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Occurrence of Mycoplasmas in Galapagos Sea Lions (*Zalophus wollebaeki*) and their Association with Other Respiratory Pathogens

María S. Sarzosa,[1] Pádraig Duignan,[2] Eugene J. DeRango,[3] Cara Field,[2] Carlos Ríos,[2] Susan Sanchez,[4] Eduardo Espinoza,[5] Andrea Loyola,[5] Danny Rueda,[5] and Diego Páez-Rosas[1,6] [1]Galapagos Science Center, Universidad San Francisco de Quito, Isla San Cristóbal EC200150, Islas Galápagos, Ecuador; [2]The Marine Mammal Center, 2000 Bunker Road, Sausalito, California 94965, USA; [3]Department of Animal Behavior, Bielefeld University, Morgenbreede 45, Bielefeld 33501, Germany; [4]Department of Infectious Diseases, and the Athens Veterinary Diagnostic Laboratory, University of Georgia, Tucker Hall, Athens, Georgia 30602, USA; [5]Dirección de Ecosistemas, Dirección del Parque Nacional Galápagos, Isla Santa Cruz EC200350, Islas Galápagos, Ecuador; [6]Corresponding author (email: dpaez@usfq.edu.ec)

ABSTRACT: During the 2018 breeding season, an outbreak of respiratory disease occurred among Galapagos sea lions (*Zalophus wollebaeki*) that inhabit rookeries near urban areas with introduced fauna such as dogs and cats. Several sea lions had nasal discharge and respiratory distress and were in poor body condition. Eighteen sea lions were captured for a general health assessment including collection of blood for serology and nasal discharge for culture and PCR. Samples were analyzed for 15 respiratory pathogens known to infect cats, dogs, and marine mammals. There was no evidence for interspecies pathogen transmission between Galapagos sea lions and domestic animals. Several bacterial pathogens associated with respiratory tract infection in the California sea lion (*Zalophus californianus*) were isolated. *Mycoplasma* spp. were identified by PCR in nasal discharge samples but were not the species commonly found in cats and dogs.

Key words: Galapagos sea lions, introduced fauna, *Mycoplasma* spp., respiratory pathogens.

The Galapagos sea lion (GSL; *Zalophus wollebaeki*) is an emblematic species in the Galapagos archipelago and is listed as Endangered by the *International Union for Conservation of Nature Red List* (Trillmich 2015). Natural factors that affect the GSL population are interannual warm events such as the El Niño–Southern Oscillation (Trillmich and Dellinger 1991), while anthropogenic threats include fishery interactions and illegal hunting (Denkinger et al. 2017). The role of climate change on the abundance and quality of prey and the role of novel pathogens through contact with introduced fauna (i.e., dogs, cats, and rats) is a major conservation issue for this species (Páez-Rosas and Guevara 2017). As the number of domestic animals and humans increases on inhabited islands, so does the potential for transmission of novel pathogens to endemic species (Kilpatrick et al. 2006). Under this premise, health assessments are crucial for understanding the response mechanisms of endemic Galapagos species to novel pathogens (Páez-Rosas et al. 2016).

Disease surveillance in GSL has been limited when compared to that for the closely related California sea lion, *Zalophus californianus*. However, a survey of pup mortality at GSL rookeries on islands with human inhabitants reported a low level of exposure, without clinical disease, to pathogens such as canine distemper virus (CDV) and *Leptospira* spp. (Denkinger et al. 2017). The source of infection was not determined but was suspected to be introduced terrestrial mammals. Transmission of CDV to pinnipeds leads to high mortality (Duignan et al. 2014), while leptospirosis is a significant cause of seasonal mortality in otariids from Alaska, US to Chile (Sepúlveda et al. 2015). For a highly gregarious pinniped such as the GSL that inhabits areas close to humans and introduced fauna, there is a high potential for interspecies transmission of novel pathogens and the emergence of disease.

