significant disease in pups of the Australian sea lion (*Neophoca cinerea*), an endemic, endangered Australian pinniped (IUCN Red List, 2008). This study explores the hypotheses that serum concentrations of maternally derived antibodies predict hookworm disease outcomes in pups; that pups with low levels of maternally derived antibodies have a compensatory elevated acute phase protein response to hookworm infection; and that increasing IgG concentration, associated with the development of active immunity in the pup, is temporally associated with eventual elimination of hookworm. Archived serial serum samples from known-age Australian sea lion pups (n=58) from Seal Bay, Kangaroo Island, South Australia were selected for analysis by indirect protein A ELISA and serum protein electrophoresis, and results compared to disease outcomes, as defined by presence of anaemia (PCV < 35%) and hypoproteinaemia (TPP < 60g/L).

Pups with relatively low serum concentrations of maternally-derived IgG and gamma globulins at less than 30 days of age were more likely to develop hookworm-associated anaemia and hypoproteinaemia. Unexpectedly, it was mildly affected pups that had a more pronounced acute phase response, based on elevated α_2 -globulin and β_1 -globulin fractions, than those pups that were severely affected. Serum IgG increased steadily until two to three months of age, the approximate period of hookworm elimination. To determine whether the rate of increase in serum IgG over time was related to hookworm infection, rates of increase were compared among those pups treated with the parasiticide, ivermectin, and those administered a placebo at Dangerous Reef, Spencer Gulf, South Australia, during high and low hookworm intensity seasons; those administered the placebo demonstrated a greater rate of increase in serum IgG over time during the high intensity season, indicating that hookworm infection elicits a significant immune response in these pups.

Detection of *Chlamydia psittaci* and psittacine beak and feather disease virus in sympatric passerines and an invasive psittacine species

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Invasive species disrupt native ecosystems through consumption of resources, displacement of native species from habitat niches, and introduction of novel pathogens. Rosy-faced lovebirds (*Agapornis roseicollis*) have been observed in the Phoenix area for 25 years. In 2013, *Chlamydia psittaci* (CP) was identified in a mortality event of 20 lovebirds. In 2014, the Arizona Game and Fish Department received multiple reports of similar mortality events in lovebirds. Testing confirmed systemic CP infections in the affected birds. Due to concerns for spread to native species interacting with the lovebirds at feeders and potential public health risks, a live bird surveillance project was conducted. Swab samples were collected from 47 lovebirds and 143 psittacine birds captured at backyard feeders at 4 mortality and 2 control locations. Samples were screened for CP, and a viral infection of lovebirds, psittacine beak and feather disease (PBFD). *Chlamydia psittaci* and PBFD nucleic acids were detected in 94% (44/47) and 81% (38/47), respectively, of lovebirds at all sites. Detection of CP and PBFD DNA occurred more frequently in sympatric species sampled at mortality sites than control sites (CP: mortality 13% [14/108], control 3% [1/35]; PBFD: mortality 34% [37/108], control 9% [3/35]). *Chlamydia psittaci* and PBFD DNA were more likely to be detected among rock doves (*Columba livia*, OR_{CP} 72.1, 95% CI 11.8–440; OR_{PBFD} 4.25, 95% CI 1.0–17.3) than other non-psittacine species. Although mortality of native species associated with these diseases has not been documented, such an event may be difficult to detect in a species that is less common or noticeable than lovebirds. Because PBFD causes immunosuppression, the co-infection of birds with PBFD and CP may increase the probability of a disease event in native birds. Investigation of population declines, mortality, and feather abnormalities in native birds in the area is warranted.

Multidisciplinary investigation of Sea Star Wasting Disease (SSWD)

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Sea star mortality in several genera including Pycnopodia and Pisaster was unusually high along the west coast of the North America, from Alaska to southern California from September 2013 -current (March, 2015). The number of dead sea stars is estimated in the millions and the mortality rates in affected regions continue to be high with upwards of 100% mortality documented in certain sea star species¹. A similar but smaller event also occurred along the east coast earlier in 2013. An international and multidisciplinary team of scientists at various institutions including, but not limited to, Cornell University, Wildlife Conservation Society, SeaDoc Society, Monterey Bay Aquarium, Vancouver Aquarium, Seattle Aquarium, USGS National Wildlife Health Center, and Northwest ZooPath, are participating in the ongoing efforts to determine the cause of this unusual wildlife mortality event. Captive and free-ranging sea stars of various species and states of health were collected in British Columbia, Washington, and California. Sea star samples were analyzed using a wide array of diagnostic techniques including cytology, microbiology utilizing bacterial and viral genomics, toxin analysis, water and sediment evaluation, histopathology using traditional and non-traditional polymer embedding techniques, and transmission and scanning electron microscopy. To date, 147 samples, representing 14 asteroid species have been examined histologically by members of a SSWD pathology working group. Consistent histologic changes include: 1) epidermal degeneration, necrosis and ulceration; 2) dermal edema, necrosis and inflammation. Comparative body wall composition analysis and mineral staining were also pursued to investigate the pathogenesis of the clinical body wall dissolution. Community fingerprinting and bacterial metagenomics identified 3 candidate disease-associated bacterial families including Bacteroidetes, Gammaproteobacteria and Spirochaetes. Viral metagenomes prepared from symptomatic (N=16) and asymptomatic sea stars (N=16) identified several candidate disease-associated metazoan viruses including a sea star-associated densovirus (Parvoviridae)². The results of transmission and scanning electron microscopy by wildlife veterinary pathologists at the University of Connecticut and the Wildlife Conservation Society will be reviewed. Lastly, the challenges and importance of investigating a large-scale wildlife mortality event in a marine species with little previous baseline health data through the efforts of a large team of scientists will be discussed with the summary of current diagnostic results and future directions.

Electrical Shock Injury in a Curve-billed Thrasher (*Toxostoma curvirostre*)

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This is the first documented case of electric shock injury in a Curve-billed Thrasher (*Toxostoma curvirostre*), the first record of survival of such injury, and the first record of successful treatment of electric shock injury in this species with release of the bird back to the wild. An electric shock strong enough to damage a songbird is likely to be explosively lethal. There are no records of Curve-billed Thrashers suffering ESI, under treatment for, nor surviving such a trauma. The clinical presentation, treatment and progress are discussed.

