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SEROLOGIC SURVEY FOR POTENTIAL PATHOGENS AND ASSESSMENT OF DISEASE RISK IN AUSTRALIAN FUR SEALS

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ABSTRACT: The introduction of pathogens into populations of animals with no previous exposure to them and, therefore, no immunologic protection, can result in epizootics. Predicting the susceptibility of populations to infectious diseases is crucial for their conservation and management. Australian fur seals (*Arctocephalus pusillus doriferus*) have a relatively small population size, a restricted range, and form dense aggregations. These factors make this species vulnerable to epizootics of infectious diseases that spread by direct animal-to-animal contact. Blood samples were collected from 125 adult female Australian fur seals between 2007 and 2009 and tested for exposure to selected pathogens. The testing protocol was based on pathogens important to marine mammal health or those significant to public and livestock health. No antibodies were detected to morbilliviruses, influenza A viruses, six *Leptospira* serovars, *Mycobacterium tuberculosis*-complex species, or *Toxoplasma gondii*. Overall antibody prevalence to an unidentified *Brucella* sp. was 57% but varied significantly ($P<0.02$) between 2007 (74%) and 2008 (53%). The findings indicate *Brucella* infection may be enzootic in the Australian fur seal population. Further investigations are required to isolate the bacteria and establish if infection results in morbidity and mortality. Australian fur seals remain vulnerable to the threat of introduced disease and should be managed and monitored accordingly.

Key words: Australian fur seals, *Brucella*, influenza, *Leptospira*, morbillivirus, serology, *Toxoplasma*, tuberculosis.

INTRODUCTION

Infectious diseases regulate wild populations by reducing reproductive success and increasing mortality rates (Delahay et al., 2009). In marine mammals, pathogens have caused epizootics resulting in significant changes to population size and structure (Heide-Jorgensen et al., 1992; Wilkinson et al., 2006). As well as directly causing the death of the individual, disease morbidity can decrease individual foraging ability, increase susceptibility to predation, and reduce life-time reproductive output by causing gestational failure or inhibiting growth and development (Colagross-Schouten et al., 2002; Hone et al., 2010). The epizootiology of marine mammals can also involve interaction with humans and domesticated animals. In some instances, marine mammal pathogens may pose a risk to public health and to the health of domesticated livestock or, alternatively, disease transmission to marine mammals from domestic animals may occur (Dubey et al., 2003; Fenwick et al., 2004).

Australian fur seals (*Arctocephalus pusillus doriferus*) are an Australian endemic species that breed mainly on small islands in Bass Strait, a shallow marine basin between the south-eastern Australian mainland and Tasmania (Warneke, 1982). Compared with other fur seal species, the Australian fur seal has a restricted range, a relatively small population, and has recovered slowly following the cessation of commercial sealing in the early 1900s. The population size in 2007 was estimated to be approximately 120,000 seals (Kirkwood et al., 2010). Slow population growth in the Australian fur seal, in part, results from low fecundity, as a high incidence of

spontaneous abortions occurs in the second half of gestation (Gibbens et al., 2010).

It is largely unknown whether infectious diseases, particularly bacterial, viral, and protozoal pathogens significant in pinniped species elsewhere, are present in Australian fur seals and affect their population dynamics. Two potential pathogens have been identified: *Mycobacterium pinnipedii*, a novel member of the *M. tuberculosis* complex (Cousins et al., 2003), is very sporadically isolated from diseased Australian fur seals (Woods et al., 1995); and antibodies to an unidentified *Brucella* spp. were found in two of 15 wild-caught, captive, and apparently healthy individuals (Dawson 2005). Tuberculosis is a well-documented cause of morbidity and mortality among free-living fur seals and sea lions in Australia, New Zealand, and South America, with the potential to infect people and livestock (Bernardelli et al., 1996; Cousins et al., 2003). Marine mammal brucellosis also may pose a risk to public and livestock health and, in pinnipeds, *Brucella* infections have been associated with pathologies including abscessation and bronchopneumonia (Foster et al., 2002; Prenger-Berninghoff et al., 2008).

The dense aggregations of Australian fur seals at their breeding sites is an ideal environment for the transfer of pathogens between individuals, providing one of the optimal factors needed for the development of disease epizootics (Norman, 2008). In addition, genetic and foraging studies suggest high levels of intercolony movement of animals (Arnould and Kirkwood, 2008; Lancaster et al., 2010), which would facilitate the spread of infectious disease throughout the entire Australian fur seal population once introduced at a single location. The range of Australian fur seals overlaps with those of several other pinniped species, notably the New Zealand fur seal (*Arctocephalus forsteri*) and the Australian sea lion (*Neophoca cinerea*), providing opportunities for disease transfer between these species. Their restricted range and small population size make the Australian fur seal susceptible to the impact of stochastic events, including epizootics. Therefore, investigation of the role of disease in the ecology of this species has been identified as a key conservation objective (Shaughnessy, 1999).

Our aim was to establish, by antibody survey, if Australian fur seals have been exposed to pathogens recognized elsewhere as significant causes of mortality or morbidity in pinnipeds or as important due to their livestock or public health implications. Results from this testing could subsequently be used to perform a qualitative risk assessment describing the infectious disease threats to this species. Additionally, the results will enable further clarification of disease transfer risks resulting from contact between seals and humans and domesticated animals.