Bacteria from the genus *Mycoplasma* are common commensal flora of mucosal surfaces within the respiratory and genital tracts (Razin et al. 1998), but some are recognized as either opportunistic or primary pathogens in a wide range of vertebrate species including pinnipeds (Geraci et al. 1984; Giebel et al. 1991). *Mycoplasma phocae* was first isolated in respiratory tracts of harbor seals (*Phoca*

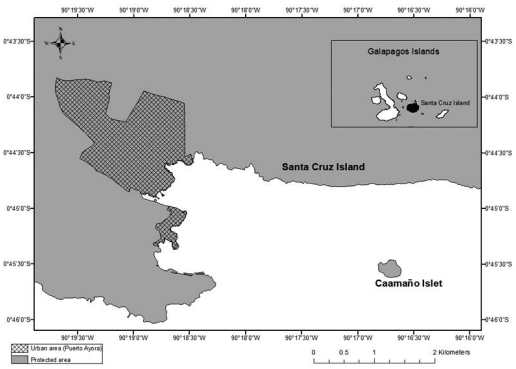

FIGURE 1. Geographic location of the Caamaño Islet and surrounding areas on Santa Cruz Island, Galapagos Islands, Ecuador, where respiratory disease was seen in Galapagos sea lions (*Zalophus wollebaeki*). The sea lions of this rookery constantly move to urban and protected areas ashore on Santa Cruz.

FIGURE 2. Subadult male Galapagos sea lion (*Zalophus wollebaeki*) resting on the tourist docks of Puerto Ayora, Santa Cruz Island, Galapagos Islands, Ecuador. The animal had lethargy, respiratory distress, and nasal discharge.

vitulina) that died during an epidemic of respiratory disease in New England (Geraci et al. 1984). While the primary pathogen was Influenza A virus, it was thought that respiratory pathology was exacerbated by the presence of these bacteria. During the phocine distemper virus epidemics in Europe, *Mycoplasma phocacerebrale* and *Mycoplasma phocirhinis* were isolated from harbor seals that died with severe pneumonia (Giebel et al. 1991). It is reasonable to expect that these bacteria could cause or exacerbate respiratory disease in otariids such as GSL.

During the reproductive season of 2018, adult GSLs from the Caamaño rookery (0°45′S, 90°16′W) near Puerto Ayora, Santa Cruz Island (Fig. 1) began to show respiratory signs including dyspnea, coughing, copious mucopurulent nasal discharge, and lethargy, and they were in poor body condition (Fig. 2). Several adult mortalities from unknown causes were also found within this rookery. Due to these unusual events in December 2018, Galapagos National Park Directorate decided to capture 18 symptomatic animals (14 males and four females) for a general health assessment. All animals were captured with hoop nets and manually restrained in a prone position without chemical immobilization. Blood was collected using standard techniques for otariids (Páez-Rosas et al. 2016), and basic physiologic parameters such as temperature and heart and respiratory rates were recorded. Our ethics and wildlife management protocols were based on the stringent regulations that the Galapagos National Park Directorate and the Ministry of the Environment of Ecuador use to regulate research in the Galapagos Islands.

Caudal gluteal venipuncture was performed within 3 min of capture using a heparinized 20-ga, 4-cm needle to collect up to 10 mL of blood per sea lion. The blood was immediately divided into one ethylenediaminetetraacetic acid tube and one serum tube. From each ethylenediaminetetraacetic acid tube, two blood smears per individual were prepared. The serum tube was centrifuged at 1800 × G for 10 min to separate cells, and serum was frozen (–20 C) for future analyses. Hematology was conducted at the Universidad San Francisco de Quito's veterinary clinic (see Supplementary Material Table S1). Mucopurulent samples were collected directly from the mouth or nose of three symptomatic males using polyester swabs. Serum samples, blood smears, and swabs were shipped to The Marine Mammal Center and Athens Veterinary Diagnostic Laboratory, University of Georgia (Athens, Georgia, USA), for serology and PCR, respectively (Table S1). Serology was conducted to look for antibodies to CDV and phocine distemper virus (Duignan et al. 2014). Swabs were plated onto blood agar,

colistin blood agar, and MacConkey lactose agar and incubated at 37 C for 24 h. The PCR for influenza virus was performed at University of California Davis (Davis, California, USA; Boyce et al. 2013; Table S1). Three mucopurulent samples of three symptomatic individuals were tested by PCR for known respiratory pathogens of dogs and cats (Table S1). Nucleic acids were extracted using the QIAamp Cador Pathogen Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions using a QIAcube automated nucleic acid extraction system (Qiagen). We extracted DNA for Sanger sequencing on the small amplicons obtained from 16S rRNA (Choppa et al. 1998).