Assessing viral diversity within non-human primates of Peninsular and Bornean Malaysia

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Land use change results in loss of habitat for non-human primates (NHPs) and increasing potential contact and conflict between NHPs and people¹²³. The result of our viral surveillance program serves as an initial step towards understanding viral diversity in NHPs and potential spillover to people⁴. Oropharyngeal, urogenital and rectal swabs were collected from 6 species of NHPs, (Peninsular = 632 animals; Sabah, Bornean state = 86 animals) from areas of close human contact. Samples were screened using family-level PCR assays for 17 viral families and were confirmed by sequencing. On Peninsular, three types of herpesviruses, including *Macacine herpesvirus 1*, were found in 26% of sampled animals, while in Sabah, 8 herpesviruses were found in 90% of sampled animals. Collectively, 34% animals had herpesviruses. On Peninsular, 10% had macaque foamy virus and 0.3% had human adenovirus. In Sabah, two foamy viruses were found in 22% of sampled animals, one astrovirus in 2.3% of sampled animals, one adenovirus in 2.3% of sampled animals and one human paramyxovirus in 1.2% of sampled animals. Our results indicate the potential for zoonotic viral transmission from wildlife in areas with NHP-human contact. Certain types of NHP herpes can be hazardous to people. The human paramyxovirus and adenovirus found in wildlife demonstrates anthrozoönotic transmission can occur and could be a health threat to protected species. Land use change impacts on disease emergence needs to be considered when expanding urban or agricultural areas, to minimize conflict and potential viral transmissions between NHPs and people.

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Discoveries and Lessons Learned from Twenty Years of Sampling Wild Birds for *Mycoplasma gallisepticum*

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Mycoplasma gallisepticum was a well characterized bacterial pathogen of chickens and turkeys worldwide and apparently relatively host-specific, until 1994 when an outbreak was identified in Eastern USA house finches. The opportunity to collect samples and study this emergent disease with a multi-institutional, multi-disciplinary team has yielded a wealth of knowledge and been a model of collaborative research. Key to the success and productivity of this effort has been isolation of *M. gallisepticum* spanning the wild bird host, temporal, and geographic ranges of the disease from emergence to endemicity. Faced with the challenges and costs of sample collection for mycoplasma culture from remote locations over time, we tried various protocols to arrive at a practical optimum. Wild bird sampling for mycoplasma culture allowed us to identify *M. sturni* associated with conjunctivitis in cliff and barn swallows, and *M. corogypsi* associated with polyarthrititis and tenosynovitis in black vultures. *M. gallisepticum* isolates in experimental infections of house finches showed evolution of virulence with parallel patterns of increased virulence in both western and eastern isolates. Phylogenetic studies using isolates from wild birds and poultry suggested multiple host transfers from poultry to house finches, but only one successful lineage accounting for the continent-spanning epidemic. Genomics showed extensive variation in surface lipoprotein gene content, phenotypic plasticity, and

genomic changes associated with virulence evolution. Using serology and PCR, we recently found that a diverse range of wild bird species may carry or have been exposed to *M. gallisepticum* in the USA, as in Europe and Asia. This evidence for a diverse wild bird host range exposed to *M. gallisepticum* exemplifies a transmissible subclinical mycoplasmosis: achievement of an ideal host/parasite relationship. Emergence of a pathogenic *M. gallisepticum* strain in house finches may actually be the exception that has allowed us to identify the broader epidemiologic picture.

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Developing immune reagents and assays to define Koala (*Phascolarctos cinereus*) immune responses to disease and vaccination

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The koala (*Phascolarctos cinereus*) is an arboreal herbivorous marsupial that is an Australian icon. It is widely accepted that koala populations are declining at an increased rate with recent studies suggesting that the strongest contributing factor to this population decline is the prevalence of disease. *Chlamydia pecorum* and *Chlamydia pneumoniae* are obligate intracellular Gram-negative bacteria that cause abortion, infertility, arthritis, conjunctivitis, respiratory disease, and in some cases death in koala populations. Due to a lack of koala-specific immune reagents and assays there is no way to adequately analyse the immune response in diseased, healthy or vaccinated animals. Using sequence homology and available transcriptome data cluster of differentiation markers expressed by key lymphocyte subsets and key soluble koala immune molecules were identified and expressed as recombinant proteins to produce monoclonal antibodies. Following the collection of primary koala spleen and lymph node cells the verification of each antibody was performed through Western Blot, ELISA, Immunohistochemistry, qPCR and Flow Cytometry analysis. The koala specific monoclonal antibodies were then used to investigate the cellular response to infection/vaccination of chlamydia through flow cytometry and ELISA assays. This research project will provide the first in depth understanding of the koala immune system in response to chlamydial infection by developing a suite of species specific immune reagents and characterising immune profiles of healthy, diseased and vaccinated animals. The reagents and techniques developed from this project are applicable to further understand the koala immune response to other diseases and injury.

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Clinical findings in free-ranging elk (*Cervus elaphus*) affected with treponeme-associated hoof disease (TAHD) in southwest Washington state, USA

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Treponeme-associated hoof disease (TAHD) has emerged as a significant disease of elk (*Cervus elaphus*) in southwest Washington state during the past decade. While severely affected individuals and an apparently high prevalence of disease are readily observable in the TAHD-endemic area, the population effects of this disease are unknown. In order to inform management of elk in the TAHD-endemic area, during February 2015 the Washington Department of Fish and Wildlife initiated a 5-year study to investigate the potential effects of TAHD on adult female (>2 years old) elk survival and reproduction. A total of 78 animals were radio-collared: 58 with visual evidence of TAHD, and 20 with normal-appearing hooves and no evidence of lameness at the time of capture. In conjunction with marking the animals for this study, samples were collected for the following analyses: whole blood for measurement of lymphocyte response to known hoof disease pathogens and for complete blood counts; serum for measurement of antibodies to known hoof disease pathogens; and hoof swabs for bacterial culture, polymerase chain reaction, and metagenomic microbial studies. In addition, nutritional status was assessed using elk-specific body condition scoring methods and pregnancy status was determined via transrectal ultrasound. Lymphocytes from affected elk exhibited significant proliferation to pathogenic *Treponema* sp. antigens compared to elk from outside the TAHD-endemic area, but did not differ in response to *Fusobacterium necrophorum* or *Dichelobacter nodosus* antigens. Within the endemic area, preliminary analyses suggest that elk with TAHD had higher neutrophil/lymphocyte ratios than unaffected elk. Remaining analyses are ongoing at the time of abstract submission and will be included in the conference presentation.

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Horse behaviour, paddock structure and frequency of contact with Hendra virus

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Hendra virus (HeV) spillover from Australian flying fox (FF) bats to horses is still a rare event. To understand some of the mechanisms driving the risk of spillover we developed a mechanistic model that we used to design an observational study in the interface where spillover occurs, the paddock. We selected a few of the model parameters

to directly observe in the field and provide an estimate of how often Horses might interact with HeV. To do so we attached GPS trackers on horses vaccinated against HeV to track their movements. To record bat activity we deployed infra red cameras under trees that had bat activity and matched the information recorded by the cameras with live counts. Bat activity was used to simulate HeV excretion and decay with a published model (Martin et al 2015). With these data we estimated how often horses can interact with bats or HeV and identified areas of intervention to reduce the risk of contact with areas potentially contaminated with HeV.

Investigating regent parrot declines in South Australia – biomedical survey and telemetry studies.