MATERIALS AND METHODS

Sampling

The study was conducted between 2007 and 2009 at the Australian fur seal colony on Kanowna Island (39°10′S, 146°18′E), northern Bass Strait. Capture efforts were concentrated at one colony to limit potential between-colony confounding factors that might affect pathogen antibody presence and prevalence. Adult animals were selected for initial antibody screening because, for many infectious diseases, the probability of antibody-positive status increases with age (Smith et al., 1977; Burek et al., 2005). Females were sampled in preference to males as they are more tractable for large-scale sampling. Animals were sampled over 3 yr to investigate interannual variation in antibody prevalence and to obtain sufficient numbers to detect antibody-positive animals even at a low prevalences. Desired sample sizes were calculated by WinPepi epidemiologic software, Version 10.5 (Abramson, 2004). The estimated population size of Australian fur seals on Kanowna Island is 15,000 individuals, which includes an annual production of >3,000 pups (Kirkwood et al., 2010). A sample size of at least 59 females was needed in order to detect, with a 95% probability, at least one positive individual if antibody prevalence was ≥5%.

Animals were selected at random and captured using a large hoop net (Research Nets Inc., Kirkland, Washington, USA and Fuhrman Diversified Inc., Seabrook, Texas, USA). Blood samples were collected from the interdigital hind flipper veins, or from a vein lying in the webbing of the fore flipper, in either manually restrained or anesthetized seals. Anesthesia was induced when seals were subjected to procedures other than just blood sampling, such as attachment of dive-recording devices, and was achieved using isoflurane (100% vol./vol. Forthane®, Abbott Australia Pty, Ltd., Botany, New South Wales, Australia) supplied via a closed anesthetic circuit (Stinger, Advanced Anaesthesia Specialists, North Ryde, New South Wales, Australia). Samples were transferred into untreated tubes (BD Vacutainers, Becton, Dickinson and Co., North Ryde, New South Wales, Australia) and allowed to clot for at least 1 hr prior to centrifugation. Serum aliquots were stored at −20 C or lower until analysis.

Antibody assays

Testing for antibodies to influenza A viruses, *Leptospira* serovars, and *Brucella* spp. was conducted at the laboratories of the Veterinary Diagnostic Services, Department of Primary Industries, Attwood, Victoria 3049, Australia. *Toxoplasma gondii* antibody testing was performed at the Department of Primary Industries and Water, Mt. Pleasant Laboratories, Launceston, Tasmania 7250, Australia.

Exposure to morbilliviruses was investigated by serum neutralization testing utilizing canine distemper virus (CDV) as the antigen and VeroDogSLAMtag cells as described by Nielsen et al. (2008). This was judged reasonable to use as an initial screening test because antibodies to specific morbilliviruses will cross-react with all virus species, with the highest titers being obtained against the homologous species (Liess et al., 1989). Neutralization titers were expressed as the reciprocal of the highest dilution of serum that gave an 80% reduction in the number of virus plaques in comparison with the quadruplicate plates inoculated with virus and diluent alone. Antibody titers of 16 or greater were considered positive.

Serum was tested for antibodies to influenza A viruses by an equine influenza virus, competitive enzyme-linked immunosorbent assay (EIV cELISA; Selleck, 2008). The test detects antibodies against all subtypes of influenza A viruses by competitive binding between test sera and a monoclonal antibody specific for a highly conserved epitope on the influenza A nucleotide. Seal sera giving a percentage inhibition of less than 40% were considered negative.

Testing for antibodies to five *Leptospira* serovars; *L.* Australis, *L.* Copenhageni, *L.* Grippotyphosa, *L.* Hardjo, *L.* Pomona, and *L.* Tarassovi was by a microscopic agglutination test (MAT). The technique followed protocols outlined in the Australian and New Zealand Standard Diagnostic Procedures Schedule (SCAHLS, 2010). Serial dilutions of 1:50 to 1:6,400 were utilized and agglutination at ≥1:100 was considered positive based on the validation of the MAT in Californian sea lions (Colagross-Schouten et al., 2002).

Sera were tested for evidence of exposure to *Brucella* spp. using a commercially available competitive ELISA kit (Svanovir®, Svanova Biotech, Uppsala, Sweden). Briefly, test serum is exposed to *B. abortus*-coated wells together with a mouse monoclonal antibody specific for an epitope on the o-polysaccharide portion of the S-LPS antigen. Based on the manufacturer's recommendations for bovine sera, inhibition values of ≥30% were considered positive. Sera that fell within 5% of this cut-off were retested, and the mean value inhibition value was used to determine the final result. The cut-off values have not been validated in marine mammals.

A rapid lateral-flow test (Elephant TB STAT-PAK, Chembio Diagnostic Systems, Inc. Medford, New York, USA) that employs a mixture of recombinant antigens from *M. tuberculosis* and *M. bovis*, including ESAT-6, CFP10, and MPB83, was used to test seal serum for exposure to species from the *M. tuberculosis* complex. Serum and diluent buffer are applied to a test pad, and capillary action drives this mixture across the fixed antigens (including a positive control) that contain a blue latex bead signal detection system (Lyashchenko et al., 2006).

The presence of agglutinating antibodies to *T. gondii* in seal sera was investigated using a modified agglutination test (ModAT; Antigen Toxo AD kit, BioMerieux, Charbonnieres les Bains, France). Formalin-treated *T. gondii* tachyzoites were used as the antigen, and sera were tested at serial dilutions, with the results expressed as the reciprocal of the highest dilution giving a positive reaction. A result of ≥64 was considered positive.

RESULTS

Between 2007 and 2009, 125 adult female Australian fur seals were captured and sampled on Kanowna Island. Seals

sampled in 2007 ($n=43$) and 2008 ($n=61$) were tested for antibodies to all six pathogens. An additional 21 animals captured in 2009 were only tested for antibodies using the morbillivirus serum neutralization test and the *Brucella* cELISA. Assuming a test sensitivity of 100%, the sample size of 125 animals (morbillivirus and *Brucella*) gave a 95% confidence level of detecting at least one positive case if the true antibody prevalence was 3.6% or higher. The sample size of 104 animals would result in at least one positive animal being detected if the antibody prevalence (influenza A, *Leptospira*, *Mycobacteria*, and *T. gondii*) was 4.2% or higher.

