LETTERS

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Postmortem Findings in Four South American Sea Lions (*Otaria byronia*) from an Urban Colony in Valdivia, Chile

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ABSTRACT: We performed postmortem examination on four South American sea lions (*Otaria byronia*) from an urban colony in Valdivia, Chile. Chronic leptospirosis and suspected morbillivirus-like infection were diagnosed in one individual. Antibodies against *Toxoplasma gondii* and the zoonotic helminthes *Contracaecum* sp., *Pseudoterranova* sp., and *Diphyllobothrium* sp. were also detected.

A resident, nonbreeding, small group of South American sea lions (SASLs; *Otaria byronia*) has been present since the mid-1970s in the center of Valdivia, southern Chile (39°48′S, 73°14′W) (Schlatter 1976). This small group consists of approximately of 50 juvenile, subadult, and adult males. Because SASLs use public spaces, there is a risk of pathogen transmission between SASLs and humans and their pets. We performed postmortem examination in four SASLs to determine cause of death and assess presence of relevant pathogens.

Between March 2008 and December 2010 we necropsied four SASLs and performed ancillary diagnostic studies. Two SASLs (SASLs 1 and 2) were part of an ecologic study and died during routine anesthesia (1.2 mg/kg of tiletamin/zolazepam, Zoletil®, delivered intramuscularly) (Haulena 2008). Both animals died despite being apparently healthy at the time of darting. The two additional sea lions (SASL 3 and 4) were found emaciated on the shore of the Valdivia River and died within 24 h of discovery. Serum samples to detect antibodies against canine distemper virus (CDV), canine parvovirus-2 (CPV-2), *Toxoplasma gondii*, *Leptospira interrogans* serovars Pomona, Icterohaemorrhagiae, Hardjo, Bratislava, Copenhageni, Canicola; *Leptospira kirschneri* serovar Grippotyphosa; and *Leptospira biflexa* serovar Patoc were collected premortem from SASLs 1, 2, and 4. Blood from SASL 3 was plated on MacConkey and blood agars.

Complete necropsies were performed on all SASLs within 24 h of death. Sections from major organs and tissues (including brain) were fixed in 10% neutral buffered formalin and processed for histopathology. Samples of lung, liver, and mesenteric, bronchial, and mediastinal lymph nodes were collected from SASLs 3 and 4 and were processed and cultured, and the microorganisms were identified by standard bacteriologic techniques (Barrow and Feltham 2004). Gastrointestinal parasites were placed in 70% ethanol and identified by light microscopy (Carvajal et al. 1983; Mercado et al. 2010). Immunohistochemistry (IHC) using a monoclonal antibody against CDV nucleoprotein was performed on lung, mediastinal lymph node, brain, spleen, and bladder (Stone et al. 2011). We also performed IHC on kidney samples using a *Leptospira*-specific polyclonal antibody against *L. interrogans* serovars Bratislava,

TABLE 1. Antibody titers against selected pathogens in three South American sea lions (*Otaria byronia*), Valdivia, Chile, between 2008–10.

Pathogen[a]	Antibody titers[b]			Test performed
	Sea lion 1	Sea lion 2	Sea lion 4	
CDV[c]	1:8	NR	1:8	Viral seroneutralization
CPV-2[d]	1:64	1:64	1:8	Hemagglutination Inhibition
Leptospira interrogans serovar Bratislava[d]	1:800	NR	NA	Microagglutination
L. interrogans serovar Hardjo[c,d]	NR	NR	NR	Microagglutination
L. interrogans serovar Icterohaemorrhagiae[d]	NR	NR	NA	Microagglutination
L. interrogans serovar Pomona[c,d]	1:400, 1:800[e]	NR	NR	Microagglutination
L. interrogans serovar Copenhageni[c]	NR	NR	NR	Microagglutination
L. interrogans serovar Canicola[c,d]	NR	NR	NR	Microagglutination
Leptospira kirschneri serovar Grippotyphosa[c,d]	NR	NR	NR	Microagglutination
Leptospira biflexa serovar Patoc[d]	1:200	1:100	NA	Microagglutination
Brucella abortus[c]	NR	NR	NR	Bengal rose
Brucella canis[d]	NR	NR	NR	Plaque agglutination
Brucella sp.[d]	NR	NR	NR	Plaque agglutination
Toxoplasma gondii[d]	NR	1:256	NA	Latex agglutination

[a] CDV = canine distemper virus; CPV-2 = canine parvovirus-2.