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The eastern regent parrot (*Polytelis anthopeplus monarchoides*) is an endangered subspecies ranging along the Murray River in south-eastern Australia. There may be as few as 800 breeding individuals in South Australia. Clearing and fragmentation of native habitat, the continuing decline in riparian trees due to changes in the water table and river regulation, and persecution as a perceived agricultural pest, are all postulated as significant threatening processes. Aspects of the natural history of this population remain incompletely understood, and efforts are ongoing to determine the principle drivers of the decline.

During the 2012-14, 69 birds were captured in mist nets at a number of feeding flyways between Berri and Waikerie, SA. All birds were examined under isoflurane anaesthesia. Each bird was weighed, measured, and leg banded. Blood samples were collected for DNA sexing, routine haematology and biochemistry, and nutritional analyses. Feathers and blood spots were collected for Psittacine Circovirus testing, and swabs were collected for *Chlamydia* sp., Psittacid Herpesvirus-1 and Avian Bornavirus PCR.

Five parrots had proliferative cutaneous foot lesions, previously identified but not investigated in regent parrots in Victoria. Histology and molecular testing to date indicates a papillomavirus aetiology.

To enhance understanding of regent parrot biology, we are beginning to deploy telemetry devices. A backpack style harness design has been refined for this species, trialled on captive birds, and has successfully been fitted to a number of free-ranging birds. Three different transmitters have been used to date. The relative merits of each are currently being evaluated to determine the most efficient tool to answer prescribed questions.

This presentation will discuss results of the biomedical survey, and give an update of telemetry studies to date.

Recreational fishing debris: sinking our coastal wildlife?

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It is difficult to quantify the impacts of recreational fishing debris on Australian coastal wildlife, consequently hindering targeted management and public awareness messages. Taronga Wildlife Hospital receives over 700 injured wild animals annually, allowing a unique snapshot into the health status of local wildlife. Taronga's Australian Registry of Wildlife Health and Taronga Wildlife Rehabilitation Database are valuable data sources allowing a longitudinal, objective study of the potential impact of recreational fishing. Recreational fishing debris (fishing line, small hooks, spears and nets but not trawl or shark nets) cause accidental death and injury to marine mammals, birds, fish and reptiles, with more than one admission every month on average. Over the past decade, 156 animals have been admitted to the hospital with fishing related injuries, with only 22% surviving to be released. Hook ingestion is the most common injury and has the lowest survival outcome.

Marine and aquatic birds are the focus of this analysis, comprising 72% of animals admitted with fishing related injury. The remaining affected animals include turtles (17%) and marine mammals (11%). Birds are more vulnerable to hook ingestion, while turtles and marine mammals are more likely to be affected by entanglements from line and nets. Birds are most affected by hooks because of their exclusively piscivorous diet, their foraging methods and their size. Admissions of birds with fishing related injury has increased over the past decade ($r^2=0.792$, $p=0.000$). Twice as many male as female birds are admitted with fishing related injury possibly attributable to differences in life histories and hunting strategies. Longitudinal analysis reported here highlights the most vulnerable species and individuals, the most hazardous debris types and guides decisions regarding the best care of injured wildlife. Cormorants and pelicans make up the majority of bird admissions, making them important target species for public conservation messaging.

TRAIL to death: Is TNF related apoptosis inducing ligand (TRAIL) a common antiviral mechanism employed by reservoir species?

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While certain species (e.g. bats and ducks) are capable of carrying viruses asymptotically, other species (e.g. humans and chickens) succumb to infection and develop symptoms of disease. The reason why certain species are subject to disease though other species are resistant remains an enigma and a question that urgently needs answering. Mechanisms that reservoir species use to regulate viral infections are largely unknown. Previous research has shown apoptosis, or programmed cell death, may play an important role in the antiviral response of reservoir hosts (Wynne et al, 2014, Kuchipudi et al, 2012). Apoptosis is a naturally occurring mechanism that is critical in biological development and the immune system. Apoptosis can be induced through a number of signalling molecules, including TRAIL. TRAIL, is a secreted molecule that can bind to viral infected cells, through their interaction with death receptors, to induce apoptotic cell death. Our laboratory has previously shown that bat kidney, but not human kidney cells, undergo TRAIL mediated apoptosis in response to Hendra virus, a highly pathogenic bat borne virus. In the present study we investigated the induction of TRAIL mediated apoptosis across a range of bat and human cells. By using enzymatic assays combined with real-time PCR we demonstrate that bat cells are hypersensitive to apoptosis, evidenced through increased caspase 3/7 activity. We also showed that bat cells have a significant induction of TRAIL mRNA and its associated receptor. Our findings highlight the important antiviral response of TRAIL in bats. We hypothesise that the rapid induction of apoptotic cell death in viral infected cells, may be mechanisms by which bats control viral replication. Future research in our laboratory will examine this pathway in other reservoir host models, including influenza infected duck and chicken cells.

SAVEM at the Sampson Flat bushfire: data gathered during the Veterinary Response.

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South Australian Veterinary Emergency Management (SAVEM) Inc. is a formal part of the South Australian Government's State Emergency Management Plan. SAVEM sits under Primary Industries and Regions SA (PIRSA), and is a 100% volunteer agency of veterinarians, wildlife carers and others with specialist skills. SAVEM's mission is to rescue, triage, treat, reunite or rehabilitate all species of animals in emergencies. In January 2015, the Sampson Flat fire in the Adelaide Hills was the largest in South Australia for 30 years. Twenty-seven homes were lost in a fire scar of over 12,500 hectares of rural holdings, farmland, native and pine forest. Extensive domestic animal, livestock and wildlife losses occurred, with kangaroo and koala populations being severely impacted. During a six week activation, SAVEM rotated 70 volunteers who attended over 750 taskings. This Response afforded an opportunity to gather and collate data as never before in a bushfire event. Data from multiple agencies and the public now fills a comprehensive spreadsheet, logging date, district, species, injuries and final outcome for each task. Overall 234 animals received attention, with many more monitored in the field. Injuries included fractures, burns, capture myopathy, and smoke inhalation. Data analysis will explore links between the type of injury and fire intensity, terrain and escape corridor availability in each zone documented, as well as examining triage and treatment efficacy pertaining to recovery and outcome. Additional data was collected for epidemiological studies from hospitalised patients at ZoosSA, and a longitudinal cohort study is planned for a mob of rehabilitated juvenile Western Grey kangaroos to be soft released in nearby habitat. This spreadsheet allowed efficient record keeping, tracking of field teams and data-mining of the whole event, which, when merged with Geospatial Information System maps, presents a detailed overview of the bushfire, as a useful resource for future bushfire responders.

Histological assessment of captive Gouldian finches (*Erythrura gouldiae*) for evidence of mycobacteriosis

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Objective: Mycobacteriosis is an important disease of captive and wild Gouldian finches (*Erythrura gouldiae*). The aim of this study was to assess Gouldian finches from the Adelaide Zoo for mycobacteriosis and determine pathological features of the disease in this population.