There was no serologic evidence of exposure of Australian fur seals to the morbillivirus CDV, so samples were not further tested using phocine and dolphin distemper viral strains. No animals were antibody-positive for *Leptospira* serovars, influenza A viruses, *T. gondii*, or *M. tuberculosis*-complex bacteria. However, 71/125 (57%) adult females (95% CI: 48% to 66%) were positive using the *Brucella* cELISA. A chi-square test for independence indicated a significant difference in the prevalence of antibody-positive animals between years ($\chi^2=10.62$, df=2, $P<0.005$). Prevalence was highest in 2007 with 32/43 (74%) females positive (95% CI: 59–87%). By 2008, prevalence had significantly decreased ($\chi^2=5.14$, df=1, $P<0.023$) with 32/61 (53%) animals positive (95% CI: 39–65%). There was no significant difference ($\chi^2=2.29$, df=1, $P<0.13$) in prevalence between 2008 and 2009. In 2009, 7/21 (33%) animals were positive (95% CI: 15–57%). Mean percentage inhibition values from the *Brucella* cELISA were compared between 2007 and 2008 and no significant difference was found ($t=0.895$, $n=104$, $P<0.37$).

DISCUSSION

Morbilliviruses

Antibodies stimulated by morbillivirus infections are usually long lived (Schultz et al., 2010). Thus, the absence of titers in animals sampled in this study suggests that the Australian fur seal population on Kanowna Island has not been exposed to this pathogen. While distemper epizootics caused by phocine distemper virus (PDV) and CDV have occurred in northern hemisphere phocids (Osterhaus et al., 1988; Grachev et al., 1989; Kennedy et al., 2000), this has not been the case in southern hemisphere pinnipeds. Pinnipeds from Australian coastal waters have not been surveyed for morbillivirus antibodies prior to this study, but there is evidence that morbilliviruses are present in some southern hemisphere pinnipeds. Antibodies to CDV have been found in crabeater seals (*Lobodon carcinophagus*) and leopard seals (*Hydrurga leptonyx*) in Antarctica (Bengtson and Boveng, 1991). In New Zealand waters, there is serologic evidence of exposure to a PDV-like virus in both New Zealand fur seals and New Zealand sea lions (*Phocarctos hookeri*) (Duignan et al., unpubl.).

Potential sources of morbillivirus exposure in Australian fur seals are domestic dogs, wild canids, and other pinnipeds. In Australia, the prevalence of CDV in domestic dogs is low, but disease occurs sporadically (Norris et al., 2006). The prevalence of CDV is unknown in foxes, dingos, and feral dogs but likely to be low. Domestic dogs are known to have occasional close contact with Australian fur seals, particularly seals that are debilitated, providing opportunities for pathogen transfer.

Leopard seals and, to a lesser extent, crabeater seals are occasional visitors to the Australian mainland and could carry CDV or PDV to Australian fur seals. However, close contact between these individuals and local otariids would be unusual, so the risk of virus transfer is probably low. New Zealand fur seals have a broad range and are found in both New Zealand and southern Australia (Warneke and Shaughnessy, 1985). The identification of a PDV-like virus in the New

Zealand population, and evidence that individuals from New Zealand can cross the Tasman Sea to southeastern Australia (Shaughnessy, 1999), suggest New Zealand fur seals could bring morbilliviruses into the range of Australian fur seals. Furthermore, New Zealand fur seals resident in Australia often occupy the same sites as Australian fur seals (Kirkwood et al., 2009). The morbillivirus antibody status of New Zealand fur seals in Australia is unknown, although a preliminary serologic survey of 31 adult female New Zealand fur seals from Kangaroo Island found no evidence of PDV exposure (M. Lynch, unpubl.).

Influenza A viruses

Influenza A viruses can cause disease in a wide variety of avian and mammalian hosts and are significant human pathogens. Phylogenetic evidence suggests that influenza epidemics in humans and other mammals, including seals, derive from mutation and antigenic drift of viruses originating in aquatic birds (Webster et al., 1992). Influenza A viruses have caused mortality events in harbor seals along the New England coast of North America (Geraci et al., 1982; Callan et al., 1995). Infection of phocid seals by influenza A viruses appears to be sporadic with only low antibody prevalence found in populations surveyed in the North Atlantic and Arctic (Stuen et al., 1994; Nielsen et al., 2001a). A low to moderate antibody prevalence was found in South American fur seals in Uruguay (Blanc et al., 2009).

The EIV cELISA used in our study is highly sensitive and suitable for application across species (Selleck, 2008). Therefore, the negative results obtained for Australian fur seal females indicate that they have not experienced recent exposure to influenza A viruses. Influenza A viruses are known to be circulating at low prevalence in Australian aquatic birds, although they are considered to be of low pathogenic potential in their current forms (Haynes et al., 2009). Nevertheless, the possible introduction of potentially pathogenic influenza viruses from birds to seals remains, and influenza-exclusion testing is warranted for seals with pneumonia for which no other causal agent has been identified.

Leptospira serovars

Leptospirosis is a significant cause of mortality and morbidity in Californian sea lions (*Zalophus californianus*; Dierauf et al., 1985) and has been reported from a number of other northern hemisphere pinnipeds (Smith et al., 1977; Colegrove et al., 2005). Leptospirosis has not been isolated in southern hemisphere pinnipeds, but antibodies to several *Leptospira* serovars, *L.* Hardjo, *L.* Canicola, and *L.* Pomona have been found in New Zealand fur seal pups without evidence of disease (Mackereth et al., 2005).