[b] NR = not reactive; NA = not analyzed.

[c] Tests performed at the Chilean Agricultural and Livestock Department Laboratories.

[d] Tests performed in the laboratories of the College of Veterinary Medicine, University of Minnesota.

[e] Both titers are shown when results from the two laboratories were different.

Canicola, Hardjo, Icterohaemorrhagiae, and Pomona, and against *L. kirschneri* Grippotyphosa (Colegrove et al. 2005). Serologic results are shown in Table 1.

Sea lion 1 was a 200-cm, 320-kg, adult male. Antibodies to CDV (1:8), CPV-2 (1:64), and *L. interrogans* serovars Pomona and Bratislava (>1:400) were detected. At histopathology there was mild, nonsuppurative meningoencephalitis and moderate, multifocal neutrophilic and lymphoplasmacytic bronchopneumonia with epithelial necrosis and abundant deposition of fibrin in the bronchioles. Occasional bronchiolar gland epithelial cells presented mild intracytoplasmatic staining for morbillivirus antigen. There was moderate multifocal lymphoplasmacytic interstitial nephritis and a few leptospires detected in the renal tubules with the Warthin-Starry silver stain and IHC (Fig. 1). Sea lion 2 was a 210-cm, 250-kg, adult male. Antibodies against CPV-2 (1:64) and *Toxoplasma gondii* (1:256) were detected. The same pattern of bronchopneumonia observed in SASL 1 was found in SASL 2 but was milder. Sea lion 3 was a 120-cm, 65-kg, juvenile male. On postmortem examination, 70% of the lung parenchyma presented a marked multifocal to coalescing bronchointerstitial histiocytic pneumonia. Gram-negative bacilli and Gram-positive cocci were observed inside macrophages in the lung, spleen, and mediastinal and axillary lymph nodes. There was moderate lymphoid depletion in the spleen and most lymph nodes. *Proteus mirabilis* and *Staphylococcus* sp. (nonhemolytic) were isolated from lung, spleen, and mediastinal and axillar lymph nodes. Protozoal cysts (probably *Sarcocystis* sp.) were found in the skeletal muscles. Sea lion 4 was a 180-kg, 160-cm, subadult male with antibodies against CDV (1:8) and CPV-2 (1:8). This animal presented the same pattern of bronchointerstitial pneumonia found in SASL 3. *Escherichia coli* and a nonhemolytic *Staphylococcus*

