DOI: 10.7589/2016-05-092

Journal of Wildlife Diseases, 53(3), 2017, pp. 491–498

PUP MORTALITY AND EVIDENCE FOR PATHOGEN EXPOSURE IN GALAPAGOS SEA LIONS (*ZALOPHUS WOLLEBAEKI*) ON SAN CRISTOBAL ISLAND, GALAPAGOS, ECUADOR

Judith Denkinger,[1,10] Nataly Guevara,[1] Sofia Ayala,[1] Juan Carlos Murillo,[2] Maximilian Hirschfeld,[1] Ignasi Montero-Serra,[1,3] Katharina Fietz,[1,4] Tracey Goldstein,[5] Mark Ackermann,[6] Veronica Barragán,[1,9] Francisco Cabrera,[7] Cristina Chavez,[1] Edward J. Dubovi,[8] Jael Martinez,[1] and Gabriel Trueba[1]

[1] Colegio de Ciencias Biológicas y Ambientales y Galapagos Science Center, Universidad San Francisco de Quito, Campus Cumbaya, Quito, Ecuador
[2] Universidad de la Peninsula Santa Elena, Salinas, Ecuador
[3] Departament d'Ecologia, Universitat de Barcelona, 08028 Barcelona, Spain
[4] University of Hamburg, Zoological Museum and Biocenter Grindel, Martin-Luther-King Platz 3, 20146 Hamburg, Germany
[5] Wildlife Health Center, School of Veterinary Medicine, 1089 Veterinary Medicine Drive, University of California, Davis, California 95616, USA
[6] Department of Veterinary Pathology, Iowa State University, 1800 Christensen Drive, Ames, Iowa 50011, USA
[7] Universidad San Francisco de Quito, Colegio de Veterinaria, Campus Cumbaya, Quito, Ecuador
[8] Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, PO Box 5786, Ithaca, New York 14850, USA
[9] The Center of Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, Arizona 86011, USA
[10] Corresponding author (email: jdenkinger@usfq.edu.ec)

ABSTRACT: The Galapagos sea lion (*Zalophus wollebaeki*), an endangered species, experiences high pup mortality (up to 100%) in years when El Niño events reduce food supply in the Galapagos Islands. Mortality of pups in non-El Niño years is estimated to be 5% in undisturbed colonies. From 2009 to 2012 we observed high pup mortality (up to 67%) in colonies close to the Galapagos capital, Puerto Baquerizo Moreno, where contact with humans, domestic animals, and rats is frequent. Gross postmortem findings from 54 pups included hemorrhagic lesions in liver and congestion in lungs; histopathology suggested a possible association with infectious diseases. Evidence of *Leptospira* infection was found in five out of seven samples collected in 2010. Canine distemper viral (CDV) RNA was detected in tissues from six sea lions (in 2011–12), four of which were confirmed by nucleotide sequencing. The absence of CDV antibodies in 109 juvenile animals tested in 2014 at urban and remote colonies could indicate that the CDV infection observed in 2011 was likely confined to a few animals. Our results indicated that Galapagos sea lions have been exposed at least to two pathogens, *Leptospira* and CDV; however, the impact of these infections on the sea lions is unclear.

Key words: Canine distemper virus, Galapagos sea lion, leptospirosis, pup mortality, *Zalophus wollebaeki*.

INTRODUCTION

Galapagos sea lions (*Zalophus wollebaeki*) are listed as endangered by the International Union for Conservation of Nature due to the drastic 50% population decline that occurred during the past three decades (Aurioles and Trillmich 2008). Annual pup mortality of sea lions has been estimated at 5% in Galapagos sea lions (Trillmich and Limberger 1985) compared to 11% in California sea lions (*Zalophus californianus*; Aurioles and Sinsel 1988). However, mortality of Galapagos sea lion pups can increase to 100% during strong El Niño events (Trillmich and Limberger 1985). Additionally, the sea lion population did not increase in years with the absence of an El Niño event (Denkinger et al. 2015).