Methods: Post-mortem examination was performed on 11 finches culled for the purpose of diagnostic investigations, and on 16 finches following natural death. Tissues from major organs were processed for histopathology using standard techniques, and further staining with Ziehl-Neelsen, Periodic Acid-Schiff and gram stains was performed as indicated. Finches showing granulomatous inflammation associated with intracellular acid-fast bacilli (AFB) were considered positive for mycobacteriosis.

Results: In total, 27 finches have been examined, and 10 birds have confirmed mycobacterial infection. Gross lesions were rarely identified, however histopathological examination revealed granulomatous lesions in the liver and intestines of all infected birds. Intracellular AFB were found in the liver of 8/10 and intestine of 5/10 birds, and less frequently, granulomatous foci containing AFB were present in the heart, lungs, spleen, proventriculus and ventriculus. Other diagnoses included fungal (*Candida* spp., *Macrorhabdus ornithogaster*) ventriculitis or proventriculitis in 7/27 birds, apical protozoal infection consistent with *Cryptosporidium* spp. in 3/27 birds, and hepatic amyloidosis in 2/27 birds.

Conclusions: Mycobacteriosis is present in Gouldian finches at Adelaide Zoo as well as a number of other opportunistic pathogens. These findings complement previous work suggesting that Gouldian Finches may have a decreased immunocompetence caused by environmental factors or inbreeding. Research outcomes will guide further work examining for immunosuppression in captive finches and aid in the implementation of management strategies in zoos.

Paramyxoviruses in Australian flying-foxes: each to their own?

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Bats are reservoir hosts for many emerging zoonotic pathogens. In Australia, the most widely studied is the paramyxovirus Hendra virus (HeV), yet a diverse community of viruses has been detected in Australian flying-foxes (Smith et al. 2011, Vidgen et al. 2015). Although HeV or anti-HeV virus antibodies have been detected in all four flying-fox species, recent studies investigating aspects of the host-pathogen relationship suggests that the closely related black and spectacled flying-foxes (*Pteropus alecto* and *P. conspicillatus*) may be the primary reservoir host (Smith et al. 2014, Goldspink et al. 2015). Natural host-virus systems are complex and there is increasing awareness of the importance of understanding 'host communities' (a single virus may have multiple reservoir hosts, each with heterogeneous contributions to pathogen dynamics, persistence and spillover) and 'viral communities' (known zoonotic viruses are often detected alongside a wide diversity of viruses in their natural hosts, with unknown pathogenic potential) (Vasco et al. 2007; Viana et al. 2014). The species-specificity of HeV is only just beginning to be elucidated; for other Australian bat paramyxoviruses, it is unknown. A novel high-throughput multiplex PCR (Boyd et al., unpublished) was utilised to simultaneously detect up to nine known bat paramyxoviruses in individual urine samples from three of the four Australian flying-fox species collected in a related study (Edson et al. in prep). Results support the existence of specific host-viral relationships, along with synchronous shedding pulses of multiple viruses. We interpret these results in the context of a novel reservoir framework, reconsidering HeV as part of a multi-host-multi-virus community. HeV in Australian flying-foxes, and its spillover to horses and humans, is the best understood emerging bat pathogen spillover system, and by examining it in this broader context, this study will provide significant insight into both HeV dynamics and bat virus spillover in general.

Psittacine Beak and Feather Disease in endangered Australian parrots

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Critically endangered populations of animals are at an increased risk of stochastic extinction following reductions in population size due to disease. Psittacine Beak and Feather Disease (Pbfd) has been identified as a key threatening process for the critically endangered Orange-bellied Parrot (*Neophema chrysogaster*) in Australia for this reason.

Following more than a decade of intensive surveillance for the disease, Pbfd has emerged in both captive and wild Orange-bellied Parrots in recent years and appears to be causing mortalities of offspring in the tiny remnant wild population of fewer than 50 birds. The origin of this emergent pathogen in this species remains unknown, but its potential affect on recruitment is of particular concern.

We have generated a significant database of whole-genome beak and feather disease virus (BFDV) genotypes, which is beginning to shed light on the geographical and host distribution of this virus in Australia. Nevertheless sampling bias amongst wild parrots has left considerable gaps, and may explain our inability to determine the origin of the BFDV spillover into wild Orange-bellied Parrots. Future management of this virus in critically endangered parrots such as the Western Ground Parrot (*Pezoporus flaviventris*) and Swift Parrot (*Lathamus discolor*) will most likely rely on the development of an effective vaccine. Our work elucidating the structure of the capsid protein of BFDV has implications for both vaccine development and our understanding of the virulence of this highly mutable and genetically diverse virus.

Host - parasite interactions: a tool for determining human impacts to endangered wildlife

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Molecular tools have greatly expanded our understanding of host parasite interactions, including pathogen / parasite diversity, host specificity and transmission pathways. This is particularly so for the protozoan parasites *Cryptosporidium*, *Eimeria* and *Giardia*, major causes of gastrointestinal disease in many vertebrate species. For *Cryptosporidium* 70% of the currently recognised species were described over the last 10 years and molecular data has been integral to these descriptions. Molecular analyses have also revealed greater than 40 cryptic *Cryptosporidium* species in diverse wildlife hosts, for which we have limited biological information. A similar pattern is also evident for *Giardia*. For *Eimeria* the application of molecular tools has not been as rapid, and much of our understanding of the diversity of *Eimeria* stems from morphological and pathological data. We have been using recent advances in molecular understanding of parasite diversity to investigate the application of multi-parasite

targets and their interactions with endangered wildlife as a measure of human impacts. We show a significant association between parasite communities and proximity to humans, and a higher prevalence of parasites that commonly cause human infection in wildlife hosts. Our data demonstrates movement of parasitic protozoa at the interface between humans : domestic animals : wildlife and transmission of terrestrial parasites into marine ecosystems. I will discuss how the increasing knowledge of these parasites, in combination with other parasite targets, can be applied to investigate human impacts on host-parasite relationships in endangered species.

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Investigating the history of the conflict between kea (*Nestor notabilis*), an endangered parrot, and high country sheep farmers in New Zealand.