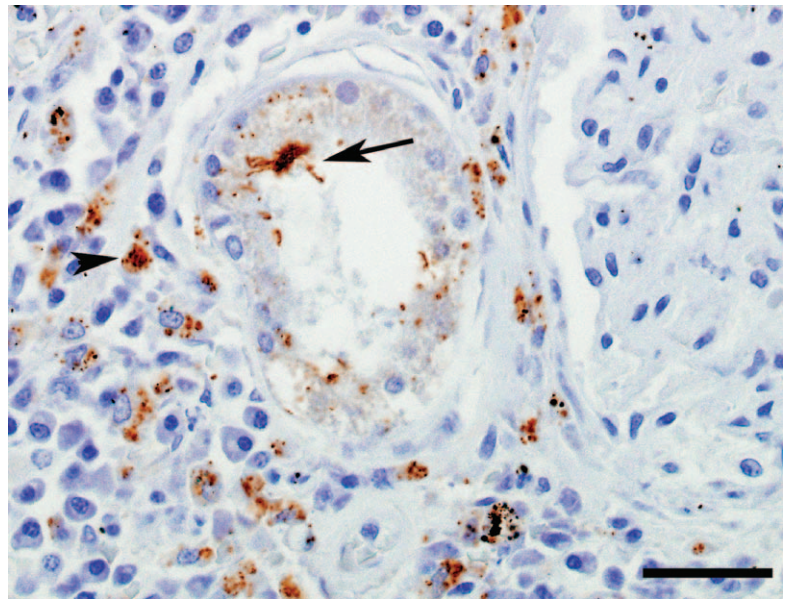

FIGURE 1. Kidney photomicrograph from a South American sea lion (*Otaria byronia*), SASL 1. There are occasional aggregates of *Leptospira* sp. spirochetes within a renal tubule (arrow), and occasional macrophages that surround the affected tubule contain a large amount of partially degraded *Leptospira* sp. antigen (arrow head). Immunohistochemistry for *Leptospira* sp. antigen counterstained with hematoxylin. Bar = 25 μm.

sp. were isolated from lung, spleen, and mesenteric and mediastinal lymph nodes.

Sea lions 1, 2, and 4 had severe infections with the nematodes *Contracaecum* sp. and *Pseudoterranova* sp. A few tapeworms (*Diphyllobotrium* sp.) were found in SASLs 1 and 2. Sea lion 3 had a low number of the trematode *Ogmogaster heptalineatus*. The only histopathologic change associated with these parasites was moderate eosinophilic gastritis in animals infected with *Contracaecum* sp. and *Pseudoterranova* sp.

Although SASLs 1 and 2 died during anesthesia, postmortem findings indicate that moderate to severe chronic infections in the respiratory and renal systems could have played a role during the anesthesia (Haulena 2008). Another possibility is an idiopathic adverse reaction to tiletamine-zolazepam, a drug combination that has caused apnea and death in otariids (Dabin et al. 2002). The most probable cause of death of SASLs 3 and 4 was pneumonia and later systemic infection with Gram-positive and Gram-negative opportunistic bacteria, one of the most prevalent causes of natural death in young otariids (Seguel et al. 2011). Serologic and immunohistochemical findings in SASL 1 and serology in SASL 4 indicate a low reaction to CDV or another related morbillivirus. In pinnipeds there have been outbreaks of CDV affecting seals (Phocidae; Kuiken et al. 2006); however, we know of no reports of CDV clinical illness in eared seals (Otariidae). Canine distemper is a common disease in dogs in Valdivia (Ernst et al. 1997); thus it is possible that dogs were the source of CDV exposure in these SASLs, as has been suggested in phocids (Kuiken et al. 2006). In the case of CPV-2, the low antibody titers (1:8 and 1:64) could represent previous exposure to or cross-reaction with an unknown parvovirus.

The serology, histopathology, and immunohistochemistry of SASL 1 are indicative of chronic leptospirosis (Gulland et al. 1996). Clinical leptospirosis is the second most-common cause of stranding in California sea lions (*Zalophus californianus*) (Greig et al. 2005). To our

knowledge this is the first report of *L. interrogans* infection in SASLs.

The presence of *T. gondii* antibodies suggests contamination of the river water with cat feces (Miller et al. 2002). This would not be surprising given the high (up to 30%) prevalence of *T. gondii* in cats in Valdivia (Ovalle et al. 2000). *Toxoplasma gondii* antibody prevalence of up to 40% has been found in California sea lions, and there are some reports of encephalitis and disseminated infection in otariids (reviewed in Dubey et al. 2003).

We have shown that SASLs are exposed to domestic animal shared pathogens (CDV, CPV-2, *T. gondii*) and are infected with agents that can represent zoonotic risk (*L. interrogans*, *Contracaecum* sp., *Pseudoterranova* sp., and *Diphyllobotrium* sp.). This highlights the potential use of marine mammals as animal and human health sentinels in an urban environment.

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