Galapagos sea lions breed on all the 11 central islands of the Galapagos Archipelago where they spend extended periods of time on land because they have a 5 mo breeding season, and pups can be nursed for 2–3 yr (Trillmich et al. 2014). One of the largest rookeries is in the town center of Puerto Baquerizo Moreno on San Cristobal Island, where sea lions are often in close contact with humans, pets, and domestic rats, which might

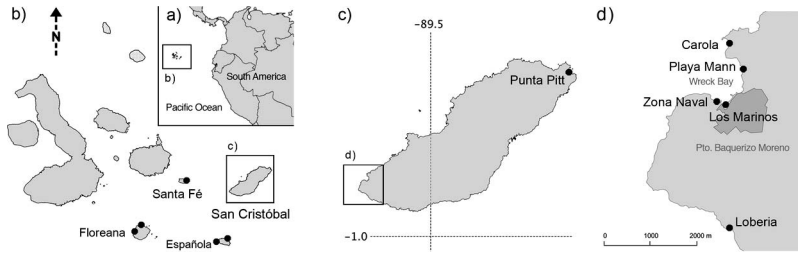

FIGURE 1. Map showing the Galapagos sea lion (*Zalophus wollebaeki*) study area on San Cristobal Island with the situation of (a) the Galapagos Archipelago, (b) San Cristobal Island in the Archipelago, and (c) the sampling area on San Cristobal Island with (d) the specific sampling sites in Wreck Bay off Puerto Baquerizo Moreno (dark gray).

be a potential source of pathogens (Brock et al. 2013).

Pathogens originating from domestic animals and that have the potential to cause disease in sea lions include canine distemper virus (CDV), *Leptospira,* parvovirus, herpesvirus, calicivirus, *Brucella* (Burek et al. 2005), and *Toxoplasma* (Dubey et al 2003; Conrad et al. 2005). The CDV is a morbillivirus that causes disease in dogs and other carnivores, including California sea lions (Barret et al. 2004), and has also been responsible for severe disease and mortality of harbor seals (*Phoca vitulina*) in the North and Baltic seas (Osterhaus et al. 1990) and Caspian seals (*Phoca caspica*) in the Caspian Sea (Kennedy et al. 2000). Leptospirosis has been associated with kidney disease and death in Californian sea lions (Gulland et al. 1996), and antibodies against various leptospiral serovars were previously reported in Galapagos sea lions using a microscopic agglutination test (Simental 2006). Antibodies against distemper virus and *Leptospira* were also recently detected in South American sea lions (*Otaria flavescens*) from an urban colony in Valdivia, Chile (Sepúlveda et al. 2015).

We examined the potential causes of increased annual pup mortality in the Galapagos Islands from 2009 to 2012. We monitored annual pup mortality, assessed tissue pathology in dead pups, and tested samples for seven pathogens that have been described to cause disease in pinnipeds.

MATERIALS AND METHODS

Study area

Samples were collected from Galapagos sea lions on San Cristobal Island from five urban rookeries (Carola 0°35′S, 89°36′W, Playa Mann 0°35′S, 89°36′W, Playa de los Marinos 0°54′S, 89°36′W, Zona Naval 0°54′S, 89°36′W, and Loberia 0°55′S, 89°36′W) located at Wreck Bay in Puerto Baquerizo Moreno. Wreck Bay is an important port for fisheries and the second most important tourist destination in the Galapagos Islands. Samples were also collected in 2014 from sea lions at remote rookeries on San Cristobal Island (Punta Pitt), Floreana, and Española Islands, where sea lions have no contact with dogs and humans. (Fig. 1).

Population census and mortality

In 2008 we began weekly surveys of the sea lion numbers on the rookeries and around Wreck Bay. Counts of dead and sick animals were also performed from 2008 to 2012. Pup mortality during each breeding season (June to January, peaking in October to November) was calculated by the number of dead pups (fetuses, newborn pups, and pups older than 2 wk) out of the total number of pups born through the end of the breeding season in November (Seguel et al. 2013). Monthly mortality was calculated by the number of dead pups out of the total pup births recorded each month during the 2011–12 breeding season.

Sample collection

Data collected upon necropsy included morphometric measurements; and pups were classified as malnourished when they had no or thin fat layers of up to 2 mm midsternal blubber thickness; as diseased when organs contained featured lesions; or injured, including when

trauma signs such as dog bites, shark attack injuries, or any other injury were present.

Samples (tissue and blood) were collected from carcasses and live animals from 2009 to 2012 and tested for infectious agents and examined by histopathology. All tissue samples were frozen at –20 C and a duplicate was stored in 10% formalin for histopathologic analysis. During the 2011–12 breeding season, tissue samples from 34 pups were examined by histopathology.