Modified agglutination tests are used for leptospirosis investigations in many species and were highly sensitive in detecting infection in Californian sea lions (Colagross-Schouten et al., 2002). Therefore, the negative results obtained from adult female Australian fur seals indicate that they have not been exposed to the *Leptospira* serovars tested. There are over 23 *Leptospira* serovars endemic in Australia and infection is acquired by direct contact with carrier animals or contaminated fresh water (Roberts et al., 2010). Australian fur seals occasionally travel up rivers and creeks that receive water runoff from cattle farming areas and, thus, could potentially be exposed to leptospires. It is also possible that Australian fur seals have antibodies to serovars not investigated in this study. A broader survey may be required in the future, particularly if seals present with pathology suggestive of leptospirosis.

Mycobacterium tuberculosis complex

Tuberculosis caused by *M. pinnipedii* has been identified in New Zealand fur seals, Australian sea lions, and Australian fur seals (Cousins et al., 2003). Tubercu-

losis of wild Australian fur seals is relevant, not only to the health of seals but also as a potential risk to the agricultural industry and as a zoonotic disease. Seal-to-cattle transmission of tuberculosis is thought to occur sporadically in New Zealand, where *M. pinnipedii* has been isolated from cattle grazed on coastal pastures (Hunter et al., 1998; Cousins et al., 2003). There is certainly the potential for seal-to-cattle pathogen transmission to occur across the range of Australian fur seals, as many of the coastal areas of southeastern Australia have cattle enterprises based on pasture grazing. In addition, human infection with *M. pinnipedii* acquired from captive seals has occurred on at least two occasions (including once in Australia from wild-caught New Zealand fur seals), highlighting the zoonotic potential of this organism (Forshaw and Phelps, 1991; Kiers et al., 2008).

Our finding of no individuals with antibodies to *M. tuberculosis*-complex antigens was notable because tuberculosis is known to occur sporadically in Australian fur seals. Whether this organism persists within the Australian fur seal population at a low prevalence, or is periodically introduced from other pinnipeds, is unknown. It is possible that the ability of the STAT-PAK test to detect latently infected animals or those in the early stages of infection is limited. This test has been validated in a range of species (Lyashchenko et al., 2008) and, like other serologic tests for tuberculosis, it relies on detecting the humoral immune response, which usually is most prominent in animals with very active and usually advanced infections (Chambers, 2009). These animals would be expected to succumb quickly, thereby reducing the chances of such individuals being sampled in the wild.

Toxoplasma gondii

Toxoplasma gondii and related protozoa occasionally cause disease in marine mammal species (Dubey et al., 2004; Dubey et al., 2008) and, in some, toxoplasmosis is emerging as a significant disease issue (Miller et al., 2008; Messier et al., 2009). In Australian waters, *T. gondii* was identified as the cause of fatal meningitis in an adult Australian sea lion (Kabay, 1996). The ModAT test used in the present study, although not validated for pinnipeds, detects antibodies against *T. gondii* in a range of species including marine mammals (Dubey et al., 2003; Dubey et al., 2005). Therefore, our negative results strongly suggest that *T. gondii* infection was not present in adult female Australian fur seals on Kanowna Island.

The life cycle of *T. gondii* requires a felid definitive host, and infected cats may excrete millions of highly resistant *T. gondii* oocysts that contaminate the environment. Infection routes for seals are poorly understood because the cold-blooded animals that most pinniped species prey on are not parasitized by *T. gondii* (Dubey et al., 2008). Possible infection sources are marine, filter-feeding invertebrates that concentrate oocysts that have contaminated the seawater (Miller et al. 2008). These may then be consumed by marine mammals or their prey. Like Australian sea lions, Australian fur seals do not prey on filter-feeding marine invertebrates (McIntosh et al., 2006; Kirkwood et al., 2008). Therefore, the frequency of exposure of Australian fur seals to infection with *T. gondii* is difficult to assess without further knowledge on the likely sources of infection. The potential impact of *T. gondii* in Australian fur seals is also unclear, as both the susceptibility of intermediate host species to disease and the virulence of parasite strains vary (Dubey, 2004).

Brucella spp.

The presence of *Brucella* antibodies in marine mammals has been reported from many geographic areas and species, indicating that *Brucella* infections affect a large number of cetacean and pinniped species and have a worldwide distribution

(Nielsen et al., 2001b; Tryland et al., 2005). A genetically distinct *Brucella* type, *B. pinnipedialis*, has been found in numerous northern hemisphere pinniped species (Foster et al., 2002; Maratea et al., 2003). Marine *Brucella* spp. have not been found in southern hemisphere pinnipeds, although they have been isolated from humans in South America and New Zealand where they were responsible for severe disease (Sohn et al., 2003; McDonald et al., 2006).

Brucella infection in cetaceans is associated with epididymitis and abortion (Ewalt et al., 1994; Foster et al., 2002). The pathologic significance of *Brucella* infections in pinnipeds, however, is unclear as it is often isolated from apparently healthy animals (Foster et al., 2002). Recently, *Brucella* was suggested as a potential cause of abortion in California sea lions (Goldstein et al., 2009).

We found a high prevalence of antibodies to a *Brucella*-like organism in the adult female Australian fur seals. False-positive results from *Brucella* serologic tests may be caused by antibodies to cross-reacting bacteria, particularly *Yersinia enterocolitica* (Kittelberger et al., 1995). However, in cattle, cELISAs have been found to have high specificity compared to indirect ELISAs and are considered reliable tests for detecting individuals naturally infected with *B. abortus* (Nielsen, 1990; Samartino et al., 1999). Whether cELISAs behave similarly in pinnipeds is unknown, but a comparison of serologic tests for *Brucella* applied in Hawaiian monk seals (*Monachus schauinslandi*) concluded that a cELISA was suitable for use as a screening test (Nielsen et al., 2005).