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Human-wildlife conflict is a growing global problem with considerable and varied impacts, e.g., on human livelihoods and endangered species, and is especially concentrated in agriculture. Although not a new problem, its conservation impacts only began to become apparent relatively recently. An unresolved example of such conflict is between kea (*Nestor notabilis*), an endangered high country parrot endemic to the South Island of New Zealand, and high country sheep farmers. Kea have been observed attacking sheep (a phenomenon referred to as "kea strike") on high country farms since 1868. Kea strike can cause illness, injury and death in sheep and was the driver for a government bounty scheme lasting approximately 100 years, which resulted in the deaths of at least 150,000 kea and a large population decline leading to their current endangered status. Kea strike has not been well studied and is poorly understood, the literature being largely historical and/or anecdotal. Kea strike still occurs, and kea are still persecuted as a result despite their conservation status. To gain a scientific understanding of kea strike, we began by investigating historical accounts from a range of sources, including government records, newspapers, and books. This enabled us to examine the commonalities between high country sheep farmers' historical accounts of kea strike, and to compare these to the experiences of farmers today. This information, in addition to other data we have collected on kea strike and wild kea behaviour, will be provided to conservation managers and high country sheep farmers to help inform their policies and practices where kea strike is still a problem. We anticipate that this will help to mitigate kea strike, and therefore assist kea conservation.

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Epidemiological and evolutionary implications of seasonal birth pulses in wild mammals

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Seasonal birth pulses are frequently observed in wildlife and are expected to affect the dynamics of infectious diseases within populations. We recently showed that the range of birth pulse shapes observed across mammalian species could have a strong impact on the persistence of diseases, as measured by the critical community size¹. Here I present the predictions from our model and investigate the potential consequences of seasonal birth pulses for the evolution of virulence in pathogens. I then discuss the implications for the evolution of life history traits in host species.

1. Peel, A., J. Pulliam, A. Luis, R. Plowright, T. O'Shea, D. Hayman, J. Wood, C. Webb and O. Restif (2014). "The effect of seasonal birth pulses on pathogen persistence in wild mammal populations." *Proceedings of the Royal Society B: Biological Sciences* 281(1786): 20132962.

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Interclass transmission of avian-origin influenza A virus through common resources

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The potential role of wild mammals in avian influenza A virus (IAV) transmission cycles has received some attention in recent years and cases where birds have transmitted IAV to mammals have been documented. However, the contrasting cycle, wherein a mammal could transmit an avian-origin IAV to birds, has been largely overlooked. We experimentally tested the abilities of two mammalian species to transmit avian-origin IAV to mallards (*Anas platyrhynchos*) in simulated natural environments. Results suggested that striped skunks (*Mephitis mephitis*) can successfully transmit avian-origin IAV to mallards through indirect contact with shared resources, as transmission was noted in 1 of 4 of the mallards tested. Cottontail rabbits (*Sylvilagus* sp.) exhibited a similar pattern, as one of five cottontail rabbits successfully transmitted IAV to a mallard, likely through environmental contamination. These results suggest that certain mammal species should be taken into consideration in biosecurity plans.

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Tick Diversity in Wild Lowland Tapirs (*Tapirus terrestris*) in the Atlantic Forest and Pantanal Biomes, Brazil

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The lowland tapir (*Tapirus terrestris*) has been recognized as a very important host for several native tick species in Brazil. The goal of this study was to evaluate the diversity of ticks in wild tapirs considering the previous knowledge about other potential host species (wild and domestic). Tapirs were captured and sampled in two Brazilian biomes: Atlantic Forest - AF (22°16"S; 52°05"W) and Pantanal - PA (19°20"S; 55°43"W). Fourteen tapirs were sampled in the AF site (2006-2008), whereas 89 samples (45 tapirs + 44 recaptures) were analyzed in the PA site (2008-2014). Collected ticks were kept alive and taxonomically identified based on current literature. Overall, 7 different tick species were found on tapirs (five in the AF and four in the PA). Tick species (and potential hosts besides tapirs) in the AF site were *Amblyomma sculptum* previous synonym of *Amblyomma cajennense* (associated with several wild and domestic species) (33♂, 37♀, 56 nymphs); *Amblyomma coelebs* (only found in tapirs) (34♂, 36♀, 7 nymphs); *Amblyomma brasiliense* (strongly associated with peccaries) (6♂, 24♀, 2 nymphs); *Amblyomma ovale* (strongly associated with wild and domestic carnivores) (1♂, 2♀); *Amblyomma* sp.(3 larvae); and *Haemaphysalis juxtakochi* (strongly associated with cervids) (1♀). In the PA site, four tick species were found: *Amblyomma sculptum* (268♂, 824♀, 410 nymphs); *Amblyomma parvum* (associated with several wild and domestic species) (2♂, 13♀); *Amblyomma ovale* (2♂, 7♀); *Amblyomma* sp.(15 larvae); and *Rhipicephalus microplus* (strongly associated with cattle) (2♂, 1♀, 1 nymph). In wild animals, the relationship between ectoparasites and their hosts can be better understood when analyzed through an ecological perspective, which provides important insights about interactions between wild and domestic species in different habitat types. This is also important considering that ticks are widely recognized as vectors of a great diversity of pathogens to animals and humans.

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Intensive health screening of moose at the edge of their range

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Moose in the Adirondacks region of northern New York State represent the southern extent of the eastern moose (*Alces alces*) subpopulation. Population density estimates and health information in this area are lacking. We captured 12 moose via helicopter for health assessment and fitted them with GPS collars to document spatial patterns and conduct sightability surveys. On site, animals were checked for ectoparasite presence and load, body condition was estimated, and whole blood, serum, feces, ectoparasites, and hair samples were collected. Samples from 9 females and 3 males were examined for complete blood count, blood chemistry, selenium, fecal parasites, and infectious diseases (bovine viral diarrhea, eastern equine encephalitis, West Nile virus, and *Neospora*). Females were additionally screened for serum and fecal progesterone and fecal glucocorticoid metabolites using both cortisol and corticosterone immunoassays. Hands-on evaluation found fewer ectoparasites (e.g., winter tick, *Dermacentor albipictus*) than other moose in eastern states. However, most moose had high eosinophil counts (mean = 33%) and eight of 11 were positive and the remaining three were suspicious for *Neospora caninum*. Eosinophilia has been linked previously to migrating parasites and could be related to the *Neospora* infection. However, *Parelaphostrongylus tenuis* (i.e., white-tailed deer brainworm or meningeal worm) and *Fascioloides magna* (i.e., giant liver fluke) have been found before in New York moose mortalities. These species were not detectable in moose fecal exams. Additionally, selenium values in blood were below detectable limits. This area is known to be selenium limited, but also high in mercury that may limit bioavailable selenium. Low circulating selenium and *Neospora* may be impacting reproductive potential. We contrast our findings with other moose populations across their range to determine if New York moose face unusual health challenges that impede formation of a robust population or further southern expansion of their range.

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Live genome resource banking to aid koala conservation in South-east Queensland

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The increased morbidity and mortality rates associated with Chlamydia are putting significant strain on koala populations, which already face the detrimental concerns associated with fragmentation; such as loss of genetic variation, inbreeding depression and reduced population fitness. My PhD research is a pilot study for how we can utilise the accessibility of captive koala populations to improve the population health, genetic variation and reproductive capacity of wild koala populations that are threatened by disease. This pilot study will determine the logical application, and establishment, of a Living Genome Resource Bank for aiding koala conservation.