Serum samples were collected from 109 apparently healthy juveniles live-captured in 2014 at urban rookeries: Wreck Bay (n=30); and remote sites Punta Pitt (n=20), Española (Playa Gardner [1°21′S, 89°39′W, n=10], Punta Suarez, [1°22′S, 89°44′W, n=9], Santa Fe [0°48′S, 90°02′W, n=20]), and Floreana (Mirador de la Baronesa [1°14′S, 90°26′W, n=20]). Blood samples were drawn from the caudal gluteal vein of manually restrained animals (Brock et al. 2013; Paez-Rosas et al. 2015).

Pathogen analysis

We extracted DNA and RNA from tissue samples following the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) and RNeasy kit (Qiagen, Boston, Massachusetts, USA) respectively, following the manufacturer specifications.

Samples were tested by PCR for the presence of *Leptospira* by amplifying the 298-base pair (bp) fragment of the 16S rARN gene (Mérien et al. 1992). All positive samples were sequenced and results were compared to sequences using BLAST GenBank (National Center for Biotechnology Information 2016) and MEGA software, version 5.0 (Tamura et al. 2011). Sequences were aligned using available sequences in the GenBank by Neighbor Joining and Maximum Parsimony methods and evolutionary distances were computed with the Kimura 2-parameter method using MEGA software version 5.0 (Tamura et al. 2011).

Samples were also tested by PCR for *Brucella* by targeting the *bcsp31* gene (Kumar et al. 2011) and *Toxoplasma gondii* (Lin et al. 2000). We tested for the presence of viruses using panherpesvirus assay targeting a fragment of the DNA polymerase gene (VanDevanter et al. 1996); pan-calicivirus PCR targeting the RNA-dependent RNA polymerase region (Reid et al. 2007), influenza A virus PCR targeting a fragment of the matrix protein gene (Spackman et al. 2003), and CDV PCR targeting a 149-bp fragment of the phosphoprotein gene (Stanton et al. 2002).

Amplicons from all CDV positive samples were sequenced at Functional Biosciences (Madison, Wisconsin, USA). Sequences were aligned using available CDV and phocine distemper virus sequences in using Neighbor Joining and Maximum Parsimony methods with MEGA software version 5.0 (Tamura et al. 2011). The presence of CDV antibodies was measured by virus neutralization assay (Appel and Robson 1973) using twofold serum dilutions in duplicate and 100-300 TCID50 of CDV (Onderstepoort strain, obtained from Baker Institute, Ithaca, New York, USA).

RESULTS

Population census and pup mortality counts

Pup mortality increased from 2% in 2008 to 52% in the 2009, 45% in the 2011, and 43% in the 2012 breeding seasons (Table 1). In 2009, overall mortality in Wreck Bay was highest (52%), and Loberia was the most affected site (67%) followed by Playa de los Marinos (56%). In 2010, overall mortality decreased to 30% but increased again in the 2011 and 2012 breeding seasons to 45% and 43%, respectively. Site-specific mortality in 2011 and 2012 was highest at Carola (54% and 53%, respectively) and Playa Mann (51% and 49%, respectively).

The increase in pup mortality in 2011 began in April, and aborted fetuses were found in May. The frequency of abortions and stillbirths increased until August, and then decreased from September to December (Fig. 2). Most of the newborns (up to 2 wk of age) and pups (2 wk to 6 mo of age) died shortly after birth (n=28); 15 (pups) died after 2 wk, and 11 (fetuses) were born prematurely or were aborted. Fetuses or premature pups had a mean standard length of 45 cm and an average weight of 3 kg, whereas newborn pups measured 64 cm and weighed 5 kg. Older pups died with an average length of 67 cm and average weight of 5 kg with an extremely thin fat layer (0–2 mm; Fig. 3).

Gross pathology and histopathology

We examined 54 carcasses found during the 2011–12 breeding season and sampled 34 for histopathology (six aborted fetuses, 25 newborn pups <2 wk old, and three pups of at least 2 wk of age). Livers and lungs appeared to be the most commonly affected (55% and 49% of the animals necropsied) showing hemorrhage (n=7) and congestion (n=9) in

TABLE 1. Total number of Galapagos sea lion (*Zalophus wollebaeki*) pups born, percent pup mortality rates at four rookeries, and totals from Wreck Bay, San Cristobal Island, Galapagos Islands, at the end of the breeding season in November from 2008 through 2012.