The significant decrease in the prevalence of *Brucella* antibodies in Australian fur seal females between 2007 and 2008 cannot be explained with our data. It may indicate that exposure of the population to *Brucella* occurred immediately prior to 2007 and that antibody levels in animals were waning, but this is not supported by our finding of no significant difference in mean antibody titers between the 2 yr. A high prevalence of antibody-positive animals in a population, as seen with *Brucella* in this study, often indicates enzootic infection where animals are frequently exposed to a pathogen or where it persists within the population.

It is unknown whether Australian fur seals in our study were actively infected with *Brucella* when sampled; all but three animals appeared healthy. One of these three (observed to abort just prior to sampling) was antibody-negative and a second (suspected of recently aborting due to a bloody, vulval discharge) was positive. Aborted fetuses and premature births are often observed in Australian fur seals, and the rate of gestational failure has been estimated to vary between 21% and 37% (Gibbens et al., 2010). Based on the findings of a high prevalence of antibody-positive adult female Australian fur seals reported here, and potential links of infection to infertility and abortion, further investigation of *Brucella* epizootiology in Australian fur seals is required.

In summary, we report the first serologic survey for infectious disease in free-ranging Australian fur seals. The results suggest that, in the Kanowna Island colony, five of six potentially important pathogens are either not present or are at low prevalence. The importance of the high prevalence of antibodies to an unidentified *Brucella* sp., as measured by the cELISA, is unclear and investigations to establish this are currently being conducted. The disease status of other Australian fur seal colonies remains unknown but may be expected to be similar, as the frequent movement of individuals between sites would facilitate the spread of infectious disease throughout the population.

Should pathogens capable of inducing high morbidity and mortality be introduced into the Australian fur seal population, the potential exists for propagation of an epizootic. Their susceptibility to epizootics is contributed to by the density of the

animals at haul-out sites, the local distribution of the species in Bass Strait, and the apparent lack of immunity to some infectious agents known to cause disease in other otariids.

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LITERATURE CITED

ABRAMSON, J. H. 2004. WinPepi (PEPI-for-Windows): Computer programs for epidemiologists. Epidemiological Perspectives and Innovations 1: 6.

ARNOULD, J. P. Y., AND R. KIRKWOOD. 2008. Habitat selection by female Australian fur seals (*Arctocephalus pusillus doriferus*). Aquatic Conservation: Marine and Freshwater Ecosystems 17: S53–S67.

BENGTSON, J. L., AND P. BOVENG. 1991. Antibodies to canine distemper virus in Antarctic seals. Marine Mammal Science 7: 85–87.

BERNARDELLI, A., R. BASTIDA, J. LOUREIRO, H. MICHELIS, M. I. ROMANO, A. CATALDI, AND E. COSTA. 1996. Tuberculosis in sea lions and fur seals from the south-western Atlantic coast. Revue scientifique et technique. Office International des Epizooties 15: 985–1005.

BLANC, A., D. RUCHANSKY, M. CLARA, F. ACHAVAL, A. LEBAS, AND J. ARBIZA. 2009. Serologic evidence of influenza A and B viruses in South American fur seals (*Arctocephalus australis*). Journal of Wildlife Diseases 45: 519–521.

BUREK, K. A., F. M. D. GULLAND, G. SHEFFIELD, K. B. BECKMEN, E. KEYES, T. R. SPRAKER, A. W. SMITH, D. E. SKILLING, J. F. EVERMANN, J. L. STOTT, J. T. SALIKI, AND A. W. TRITES. 2005. Infectious disease and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska, USA: Insights from serologic data. Journal of Wildlife Diseases 41: 512–524.

CALLAN, R. J., G. EARLY, H. KIDA, AND V. S. HINSHAW. 1995. The appearance of H3 influenza viruses in seals. Journal of General Virology 76: 199–203.

CHAMBERS, M. A. 2009. Review of the diagnosis and study of tuberculosis in non-bovine wildlife species using immunological methods. Transboundary and Emerging Diseases 56: 215–227.

COLAGROSS-SCHOUTEN, A. M., J. A. K. MAZET, F. M. D. GULLAND, M. A. MILLER, AND S. HIETALA. 2002. Diagnosis and seroprevalence of leptospirosis in California sea lions from coastal California. Journal of Wildlife Diseases 38: 7–17.

COLEGROVE, K. M., L. J. LOWENSTINE, AND F. M. D. GULLAND. 2005. Leptospirosis in northern elephant seals (*Mirounga angustirostris*) stranded along the California coast. Journal of Wildlife Diseases 41: 426–430.

COUSINS, D. V., R. BASTIDA, A. CATALDI, V. QUSE, S. REDROBE, S. DOW, P. DUIGNAN, A. MURRAY, C. DUPONT, N. AHMED, D. M. COLLINS, W. R. BUTLER, D. DAWSON, D. RODRIGUEZ, J. LOUREIRO, M. I. ROMANO, A. ALITO, M. ZUMARRAGA, AND A. BERNARDELLI. 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. International Journal of Systematic and Evolutionary Microbiology 53: 1305–1314.

DAWSON, C. E. 2005. Anti-*Brucella* antibodies in pinnipeds of Australia. Microbiology Australia 26: 87–89.

DELAHAY, R. J., G. C. SMITH, AND M. R. HUTCHINGS. 2009. The science of wildlife disease management. *In* Management of disease in wild mammals, R. J. Delahay, G. C. Smith, and M. R. Hutchings (eds.). Springer, Tokyo, Japan. pp. 1–8.