The primary methodology for this study is utilising genetic analysis associated with SNP technology, which has not yet been applied to koala genetics. SNP data will not only determine population structure and dynamics, but also levels of inbreeding which have occurred as a result of disease-associated population decline and fragmentation. This data can also establish a heterozygosity fitness correlation between the genetic profile and reproductive capacity of a population, by using captive colonies as a model.

The secondary methodology for this study is the incorporation of assisted reproductive technologies into the LGRB to aid in genetic recovery and exchange. We have successfully implemented gamete recovery from deceased males in an effort to retain their genetic information. Parallel to this, previous efforts to extend chilled koala semen for the purpose of artificial insemination have recorded a 72hr success rate in producing pouch young. Part of this current study aims to extending this time to 35 days, to sync with the females oestrous cycle, so that regardless if a male is alive or not, his genetics can still be represented within the population.

Genetic identification, recovery and exchange can, and will, aid in improving population fitness and increasing population numbers, which are currently threatened by disease.

***Thelazia callipaeda* in wild animals in the Russian Far East**

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Thelazia callipaeda is a spirurid nematode selectively parasitic in eyes of carnivores and humans. It usually localizes in a conjunctival sac causing medium to severe inflammation of conjunctiva such as conjunctivitis, keratitis and ulcers of cornea. Within the Russian Federation, the infection of *T. callipaeda* was reported in dogs, cats, foxes and racoon dogs in the Russian Far East. Wild carnivores sampled in Primorskiy Krai were examined posthumously in the period from the winter 2012 to the summer 2014. Conjunctival sacs of the animals including the space under a nictitating membrane were examined for the presence of thelaziids. Totally, 544 animals were examined including 492 specimens of sables (*Martes zibellina*), 25 Siberian weasels (*Mustela sibirica*), 11 racoon dogs (*Nyctereutes procyonoides*), 4 American minks (*Neovison vison*), 3 Amur leopard cats (*Prionailurus bengalensis euphilurus*), 3 foxes (*Vulpes vulpes*), 2 yellow-throated martens (*Martes flavigula*) and one specimen each of lynx (*Lynx lynx*), European badger (*Meles meles*), brown bear (*Ursus arctos*) and Asian black bear (*U. thibetanus*). Morphological study of helminthes and their species identification were performed under light microscope. Totally, 6,8% (37 out of 544) of examined animals were found infected by *T. callipaeda*. Helminths were detected in 28 sables from 492 examined, in 5 racoon dogs from 11 in 2 foxes from 3 in one lynx and Asian black bear examined. Sable, Asian black bear and lynx were registered as hosts of *T. callipaeda* for the first time (until present, the only record of *T. callipaeda* infection in the lynx was related to the zoo). Our own data as well as the analysis of the previous research indicate on a presence of the persistent infection nidus in the Russian Far East which poses threats for the epidemiological situation in the area. Supported by the grant 14-16-00026 from Russian Scientific Foundation.

Clinical investigation into urolithiasis in yellow-footed rock wallabies (*Petrogale xanthopus*)

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Urolithiasis has been documented as a cause of mortality in captive populations of yellow-footed rock wallabies, yet prospective investigation into potential risk factors for development of uroliths in wallabies is lacking. Following the death of two adult male yellow-footed rock wallabies from calcium carbonate obstructive urolithiasis, the remaining 26 resident adult wallabies were screened for the presence of uroliths and for potential risk factors for development of calcium-based urolithiasis. Comprehensive health screening included physical examination, abdominal radiographs, abdominal ultrasonography, complete blood count, serum biochemistry, serum 25-hydroxyvitamin D, urinalysis, urine chemistry analysis, and diet analysis. Urine was alkaline, and calcium carbonate crystalluria was present in several individuals. Serum calcium and 25-hydroxyvitamin D were within reference range of many mammal species. However, spot fractional excretion of calcium was highly variable, with mean values significantly higher than reported for greyhound dogs. Selected dietary components were discovered to have a high calcium level, high calcium:phosphorus ratio, and high dietary cation:anion ratio. The results of this investigation provide valuable data to help better understand calcium status and urolith formation in yellow-footed rock wallabies and macropods in general.

Bat flies (genus *Cyclopodia*): an unlikely vector for the transmission of Hendra virus.

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Hendra virus (HeV) can cause potentially fatal respiratory and neurological disease in horses and humans. Australian flying-foxes (genus *Pteropus*) are the natural reservoir host for HeV. There is limited understanding of the route of transmission for the spillover of HeV from flying-foxes to horses and also for the route of transmission for the enzootic maintenance of HeV within flying-foxes; although exposure to flying-fox fluids (i.e. urine) contaminated with HeV is hypothesised to play a role. Flying-foxes also host blood feeding ectoparasites known as bat flies (genus *Cyclopodia*). Whilst there are no examples of paramyxoviruses that utilise insect vectors for transmission, there has been limited investigation of these haematophagous insects as potential vectors of HeV. A total of 186 bat flies were collected from flying-foxes (*Pteropus alecto*, *P. poliocephalus* and *P. scapulatus*) between December 2013 and June 2014, when the average prevalence of HeV in the flying-fox roost was 3%. Bat flies were sexed and then dissected to expose the internal organs with specific attention to the crop, gut and when available, the larvae. RNA from dissected tissues and larvae was extracted using the MagMAX Viral RNA Isolation Kit and tested for HeV using a qRT-PCR targeting the M gene. All bat flies and larvae from this study tested negative for HeV. The probability of failure to detect HeV in these bat flies at a prevalence of at least 3% was <0.004. This study provides the first molecular based evidence that bat flies are unlikely to be a transmission vector for HeV between flying-foxes and in spillover events. This study also demonstrated the utility of studying a potential vector in tandem with the natural reservoir host to allow appropriate interpretation of results.

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Twenty years of Hendra virus in Queensland (1994-2014): laboratory submission trends and risk factors for infection in horses.

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Hendra virus (HeV) produces high mortality rates amongst both horses, and subsequently in-contact humans, when it spills over from its natural host (flying-foxes, genus *Pteropus*). Over a period of twenty years (1994-2014) there have been 52 recorded HeV incidents, with a total of 90 horses and 7 humans infected; 4 fatally. The Australian winter of 2011 saw an "unprecedented" number of HeV incidents with 18 occurring in the states of Queensland and New South Wales. This represented a major departure from the annual number and distribution of HeV incidents previously seen. The years 2012 and 2013 continued to see relatively high numbers of HeV incidents. In order to explain the high number of HeV incidents in recent consecutive years we undertook a unique retrospective analysis of 2,552 horses submitted for HeV testing at Queensland's state veterinary reference laboratory, Biosecurity Sciences Laboratory (BSL, Biosecurity Queensland, Department of Agriculture and Fisheries) between 1994-2014. We examine case characteristics provided to the laboratory, such as age, breed, clinical signs (neurological and respiratory), location (north or south of the Tropic of Capricorn), season, sex, year and look for trends in submission rates or risk factors for infection in horses. The adjusted mean prevalence of HeV in horses submitted to BSL was 2.1%. The number of horses submitted for HeV testing increased dramatically after the availability of a local diagnostic PCR (2008, from 1/2 months to 9/week) and after the first detection of HeV in each calendar year from 2008-2011 (from 3 to 22/week, $P < 0.01$) but not for 2012-2014, where the submission rate remained steady (9/week, $P = 0.10$). Significant variables identified in the generalised linear model included breed, clinical signs, location, the interaction between location and year, and season. This is the first comprehensive study to examine risk factors for HeV infection in horses.