Year	Pup production[a]	Rookery				Wreck Bay total
		Loberia	Playa de los Marinos	Playa Mann	Carola	
2008	Total pups born	17	16	8	4	45
	% mortality	6	0	0	0	2
2009	Total pups born	12	40	8	4	64
	% mortality	67	56	25	29	52
2010	Total pups born	11	24	8	4	47
	% mortality	36	37	12	0	30
2011	Total pups born	50	57	32	30	169
	% mortality	32	49	51	54	45
2012	Total pups born	44	43	25	17	129
	% mortality	34	39	49	53	43

[a] Pup production was calculated as mean of all November pup counts.

the lungs. Additionally, gross pathology showed that tracheal hemorrhage was present in the mucosa of 42% of the animals, and stomachs were hemorrhagic and/or ulcerated in 31% of the animals.

Lesions in the lungs (n=25) showed increased alveolar septal thickening with infiltrating lymphocytes and plasma cells (n=13). Four of the aborted fetuses had increased fetal connective tissue stroma in alveolar septae and increased alveolar septal thickening. Eight out of 25 newborns and one out of three pups had increased septal thickening with mild to moderate lymphocytic and/or plasma cell infiltration in septal walls. Three (two newborns and one pup) had increased numbers of alveolar macrophages in the alveolar lumen (alveolar histiocytosis). Three newborn pups had large alveolar cells that were occasionally multinucleate and thus had features of syncytial cells. Congestion and hemorrhage was present in 20 lungs. The livers from 21 pups were examined, 13 of which (one fetus, 10 newborns, and two pups) had vacuolar change that was suggestive of fat (or glycogen) accumulation in hepatocytes, indicative of metabolic stress and consistent with reduced subcutaneous adipose tissue present grossly in many pups. Two pups had bile stasis within hepatocytes. Kidney tissue was collected from 21 pups, of which four fetuses and 16 newborns had congested blood vessels. Stomach tissue was collected from 11 animals, of which one fetus, two newborns, and one pup had congestion or hemorrhage. Additionally, 25% of live animals observed during colony counts in the 2011–12 breeding season showed signs of disease, including

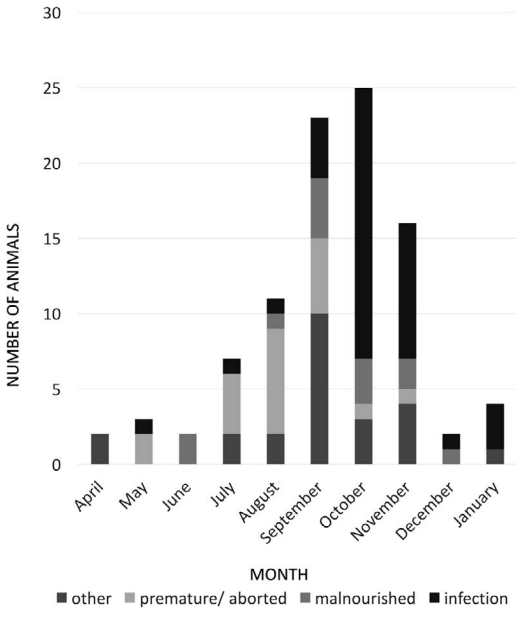

FIGURE 2. Cause of death by month of Galapagos sea lion (*Zalophus wollebaeki*) pups necropsied during the pupping season of 2011–12 at San Cristobal Island, Galapagos Islands.