DIERAUF, L. A., D. J. VANDENBROEK, J. ROLETTO, M. KOSKI, L. AMAYA, AND L. J. GAGE. 1985. An epizootic of leptospirosis in California sea lions. Journal of the American Veterinary Medical Association 187: 1145–1148.

DUBEY, J. P. 2004. Toxoplasmosis—A waterborne zoonosis. Veterinary Parasitology 126: 57–72.

———, R. ZARNKE, N. J. THOMAS, S. K. WONG, W. VAN BONN, M. BRIGGS, J. W. DAVIS, R. EWING, M. MENSE, O. C. H. KWOK, S. ROMAND, AND P. THULLIEZ. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. Veterinary Parasitology 116: 275–296.

———, T. P. LIPSCOMB, AND M. MENSE. 2004. Toxoplasmosis in an elephant seal (*Mirounga angustirostris*). Journal of Parasitology 90: 410–411.

———, P. A. FAIR, G. D. BOSSART, D. HILL, R. FAYER, C. SREEKUMAR, O. C. H. KWOK, AND P. THULLIEZ. 2005. A comparison of several serologic tests to detect antibodies to *Toxoplasma*

gondii in naturally exposed bottlenose dolphins (*Tursiops truncatus*). Journal of Parasitology 91: 1074–1081.

———, ———, N. Sundar, G. Velmurugan, O. C. H. Kwok, W. E. McFee, D. Majumdar, and C. Su. 2008. Isolation of *Toxoplasma gondii* from bottlenose dolphins (*Tursiops truncatus*). Journal of Parasitology 94: 821–823.

Ewalt, D. R., J. B. Payeur, B. M. Martin, D. R. Cummins, and W. G. Miller. 1994. Characteristics of a *Brucella* species from a bottlenose dolphin (*Tursiops truncatus*). Journal of Veterinary Diagnostic Investigation 6: 448–452.

Fenwick, S. G., P. J. Duignan, C. M. Nicol, M. J. Leyland, and J. E. B. Hunter. 2004. A comparison of *Salmonella* serotypes isolated from New Zealand sea lions and feral pigs on the Auckland Islands by pulsed-field gel electrophoresis. Journal of Wildlife Diseases 40: 566–570.

Forshaw, D., and G. R. Phelps. 1991. Tuberculosis in a captive colony of pinnipeds. Journal of Wildlife Diseases 27: 288–295.

Foster, G., A. P. Macmillan, J. Godfroid, F. Howie, H. M. Ross, A. Cloeckaert, R. J. Reid, S. Brew, and I. A. P. Patterson. 2002. A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. Veterinary Microbiology 90: 563–580.

Geraci, J. R., D. J. St. Aubin, I. K. Barker, R. G. Webster, V. S. Hinshaw, W. J. Bean, H. L. Ruhnke, J. H. Prescott, G. Early, A. S. Baker, S. Madoff, and R. T. Schooley. 1982. Mass mortality of harbor seals: Pneumonia associated with influenza A virus. Science 215: 1129–1131.

Gibbens, J., L. J. Parry, and J. P. Y. Arnould. 2010. Influences on fecundity in Australian fur seals (*Arctocephalus pusillus doriferus*). Journal of Mammalogy 91: 510–518.

Goldstein, T., T. S. Zabka, R. L. Delong, E. A. Wheeler, G. Ylitalo, S. Bargu, M. Silver, T. Leighfield, F. Van Dolah, G. Langlois, I. Sidor, J. L. Dunn, and F. M. D. Gulland. 2009. The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. Journal of Wildlife Diseases 45: 91–108.

Grachev, M. A., V. P. Kumarev, L. V. Mamaev, V. L. Zorin, L. V. Baranova, N. N. Denikina, S. I. Belikov, E. A. Petrov, V. S. Kolesnik, R. S. Kolesnik, V. M. Dorofeev, A. M. Beim, V. N. Kudelin, F. G. Nagieva, and V. N. Sidoriv. 1989. Distemper virus in Baikal Seals. Nature 338: 209.

Haynes, L., E. Arzey, C. Bell, N. Buchanan, G. Burgess, V. Cronan, C. Dickason, H. Field, S. Gibbs, P. M. Hansbro, T. Hollingsworth, A. C. Hurt, P. Kirkland, H. McCracken, J. O'Connor, J. Tracey, J. Wallner, S. Warner, R. Woods, and C. Bunn. 2009. Australian surveillance for avian influenza viruses in wild birds between July 2005 and June 2007. Australian Veterinary Journal 87: 266–272.

Heide-Jorgensen, M. P., T. Harkonen, and P. Aberg. 1992. Long-term effects of epizootic in harbour seals in the Kattegat-Skagerrak and adjacent areas. Ambio 21: 511–516.

Hone, J., R. P. Duncan, and D. M. Forsyth. 2010. Estimates of maximum annual population growth rates (rm) of mammals and their application in wildlife management. Journal of Applied Ecology 47: 507–514.

Hunter, J. E., P. J. Duignan, C. Dupont, L. Fray, S. G. Fenwick, and A. Murray. 1998. First report of potentially zoonotic tuberculosis in fur seals in New Zealand. New Zealand Medical Journal 111: 130–131.

Kabay, M. J. 1996. Toxoplasmosis in a sea lion (*Neophoca cinerea*). Veterinary Pathology Report 43: 29–30.

Kennedy, S., T. Kuiken, P. D. Jepson, R. Deaville, M. Forsyth, T. Barrett, M. W. G. van de Bildt, A. Osterhaus, T. Eybatov, C. Duck, A. Kydyrmanov, I. Mitrofanov, and S. Wilson. 2000. Mass die-off of Caspian seals caused by canine distemper virus. Emerging Infectious Diseases 6: 637–639.