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Current status of tularemia in the Netherlands, an example of an integrated human-veterinary one health approach in practice

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Tularemia is considered an important emerging zoonosis in Europe. In the Netherlands, between 1953 and 2011 no autochthonous tularemia was reported. However between January 2011 and October 2014, five indigenous human infections with *Francisella tularensis* supsp *holarctica* have been reported, as well as three in hares (*Lepus europaeus*).

The first tularemia patient from 2011 had no history of foreign travel. Most probably, the route of transmission was via insect bites during a boat trip the North-East of the Netherlands [1].

In July 2011 a non-targeted monitoring in hares started in collaboration between veterinary and human public health institutes. The first *F. tularensis* infected hare was found in the South of the Netherlands in May 2013 [2]. In July 2013 a visitor of a nature reserve, several kilometers from the location of the positive hare, developed ulceroglandular tularemia through insect bites. The 1953 case also originated from this region.

In January 2014, a man from the South-West of the Netherlands was infected while skinning a hare that had been caught that same day by greyhounds. He developed fever and a painful swelling in the armpit. Samples of the patient as well as haunches of the hare revealed the presence of *F. tularensis*.

In the North of the Netherlands, two men became feverish in March 2014 after skinning a hare that was found dead. One of them also developed a skin ulcer resulting from a cut by a sharp bone. Sera showed high titers of antibodies against *Francisella*. Unfortunately, no material of the hare was left to confirm the source.

Above cases among humans and hares show the reemergence of *F. tularensis* in the Netherlands. An important achievement is that different organizations, with various scientific backgrounds, are actively cooperating on risk assessment, surveillance and control measures of tularemia in the Netherlands.

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Disease risk analysis for the captive management of the critically endangered Western Ground Parrot (*Pezoporus flaviventris*)

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The Western Ground Parrot (*Pezoporus flaviventris*) is a critically endangered medium-sized parrot. There are fewer than 140 Western Ground Parrots left in the wild in two conservation reserves on the south coast of Western Australia.

In 2009 and 2010, a total of 10 wild individuals were brought into captivity by the Department of Parks and Wildlife to develop husbandry techniques and explore the prospects of a breeding for release program. Although there were some breeding attempts, these were unsuccessful and in 2014, the remaining individuals (3 females and 4 males) were transferred to Perth Zoo for ongoing breeding attempts.

A disease risk analysis was undertaken in preparation for bringing the first birds into captivity. A second analysis was undertaken in 2013 in preparation for the transfer of remaining individuals to Perth Zoo. While the diseases under consideration changed little from 2009 to 2013, the relocation to a zoo environment presented a number of new potential avenues of disease risk to birds.

The 2013 review addressed a range of biosecurity risks to the parrots, including: location and health status of other psittacine species in the zoo collection; exposure to free-ranging bird species; health status of local free-ranging psittacines; keeping staff involvement with husbandry of other zoo animals. The review resulted in the implementation of a pre- and post-arrival disease screening plan, a quarantine plan and post-quarantine procedures for ongoing biosecurity.

The early involvement of personnel with expertise in psittacine health and captive husbandry, as well as the biology of the species in the wild, was instrumental in achieving a robust ongoing plan for biosecurity for this captive population. Excellent communication with all stakeholders was critical to the prompt development of the disease risk analysis and effective implementation of its recommendations.

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Strontium plesiotherapy as an adjunct treatment for squamous cell carcinoma in a Pygmy Bearded Dragon (*Pogona henrylawsoni*)

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Neoplasia is a complex process of abnormal tissue growth. As in all animals, neoplastic processes can occur in reptiles and have been documented in all orders in the class Reptilia, with snakes, lizards and chelonians most commonly affected. While cancer was once thought to be uncommon in reptiles, it is becoming a more common diagnosis with increased ownership of reptiles as pets and presentation of wildlife to veterinary clinics. With the advancement of chemotherapeutics in companion animals, animal owners now expect that chemotherapy is an available treatment option for reptiles. This case report describes a case of a four-and-a-half-year-old Pygmy Bearded Dragon (*Pogona henrylawsoni*) that presented with a mass on the skin of the right upper lip. The mass measured 5mm in diameter. The mass had grown quickly in the last week but was not invading the deep tissue or bone. A fine needle aspirate of the mass revealed abnormal epithelial cells. The mass was removed with inadequate margins due to location and submitted for histopathology. Strontium plesiotherapy at a dose of 100 gray was used by direct contact of a strontium-90 probe to the surgical site one week into recovery. At the time of writing this abstract, the mass had not regrown during the three months following therapy.

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A Two-Tier Ebola Virus Vaccination Strategy for Wild African Apes

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The 2014 Ebola virus outbreak in West Africa is a stark example of the devastating impact of ebolaviruses after establishment of human-to-human transmission. African apes (chimpanzees and gorillas) are one main source of zoonotic Ebola virus transmission into the human population, but Ebola virus is also highly lethal in apes, and has resulted in substantial reductions in wild ape populations. Our aim is to reduce the impact of Ebola virus on wild apes and therefore also reduce this source of virus introduction into the human population by using vaccination to provide immunological protection for apes against Ebola virus. The assembled multi-disciplinary team represents a unique collaboration between conservationists, wildlife veterinarians and laboratories with expertise in vaccines and ebolavirus disease. We propose a two-tiered vaccination strategy comprising a short and a long-term aim to provide immunological protection of apes against Ebola virus – initially in habituated (accustomed to close human proximity) apes and then, using a novel ‘disseminating’ cytomegalovirus (CMV)-based vaccine, potentially in all ape populations. In the short term we will pilot vaccination of captive or semi-captive western lowland gorillas using a conventional human Ebola virus vaccine, with non-invasive antibody analysis in feces. With agreement from key stakeholders including the Central African Republic (CAR) Government, and based on our prior successful field vaccination campaigns, a comparable protocol will then be used to vaccinate habituated western lowland gorillas in Dzanga-Sangha Protected Area (DSPA), CAR. This short-term vaccination will provide these habituated animals protection from Ebola virus disease, and prevent them serving as a conduit for Ebola virus transmission into the human population. It will also establish a vaccination protocol that can be considered for use in other accessible ape populations. In the long term, our previous studies in mice and ongoing studies in non-human primates indicate that CMV-based vaccines can provide immunological protection against Ebola virus. The high species specificity of CMV and its ability to disseminate through its host population regardless of prior host immunity suggest that CMV has the potential for development as a ‘disseminating’ vaccine to achieve vaccine coverage of inaccessible, non-habituated wild apes. Our immediate long-term aim is to develop gorilla CMV (GoCMV) as a ‘disseminating’ Ebola virus vaccine to vaccinate all (including non-habituated) western lowland gorillas.