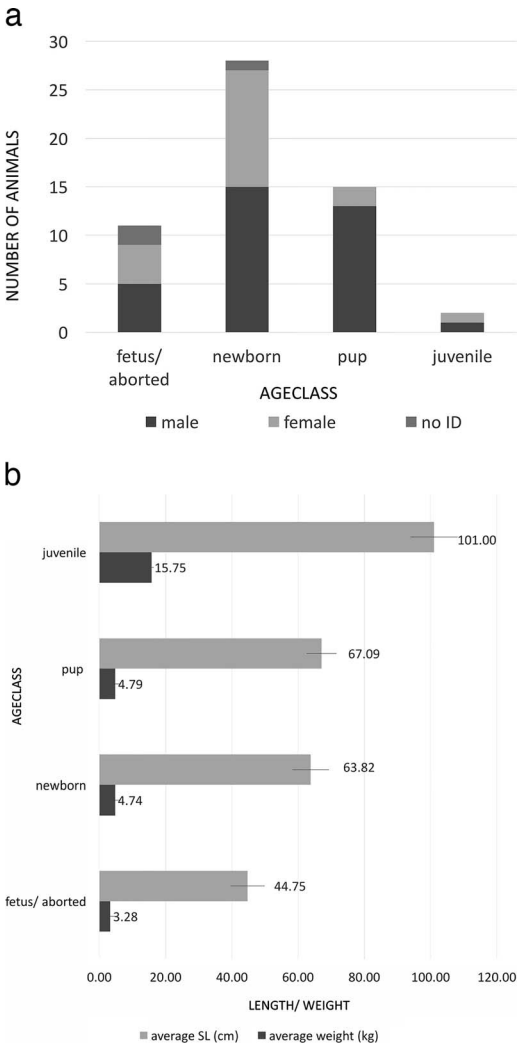

FIGURE 3. (a) Sex per age class and (b) average standard length (SL) and weight of Galapagos sea lion (*Zalophus wollebaeki*) juveniles (n=2), pups (n=15), newborns (n=27), and fetuses or aborted pups (n=11) sampled from June to December 2011 at Puerto Baquerizo Moreno, San Cristobal Island, Galapagos Islands.

increased mucus discharge from the nostrils and eyes.

Infectious disease diagnostics

Five out of seven tissue samples (three kidneys and two placentas) were positive for *Leptospira* by PCR and DNA sequences showed similarity to the genus *Leptospira* (GenBank accession no. P3: KU991657, P4: KU991658, R1: KU991659, R2: KU991660, R3: KU991661). Amplicons from two placentas and one kidney showed a high degree of identity (89%) to leptospiral pathogenic species (Fig. 4).

Six of 48 tissues (four lung and two placenta samples collected between August to November 2011) tested positive for CDV by PCR. Comparison of the 149-bp nucleotide sequences from the four samples to sequences in GenBank showed 99% similarity to CDV (GenBank accession no. Seq1: KY056763, Seq2: KY056764, Seq3: KY056765, Seq4: KY056766). Neighbor Joining analysis of the nucleotide sequences clustered with CDV and was clearly distinct from phocine distemper virus (Fig. 5). Sera from 109 apparently healthy juveniles sampled in 2014 from urban and remote rookeries on San Cristobal, Española, Santa Fe, and Floreana Island were all negative for CDV by PCR and virus neutralization (titers $\geq$1:32). All tissue samples collected in 2011–12 were negative by PCR for *Leptospira*, *Brucella*, *Toxoplasma*, San Miguel Sea lion virus, and herpes virus.

DISCUSSION

We observed an increased pup mortality in 2009, 2011, and 2012. Pup mortality in Galapagos sea lions is known to increase during El Niño Southern Oscillations (ENSO) that cause periods of food shortage (Trillmich and Limberger 1985). The 2009 ENSO resulted in 80% pup mortality in California sea lion pups (Melin et al. 2010); however, the 2009 ENSO appeared to have little effect on productivity in the Galapagos Islands (Lee and MacPhaden 2010). The high pup mortality continued in 2011 and 2012 (Fig. 2), which prompted us to investigate infectious agents; additionally, many affected animals showed signs of upper respiratory tract infection. Evidence of CDV and leptospiral infection was obtained by PCR and amplicon sequencing in four animals. The presence of respiratory signs and absence of leptospiral kidney lesions, suggested that that CDV might have been involved in some mortalities; however,

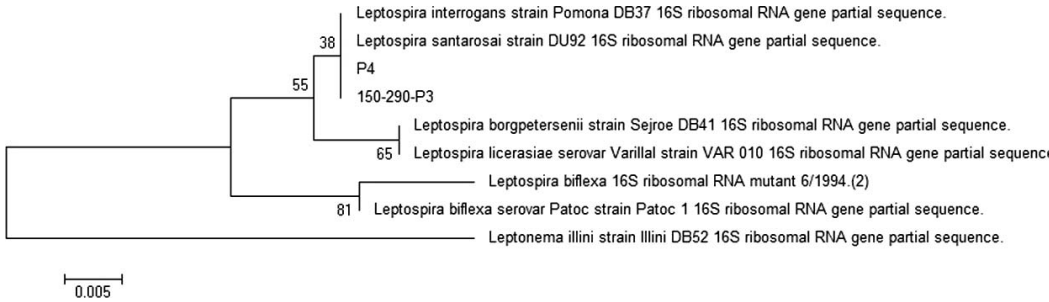

FIGURE 4. Evolutionary relationships of taxa of *Leptospira* found in Galapagos sea lions (*Zalophus wollebaeki*) on San Cristobal Island, Galapagos Islands, in 2010 using the Neighbor Joining method. The optimal tree with the sum of branch length=0.09866573 is shown with the percentage of associated taxa clustered together in the bootstrap test (500 replicates) next to the branches. Branch length reflects evolutionary distances computed with the Kimura 2-parameter method.

serologic testing of 109 juvenile animals in 2014 ruled out the presence of a major CDV outbreak in the Galapagos Archipelago. The presence of leptospiral DNA in some kidneys might suggest these were asymptomatic infections (Prager et al. 2013).