Kiers, A., A. Klarenbeek, B. Mendelts, D. Van Soolingen, and G. Koeter. 2008. Transmission of *Mycobacterium pinnipedii* to humans in a zoo with marine mammals. International Journal of Tuberculosis and Lung Disease 12: 1469–1473.

Kirkwood, R., F. Hume, and M. Hindell. 2008. Sea temperature variations mediate annual changes in the diet of Australian fur seals in Bass Strait. Marine Ecology–Progress Series 369: 297–309.

———, R. M. Warneke, and J. P. Y. Arnould. 2009. Recolonization of Bass Strait, Australia, by the New Zealand fur seal, *Arctocephalus forsteri*. Marine Mammal Science 25: 441–449.

———, D. Pemberton, R. Gales, A. Hoskins, A. Mitchell, P. D. Shaughnessy, and J. P. Y. Arnould. 2010. Continued population recovery by Australian fur seals. Marine and Freshwater Research 61: 1–7.

Kittelberger, R., F. Hilbink, M. F. Hansen, G. P. Ross, M. A. Joyce, S. Fenwick, J. Heesemann, H. Wolfwatz, and K. Nielsen. 1995. Serological crossreactivity between *Brucella abortus* and *Yersinia enterocolitica* 0:9 I immunoblot analysis of the antibody response to *Brucella* protein antigens in bovine brucellosis. Veterinary Microbiology 47: 271–280.

Lancaster, M. L., J. P. Y. Arnould, and R. Kirkwood. 2010. Genetic status of an endemic marine mammal, the Australian fur seal, following historical harvesting. Animal Conservation 13: 247–255.

Liess, B., H. R. Frey, T. Willhaus, and A. Zaghawa.

1989. Morbillivirus infection among seals (*Phoca vitulina*) during the 1988 epidemic in the bay of Heligoland. II. Serological investigations reflecting a previous phocine distemper epidemic in a seal orphanage. Journal of Veterinary Medicine Series B 36: 709–714.

LYASHCHENKO, K. P., R. GREENWALD, J. ESFANDIARI, M. A. CHAMBERS, J. VICENTE, C. GORTAZAR, N. SANTOS, M. CORREIA-NEVES, B. M. BUDDLE, R. JACKSON, D. J. O'BRIEN, S. SCHMITT, M. V. PALMER, R. J. DELAHAY, AND W. R. WATERS. 2006. Tuberculosis in elephants: Antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. Clinical and Vaccine Immunology 13: 722–732.

———, ———, ———, J. H. OLSEN, R. BALL, G. DUMONCEAUX, F. DUNKER, C. BUCKLEY, M. RICHARD, S. MURRAY, J. B. PAYEUR, P. ANDERSEN, J. M. POLLOCK, S. MIKOTA, M. MILLER, D. SOFRANKO, AND W. R. WATERS. 2008. Animal-side serologic assay for rapid detection of *Mycobacterium bovis* infection in multiple species of free-ranging wildlife. Veterinary Microbiology 132: 283–292.

MACKERETH, G. F., K. M. WEBB, J. S. O'KEEFE, P. J. DUIGNAN, AND R. KITTELBERGER. 2005. Serological survey of pre-weaned New Zealand fur seals (*Arctocephalus forsteri*) for brucellosis and leptospirosis. New Zealand Veterinary Journal 53: 428–432.

MARATEA, J., D. R. EWALT, S. FRASCA, J. L. DUNN, S. DE GUISE, L. SZKUDLAREK, D. J. ST. AUBIN, AND R. A. FRENCH. 2003. Evidence of *Brucella* sp. infection in marine mammals stranded along the coast of southern New England. Journal of Zoo and Wildlife Medicine 34: 256–261.

MCDONALD, W. L., R. JAMALUDIN, G. MACKERETH, M. HANSEN, S. HUMPHREY, P. SHORT, T. TAYLOR, J. SWINGLER, C. E. DAWSON, A. M. WHATMORE, E. STUBBERFIELD, L. L. PERRETT, AND G. SIMMONS. 2006. Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. Journal of Clinical Microbiology 44: 4363–4370.

MCINTOSH, R. R., B. PAGE, AND S. D. GOLDSWORTHY. 2006. Dietary analysis of regurgitates and stomach samples from free-living Australian sea lions. Wildlife Research 33: 661–669.

MESSIER, V., B. LEVESQUE, J. F. PROULX, L. ROCHETTE, M. D. LIBMAN, B. J. WARD, B. SERHIR, M. COULLARD, N. H. OGDEN, E. DEWAILLY, B. HUBERT, S. DERY, C. BARTHE, D. MURPHY, AND B. DIXON. 2009. Seroprevalence of *Toxoplasma gondii* among Nunavik Inuit (Canada). Zoonoses and Public Health 56: 188–197.

MILLER, M., W. A. MILLER, M. P. CONRAD, E. R. JAMES, A. C. MELLI, C. M. LEUTENEGGER, H. A. DABRITZ, A. E. PACKHAM, D. PARADIES, M. HARRIS, J. AMES, D. A. JESSUP, K. WORCESTER, AND M. E. GRIGG. 2008. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. International Journal for Parasitology 38: 1319–1328.

NIELSEN, K. 1990. The serological response of cattle immunized with *Yersinia enterocolitica* 0–9 or O-16 to *Yersinia* and *Brucella abortus* antigens in enzyme immunoassays. Veterinary Immunology and Immunopathology 24: 373–382.