We observed a small increase in the respiratory rate of some symptomatic animals (Table S1), while body temperature and heart rate were within normal ranges (Páez-Rosas et al. 2016). Animals with copious nasal discharge presented signs consistent with increased respiratory effort (i.e., increased bronchovesicular sounds). Whole blood and biochemical analyses were within normal limits for the species (Páez-Rosas et al. 2016). The three mucus samples cultured at The Marine Mammal Center had mixed growth of different bacteria species including *Pseudomonas sutzeri*, *Citrobacter freundii*, *Staphylococcus* sp., *Streptococcus* sp., and *Arcanobacterium phocae*. The CDV serology was negative, and PCR was also negative for all canine and feline pathogens except for *Mycoplasma* spp. using the feline panel (Table S1). The DNA extraction from three mucus samples yielded DNA amplicons of enough quantity and quality for analysis. Sequencing of the *Mycoplasma* PCR amplicon showed similarities to *Mycoplasma californicum*, as reported in cattle (*Bos taurus*; Jasper et al. 1981), *Mycoplasma phocidae* in seals (Rhunke and Madoff 1992), *Mycoplasma equigenitalium* in horses (*Equus caballus*; Kirchhoff et al. 1979), *Mycoplasma hominis* in humans (Anderson and Barile 1965), and *Mycoplasma arginini* in goats (*Capra aegagrus hircus*) and cats (*Felis catus*; Leach 1970).

The bacteria we isolated from GSL nasal mucus are frequently cultured from the respiratory tracts of California sea lions (Greig et al. 2005). They may be primary pathogens in some animals but are usually reported as comorbidities with parasitic pneumonia, malnutrition, trauma, or neoplasia (Greig et al. 2005). The *Mycoplasma* spp. have been previously found in California sea lions with one species, *Mycoplasma zalophi*, associated with pleuritis and necrotizing pneumonia (Haulena et al. 2006). Furthermore, in otariids, *Mycoplasma* spp. have also been observed in South American fur seals (*Arctocephalus australis*) and Australian fur seals (*Arctocephalus pusillus*) with respiratory diseases and abortions (Lynch et al. 2011; Jankowski et al. 2015). Our results showed similarities to several mycoplasmas of terrestrial animals and humans. However, the sequencing was of limited quality and only a 130–106-base pair amplicon was useful for a Blast Search. This short region of the 16S rRNA of mycoplasma was very conserved and did not allow for separation of the isolates.

Several *Mycoplasma* species are part of the normal flora of the pharynx, larynx, oral, and nasal cavities of domestic cats and dogs (Chandler and Lappin 2002). However, *Mycoplasma* species associated with dogs were not identified in the sequencing results of nasal mucus of GSL, but an unidentified species was detected in the feline PCR panel. Because mycoplasmas are common in several pinniped species worldwide where studies have been conducted, it is most likely that the *Mycoplasma* DNA extracted from the nasal mucus belongs to a species endemic to the GSL that remains to be isolated and formally identified. All GSL showing clinical signs and symptoms were found resting on docks within centers of commerce, which are easily accessible to dogs, cats, and rodents not endemic to the islands.

Alternatively, it is possible that *Mycoplasma* spp. are sylvatically maintained within the population, and outbreaks could manifest during periods of stress and increased immunostimulatory pressures from the natural environment. The strong El Niño events negatively affect the GSL population by changing the distribution of foraging areas

and creating sustained nutritional stress (Páez-Rosas et al. 2020). These environmental stressors can influence infectious disease dynamics within the marine environment and can concurrently compromise sea lion immune responses (DeRango et al. 2019). While our limited study did not determine a definitive etiology for the outbreak of respiratory disease, it did provide a baseline for pathogen exposure at the Caamaño Rookery. Ongoing surveillance will be required to fully understand the role of endemic infectious diseases in this GSL population and to facilitate the early detection of novel or emerging diseases that could further threaten this endangered pinniped.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-20-00081.

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