Additional evidence for infectious causes of the mortality was the presence of lung lesions (syncytia, alveolar septal type II hyperplasia, and histocytosis); however, airborne or hematogenous toxins can also cause type II cell hyperplasia (Kuiken et al. 2006). Another potential cause of mortality could be algal blooms (see Sellner et al. 2003); however, this possibility is unlikely because no blooms have been reported in the Galapagos Archipelago.

Domestic rats can be a common source of pathogens, including *Leptospira* (Himsworth et al. 2013). The brown rat (*Rattus norvegicus*) was introduced to the Galapagos Islands in the 1800s and is now widespread (Key and Muñoz Heredia 1994). According to the special legislation for the Galapagos Archipelago, it is prohibited to introduce any species,

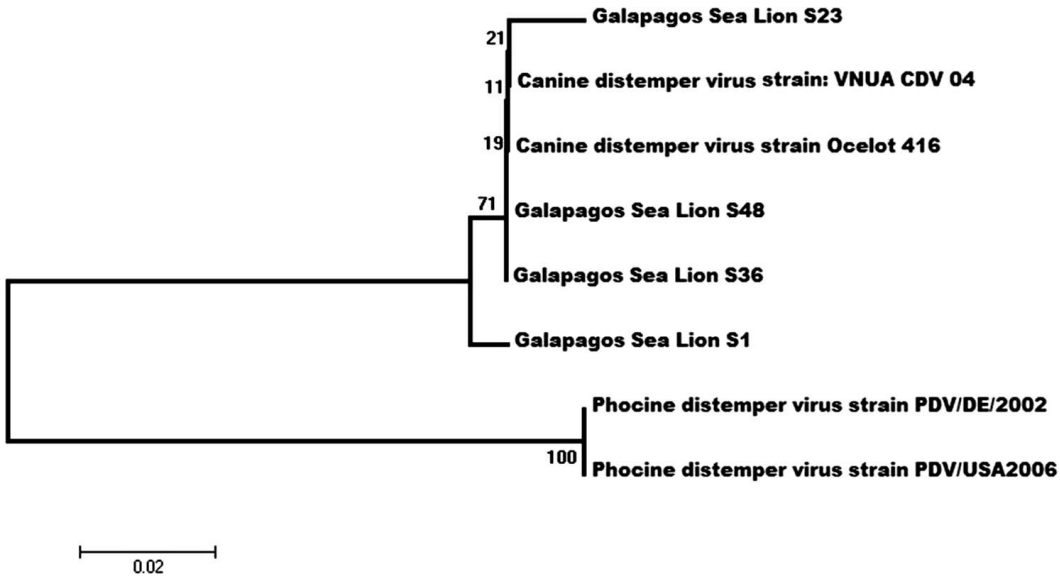

FIGURE 5. Phylogenetic tree (Neighbor Joining) of nucleotide sequences of canine distemper virus phosphoprotein gene, obtained from Galapagos sea lions (*Zalophus wollebaeki*) that died on San Cristobal Island, Galapagos Islands, in 2011–12. Numbers correspond to bootstrap values (500 replicates).

including pets, to the Islands (Ley Organica de Galapagos Registro Oficial No. 278 Marzo 1998. Art. 62); however, dogs are constantly being imported and the contact between dogs and sea lions has increased in the last 10 yr (Denkinger et al. 2015). Antibodies against potential pathogens (parvovirus, parainfluenza virus, and distemper virus) have been found in dogs on Isabela Island (Levy et al. 2008); these antibody titers might have originated from infections (vaccines for domestic animals are banned in the Galapagos Islands).

The Galapagos sea lion population is declining and so far, no obvious causes have been identified. Our results provide some evidence for the transmission of pathogens from domestic animals (or domestic rats) to Galapagos sea lions; however, more research is necessary to confirm or discard this possibility.