NIELSEN, O., A. CLAVIJO, AND J. A. BOUGHEN. 2001a. Serologic evidence of influenza A infection in marine mammals of Arctic Canada. Journal of Wildlife Diseases 37: 820–825.

———, R. E. A. STEWART, K. NIELSEN, L. MEASURES, AND P. DUIGNAN. 2001b. Serologic survey of *Brucella* spp. antibodies in some marine mammals of North America. Journal of Wildlife Diseases 37: 89–100.

———, K. NIELSEN, R. BRAUN, AND L. KELLY. 2005. A comparison of four serologic assays in screening for *Brucella* exposure in Hawaiian monk seals. Journal of Wildlife Diseases 41: 126–133.

———, G. SMITH, H. WEINGARTL, S. LAIR, AND L. MEASURES. 2008. Use of a SLAM transfected vero cell line to isolate and characterize marine mammal morbilliviruses using an experimental ferret model. Journal of Wildlife Diseases 44: 600–611.

NORMAN, S. A. 2008. Spatial epidemiology and GIS in marine mammal conservation medicine and disease research. Ecohealth 5: 257–267.

NORRIS, J. M., M. B. KROCKENBERGER, A. A. BAIRD, AND G. KNUDSEN. 2006. Canine distemper: Re-emergence of an old enemy. Australian Veterinary Journal 84: 362–363.

OSTERHAUS, A. D. M. E., J. GROEN, P. DEVRIES, F. G. C. M. UYTDEHAAG, B. KLINGEBORN, AND R. ZARNKE. 1988. Canine distemper virus in seals. Nature 335: 403–404.

PRENGER-BERNINGHOFF, E., U. SIEBERT, M. STEDE, A. KOENIG, R. WEISS, AND G. BALJER. 2008. Incidence of *Brucella* species in marine mammals of the German North Sea. Diseases of Aquatic Organisms 81: 65–71.

ROBERTS, M. W., L. SMYTHE, M. DOHNT, M. SYMONDS, AND A. SLACK. 2010. Serologic-based investigation of leptospirosis in a population of free-ranging eastern grey kangaroos (*Macropus giganteus*) indicating the presence of *Leptospira weilii* serovar Topaz. Journal of Wildlife Diseases 46: 564–569.

SAMARTINO, L., D. GALL, R. GREGORET, AND K. NIELSEN. 1999. Validation of enzyme-linked immunosorbent assays for the diagnosis of bovine brucellosis. Veterinary Microbiology 70: 193–200.

SCHULTZ, R. D., B. THIEL, E. MUKHTAR, P. SHARP, AND

L. J. Larson. 2010. Age and long-term protective immunity in dogs and cats. Journal of Comparative Pathology 142: S102–S108.

Selleck, P. 2008. Influenza A virus: A competitive ELISA for the detection of antibodies to influenza A viruses in equine sera. CSIRO Livestock Industries, CSIRO Publishing, Canberra, Australia. 20 pp.

Shaughnessy, P. D. 1999. The action plan for Australian seals. CSIRO Wildlife and Ecology, CSIRO Publishing, Canberra, Australia. 116 pp.

Smith, A. W., R. J. Brown, D. E. Skilling, H. L. Bray, and M. C. Keyes. 1977. Naturally-occurring leptospirosis in northern fur seals (*Callorhinus ursinus*). Journal of Wildlife Diseases 13: 144–148.

Sohn, A. H., W. S. Probert, C. A. Glaser, N. Gupta, A. W. Bollen, J. D. Wong, E. M. Grace, and W. C. McDonald. 2003. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. Emerging Infectious Diseases 9: 485–488.

Stuen, S., P. Have, A. D. M. E. Osterhaus, J. M. Arnemo, and A. Moustgaard. 1994. Serological investigation of virus infections in harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*). Veterinary Record 134: 502–503.

Subcommittee on Animal Health Laboratory Standards (SCAHLS). 2010. Australian and New Zealand Standard Diagnostic Procedures Schedule. www.scahls.org.au/. Accessed July 2010.

Tryland, M., K. K. Sorensen, and J. Godfroid. 2005. Prevalence of *Brucella pinnipediae* in healthy hooded seals (*Cystophora cristata*) from the North Atlantic Ocean and ringed seals (*Phoca hispida*) from Svalbard. Veterinary Microbiology 105: 103–111.

Warneke, R. M. 1982. The distribution and abundance of seals in the Australasian region, with summaries of biology and current research. *In* Mammals in the seas: Small cetaceans, seals, sirenians and otters. Food and Agriculture Organisation, Rome, Italy. pp. 431–475.

———, and P. D. Shaughnessy. 1985. *Arctocephalus pusillus*, the South African and Australian fur seal: Taxonomy, evolution, biogeography, and life history. *In* Studies of sea mammals in south latitudes, J. K. Ling and M. M. Bryden (eds.). South Australian Museum, Adelaide, Australia. pp. 53–77.

Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. 1992. Evolution and ecology of influenza A viruses. Microbiological Reviews 56: 152–179.

Wilkinson, I. S., P. J. Duignan, A. Castinel, A. Grinberg, B. L. Chilvers, and B. C. Robertson. 2006. *Klebsiella pneumoniae* epidemics: Possible impact on New Zealand sea lion recruitment. *In* Sea lions of the world, A. W. Trites, S. K. Atkinson, D. P. De Master, L. W. Fritz, T. S. Gelayy, L. D. Rea, and K. M. Wynne (eds.). University of Alaska Press, Fairbanks, Alaska. pp. 385–403.

Woods, R., D. V. Cousins, R. Kirkwood, and D. L. Obendorf. 1995. Tuberculosis in a wild Australian fur seal (*Arctocephalus pusillus doriferus*) from Tasmania. Journal of Wildlife Diseases 31: 83–86.

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