Scoping the wildlife-livestock interface – diseases, species, regions, and trends

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During recent decades, emerging pandemics of zoonotic origin have drawn attention to the interconnectedness between wild and domestic animal and human health. Although pathogen maintenance within wildlife populations and spillover to livestock has proved a common precursor to disease emergence in humans, the role of wildlife-livestock interfaces in disease ecology is a research gap.

This study is the first to utilize a scoping review framework to provide a comprehensive overview of published knowledge on infectious diseases at the wildlife-livestock interface. The specific aim was to quantitatively characterize published literature with respect to the types of diseases, the animal species involved, as well as the temporal and regional patterns.

Diseases considered in this research included all OIE-listed diseases of livestock, as well as other diseases deemed important by the OIE Working Group on Wildlife Diseases. An extensive literature search combining wildlife, livestock, disease, and geographical search strings was conducted in Web of Science™. Subsequently, a relational database was designed and dynamic queries were used to automatically link each record with its corresponding search term categories, enabling analysis on aggregated levels (e.g. continents). Overall 15,998 records were included in the final analysis.

Retrieved records were published between 1912 and 2013 and increased dramatically over time. During this period there was a shift in focus from parasitic to viral diseases. Fifteen diseases accounted for more than 50% of all records, eleven of which were zoonoses. Most commonly reported wildlife-livestock interfaces resulted largely from interaction between phylogenetically closely related and/or sympatric species.

By adapting a scoping review framework we identified the key diseases and wildlife-livestock interfaces on which research has been focused over the last century. Future work will help refine surveillance and research strategies and to inform policy makers, funding agencies, and other stakeholders about priority areas.

Preliminary investigations into neonate health and cause-specific calf mortality in a declining moose population in northeastern Minnesota

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The Minnesota moose population (*Alces alces*), like many along the southern extent of the North American moose range, has been experiencing a precipitous decline in recent years. Ongoing studies of moose on Grand Portage Indian Reservation, MN reveal high levels of disease and reduced condition associated with adult mortality and low annual calf recruitment. Specifically, calf:cow ratios have been steadily declining since 2000. To understand neonate health and causes of calf mortality on Grand Portage Reservation, we studied 19 calves of 16 moose cows from parturition since 2013. The natural mortality rate observed was 79% by November of each study year. Nine (60%) mortalities were a result of predation (bear: n = 5, wolf: n = 4), the majority of which occurred by end of summer. Remaining causes of mortality included stillbirth (n=2), septicemia (n=1), abandonment (n=1), and unknown (n=2). Neonatal health was evaluated in a subset of captured calves (n=13), 48-72 hours post-partum, by complete blood cell count (CBC), serum biochemistry panel, and total serum immunoglobulin (Ig). While general health parameters were mostly within a normal range, maternal antibody transfer as measured by total serum Ig appeared to be marginally sufficient in 5 calves and deficient in 5 calves. Unfortunately, there is a paucity of information on Ig levels in moose calves that are sufficient for immunological protection as calf immune systems develop. Further, our observations of predation as the primary cause of calf mortality is not a reliable indicator of the impact of failure of maternal antibody uptake. These findings clearly demonstrate that most calves are not surviving through their first year, many a result of predation; however, our preliminary research into neonatal health also reveals lower than expected levels of maternal-derived antibody suggesting that compromised health may play a role in calf survival.

A descriptive epidemiological study of recent Australian strandings of Risso's Dolphins (*Grampus griseus*)

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Recently, the east coast of Australia has seen a dramatic increase of Risso's dolphin (*Grampus griseus*) strandings. As of February 2015, there have been 21 strandings, involving 22 animals spread over New South Wales (NSW), Victoria and Tasmania. All animals have been in poor body condition and have ranged in both age and gender. The Tasmanian strandings are of particular significance, as prior to 2014, there has only been a single documented case of a Risso's dolphin stranding (K. Carlyon, personal communication, February 6, 2015). The objective of the report was to provide a descriptive epidemiological study of Australian Risso's dolphin strandings from August 2014 to February 2015.

Primary collection of stranding data were compiled by National Parks and Wildlife in NSW, Department of Environment, Land, Water and Planning in Victoria and Department of Primary Industries, Parks, Water and Environment in Tasmania. Full autopsies were performed on ten animals by veterinarians and others by wildlife officers where possible. Tissue samples were collected and sent off for histopathology and other testing either fixed in 10% formalin or as fresh specimens.

There seems to be no clear pattern to explain the sudden rise of Risso's dolphin strandings. Pathological abnormalities have been limited and where present have not been consistent between events. One animal (out of six) tested positive for toxoplasmosis, none (out of four) for Dolphin Morbillivirus and none (out of one) for Brucella. Four animals showed evidence of cerebral inflammation.

Based on pathological findings, an infectious cause to these strandings appears unlikely and perhaps greater focus should be placed on underlying ecological issues affecting the changing distribution of Risso's dolphins such as increasing water temperatures, shifting prey distribution and/or environmental contamination. Genetic testing would be beneficial but difficult to interpret due to an incomplete DNA database for this species.

Can oral examinations and diagnosis of soil-transmitted helminthes be combined?

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An estimated two billion people, predominately children, are infected with soil-transmitted helminthiasis (STH). *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* are the most common species. Diagnosis of infection is predominately based on fecal examination. However, mass deworming, hygiene education and sanitation of school age children is used as a prevention measure with no previous examination. Some reasons for a lack of fecal examination are cost, time and cultural sensitivities regarding fecal collection. This lack of screening means limited information on prevalence and ability to assess if prevention measures are effective. While people might be reluctant to collect their feces, many countries have international dental aid where children receive free examinations. This represents an opportunity to collect saliva and oral smears for STH testing. STH eggs have been found in oral mucosa smears and STH antibodies have been detected in saliva. African green monkeys (AGM) on St. Kitts are infected with *T. Trichuira*, providing a unique opportunity to evaluate alternative diagnostic methods. In this study, AGM will be used as an animal model to compare fecal examination, saliva antibodies and oral smears. In addition, antibodies will be tested post-treatment of AGM to

determine how antibodies correlate to fecal egg counts. Our ultimate goal would be to create a point-of-care diagnostic or screening tool to overcome sensitivities and time constraints associated with common diagnostic methods. This One-Health initiative has the potential to be used with other Neglected Tropical Diseases.