ACKNOWLEDGMENTS

This study was possible thanks to national and international collaborators. We want to thank Lenin Vinueza, Diego Quiroga, Carlos Mena, Adrien Zap, Andres Ortega, Merete Blixenkrone, Ursula Siebert, Frances Gulland, Maria Lorena Mejia Castañeda, and Jorge Torres; and all the staff of the Galapagos National Park. The project was financed with a GAIAS grant and USFQ/GAIAS emergency grant 2011 and 2014 and conducted under the permits PC-18-10, PC-27-11, and PC-24-12. Samples were imported into the US under the National Marine Fisheries Service Marine Mammal permit 932-1489-00.

LITERATURE CITED

Appel MJ, Robson DS. 1973. A microneutralization test for canine distemper virus. *Am J Vet Res* 34:1459–1463.

Aurioles D, Sinsel F. 1988. Mortality of California sea lion pups at Los Islotes, Baja California Sur, Mexico. *J Mammal* 69:180–183.

Aurioles D, Trillmich F. 2008. *Zalophus wollebaeki*. In: *IUCN red list of threatened species*, version 2016-3. IUCN SSC Pinniped Specialist Group, Cambridge, UK. www.iucnredlist.org. Accessed January 2016.

Barret T, Wohlsein P, Bidewell CA, Rowell SF. 2004. Canine distemper virus in a Californian sea lion (*Zalophus californianus*). *Vet Rec* 154:334–336.

Brock PM, Hall AJ, Goodman SJ, Cruz M, Acevedo-Whitehouse K. 2013. Immune activity, body condition and human-associated environmental impacts in a wild marine mammal. *PLoS One* 8:e67132.

Burek KA, Gulland FMD, Sheffield G, Beckmen KB, Keyes E, Spraker TR, Smith AW, Skilling DE, Evermann JF, Stott JL, et al. 2005. Infectious disease and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska, USA: Insights from serologic data. *J Wildl Dis* 41:512–524.

Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, Jessup DA, Gulland F, Grigg ME. 2005. Transmission of Toxoplasma: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. *Int J Parasitol* 35:1155–1168.

Denkinger J, Gordillo L, Montero-Serra I, Murillo JC, Guevara N, Hirschfeld M, Fietz K, Rubianes F, Dan M. 2015. Urban life of Galapagos sea lions (*Zalophus wollebaeki*) on San Cristobal Island, Ecuador: Colony trends and threats. *J Sea Res* 105:10–14.

Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, Davis JW, Ewing R, Mense M, Kwok OCH, et al. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. *Vet Parasitol* 116:275–296.

Gulland FM, Koski M, Lowenstine LJ, Colagross A, Morgan L, Spraker T. 1996. Leptospirosis in California sea lions (*Zalophus californianus*) stranded along the central California coast, 1981–1994. *J Wildl Dis* 32:572–590.

Himsworth CG, Parsons KL, Jardine C, Patrick DM. 2013. Rats, cities, people, and pathogens: A systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonosis in urban centers. *Vector Borne Zoonotic Dis* 13:349–359.

Kennedy S, Kuiken T, Jepson PD, Deaville R, Forsyth M, Barrett T, van der Bildt MWG, Osterhaus ADME, Eybativ T, Duck C, et al. 2000. Mass die-off of Caspian seals caused by canine distemper virus. *Emerg Infect Dis* 6:637–639.

Key G, Muñoz Heredia E. 1994. Distribution and current status of rodents in the Galápagos. *Not Galapagos* 53:21–25.

Kuiken T, Kennedy S, Barrett T, van de Bildt MWG, Borgsteede FH, Brew SD, Codd GA, Duck C, Deaville R, Eybatov T, et al. 2006. The 2000 canine distemper epidemic in Caspian seals (*Phoca caspica*): Pathology and analysis of contributory factors. *Vet Pathol* 43:321–338.

Kumar S, Tuteja U, Sarika K, Singh D, Kumar A, Kumar O. 2011. Rapid Multiplex PCR assay for the simultaneous detection of the *Brucella* genus, *B. abortus*, *B. melitensis*, and *B. suis*. *J Microbiol Biotechnol* 21:89–92.

Lee T, McPhaden MJ. 2010. Increasing intensity of El Niño in the central-equatorial Pacific. *Geophys Res Lett* 37:L14603.

Levy JK, Crawford PC, Lappin MR, Dubovi EJ, Levy MG, Alleman R, Tucker SJ, Clifford EL. 2008.

Infectious diseases of dogs and cats on Isabela Island, Galapagos. *J Vet Intern Med* 22:60–65.

Lin MH, Chen TC, Kuo TT, Tseng CC, Tseng CP. 2000. Real-time PCR for quantitative detection of *Toxoplasma gondii*. *J Clin Microbiol* 38:4121–4125.

Melin SR, Orr AJ, Harris JD, Laake JL, Delong RL, Gulland FMD, Stoudt S. 2010. Unprecedented mortality of California sea lion pups associated with anomalous oceanographic conditions along the central California coast in 2009. *Calif Cooperative Oceanic Fish Investig Rep* 51:182–194.

Mérien F, Amouriaux P, Perolat P, Baranton G, Saint Girons I. 1992. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. *J Clin Microbiol* 30:2219–2224.

National Center for Biotechnology Information. 2016. *Basic local alignment search tool (BLAST)*. http://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed June 2014.

Osterhaus ADME, Groen J, Spijkers HEM, Broeders HWJ, UytdeHaag FGCM, de Vries P, Teppema JS, Visser IKG, van de Bildt MWG, Vedder EJ. 1990. Mass mortality in seals caused by a newly discovered virus-like morbillivirus. *Vet Microbiol* 23:343–350.

Páez-Rosas D, Hirschfeld M, Deresienski D, Lewbart GA. 2015. Health status of Galápagos sea lions (*Zalophus wollebaeki*) on San Cristóbal island rookeries determined by hematology, biochemistry, blood gases, and physical examination. *J Wildl Dis* 52:100–105.

Prager KC, Greig DJ, Alt DP, Galloway RL, Hornsby RL, Palmer LJ, Soper J, Wu Q, Zuerner RL, Gulland FMD, et al. 2013. Asymptomatic and chronic carriage of *Leptospira interrogans* serovar Pomona in California sea lions (*Zalophus californianus*). *Vet Microbiol* 164:177–183.

Reid SM, King DP, Shaw AE, Knowles NJ, Hutchings GH, Cooper EJ, Smith AW, Ferris NP. 2007. Development of a real-time reverse transcription polymerase chain reaction assay for detection of marine caliciviruses (genus *Vesivirus*). *J Virol Methods* 140:166–173.

Seguel M, Pavés H, Paredes E, Schlatter R. 2013. Causes of mortality in South American fur seal pups (*Arctophoca australis gracilis*) at Guafo Island, southern Chile (2004–2008). *Mar Mamm Sci* 29:36–47.

Sellner KG, Doucette GJ, Kirkpatrick GJ. 2003. Harmful algal blooms: Causes, impacts and detection. *J Ind Microbiol Biotechnol* 30:383–406.

Sepúlveda MA, Seguel M, Alvarado-Rybak M, Verdugo C, Muñoz-Zanzi C, Tamayo R. 2015. Postmortem findings in four South American sea lions (*Otaria byronia*) from an urban colony in Valdivia, Chile. *J Wildl Dis* 51:279–282.

Simental RT. 2006. Detección de anticuerpos contra *Leptospira* en lobos marinos (*Arctocephalus galapagoensis* y *Zalophuscalifornianus wollebaeki*) de las Islas Galápagos, Ecuador. Zootecnician, National Autonomous University of Mexico, Mexico City, Mexico, 82 pp. [In Spanish.]

Spackman E, Senne DA, Bulaga LL, Myers TJ, Perdue ML, Garber LP, Lohman K, Daum LT, Suarez DL. 2003. Development of real-time RT-PCR for the detection of avian influenza virus. *Avian Dis* 47:1079–1082.

Stanton JB, Poet S, Frasca S Jr, Bienzle D, Brown CC. 2002. Development of a semi-nested reverse transcription polymerase chain reaction assay for the retrospective diagnosis of canine distemper virus infection. *J Vet Diagn Investig* 14:47–52.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739.

Trillmich F, Jeglinski JWE, Meise K, Piedrahita P. 2014. The Galapagos sea lion: Adaptation to spatial and temporal diversity of marine resources within the Archipelago. In: *The Galapagos Marine Reserve, social and ecological interactions in the Galapagos Islands*, Denkinger J, Vinueza L, editors. Springer, New York, New York, pp. 61–70.

Trillmich F, Limberger D. 1985. Drastic effects of El Niño on Galapagos pinnipeds. *Oecologia* 67:19–22.

VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM. 1996. detection and analysis of diverse herpesviral species by consensus primer PCR. *J Clin Microbiol* 34:1666–1671.

Submitted for publication 4 May 2016.
Accepted 20 December 2016.