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Isolation of *Leptospira interrogans* from a Bottlenose Dolphin (*Tursiops truncatus*) in the Mediterranean Sea

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ABSTRACT: The pathogenic *Leptospira* species are very widespread in nature, persisting in the renal tubules of many domestic and wild animal reservoirs. We report the isolation of *Leptospira interrogans* serovar Pomona in a bottlenose dolphin (*Tursiops truncatus*) stranded along the coast of Sardinia, Italy, in 2016.

Leptospirosis is a zoonosis with ubiquitous distribution caused by spirochetes belonging to the genus *Leptospira*. The prevalence of the disease is significantly higher in tropical and temperate regions (Everard and Everard 1993); warm-humid conditions facilitate the survival of these bacteria in the ecosystem. Heavy rains and floods increase the risk of dissemination of *Leptospira* into the environment (Kupek et al. 2000). Infection usually occurs when animals and humans come in contact with contaminated fresh water (Ko et al. 1999). *Leptospira* can survive several months in moist soil or water, unless the latter is acidic or saline (Trueba et al. 2004). However, Saito et al. (2014) demonstrated the survival of pathogenic strains of up to 4 d after immersion in seawater. To date, very little information is available in the literature on the spread of leptospirosis in marine mammals. Serologic investigations of various species of pinnipeds were carried out on the western coast of the US and showed positive results for pathogenic serogroups (Greig et al. 2014; Delgado et al. 2015; Jankowski et al. 2015; Avalos-Téllez et al. 2016). Only one isolation of a pathogenic *Leptospira* strain has been reported in a whale in Patagonia, Argentina (Loffler et al. 2015). Many aspects of leptospirosis and the role of environmental factors that increase the persistence of these bacteria in the environment are still unknown. Our studies over the last 30 yr show that Sardinia is an endemic island for leptospirosis; bovine and swine are the most affected species and the serovars Bratislava, Tarassovi, and Pomona are the most prevalent.

In accordance with the Italian Diagnostic Network on Cetaceans Strandings, a total of 16 kidneys of cetaceans—seven striped dolphins (*Stenella coeruleoalba*), four bottlenose dolphins (*Tursiops truncatus*), two Risso's dolphins (*Grampus griseus*), two sperm whales (*Physeter macrocephalus*), and one common dolphin (*Delphinus delphis*)—stranded along the coast of Sardinia in 2016 were analyzed. Selected tissues (brain, lung, heart, liver, spleen, and kidney) were stained with H&E for histopathology. The same samples were also tested for *Morbillivirus* using reverse transcriptase-PCR techniques, and for the main cetacean bacteria (*Brucella* spp., *Listeria* spp., *Salmonella* spp., *Staphylococcus* spp., and *Proteus* spp.) using media for bacterial isolation that included 5% sheep blood agar and serum dextrose agar. Immunohistochemical exams were performed on the kidney, but, because of the poor condition of the carcass, no useful responses were obtained for all samples because of advanced autolysis.

For *Leptospira* research, 25 mg of homogenated renal tissue was inoculated into three tubes containing 3 mL of semisolid Ellinghausen-McCullough-Johnson-Harris (Difco™, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) medium with 200 µg/mL 5-fluorouracil, incubated at 28–30 C for 60 d, and observed weekly by dark field

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FIGURE 1. Step of the necropsy carried out on a bottlenose dolphin (*Tursiops truncatus*) that stranded in Sardinia, Italy, in 2016. (A) The subject was in a good state of nutrition, and carcass was in an advanced decomposition stage. Measurements taken during the necropsy gave a length of 2.0 m, and an estimated weight of 150 kg. (B) Both lobes of the caudal fin had been amputated with a sharp cutting instrument. (C) Signs of scavenging showed on the right mandible.

microscopy. Out of 16 kidney samples investigated, only one positive culture was obtained from a *T. truncatus* stranded along the east coast of the island (40°22′30″N, 9°43′43″E). *Proteus* spp., characterized by API 20E (bioMerieux, Marcy l'Etoile, France), was the only non-*Leptospira* microorganism successfully isolated in the same cetacean. Histopathology failed to reveal any significant lesions. The animal showed an amputation of both lobes of the caudal fin with sharp cutting edges in which signs of bleeding and extravasation were evident, indicating that the heart had been beating when the injury occurred, which indicated an antemortem injury (Fig. 1). In several Mediterranean areas, as in many areas around the world, bottlenose dolphins have learned to follow bottom trawlers to take advantage of fish caught and often are victims of bycatch. The dolphin was in a good state of nutrition and the carcass presented an advanced stage of decomposition; nothing was known about clinical signs or other anomalies of life behavior of the animal. The carcass showed a number of skin excoriations and signs of scavenging of the right mandible.

The *Leptospira* strain (53187) that we isolated from the dolphin was identified using different molecular methodologies. First, multilocus sequence typing (MLST), including the analysis of seven housekeeping genes, was performed following the protocol described by Boonsilp et al. (2006). Sequences were aligned with the module SeqMan of the software DNASTAR (Lasergene, Madison, Wisconsin, USA) and examined on the MLST database (PubMLST 2016). Raw reads of sequenced *Leptospira* 53187 strain were submitted to the European Bioinformatics Institute (accession no. PRJEB316256). Strain 53187 corresponded to sequence type 140, which is assigned not only to *Leptospira interrogans* serogroup Pomona, but also to *L. interrogans* serogroups Grippotyphosa and Pyrogenes. Multiple-locus variable-number tandem-repeat analysis was performed using the protocol of Salaün et al. (2006) on the loci VNTR4, 7, 10, Lb4, and Lb5. The multiple-locus variable-number tandem-repeat analysis pattern for the five loci (2, 2, 10, -, and 5, respectively) was the same as *Leptospira* strains isolated in Sardinian swine (*Sus scrofa*), bovine (*Bos taurus*), and wild boar (*S. scrofa*), previously serologically identified as serovar Pomona (Tagliabue et al. 2016), but different from the strains isolated from the rest of the Italian territory. Whole-genome sequencing was performed on an Illumina (San Diego, California, USA) MiSeq platform with a 250×2–base-pair run starting from a genomic library prepared using a Nextera DNA Flex Kit (Illumina). Phylogenetic analysis was performed using a new core genome MLST scheme (Guglielmini et al. 2019). The dolphin strain shares the same core genome sequence type with other *L. interrogans* serovar Pomona strains, with sequence similarities within the range 97–100%. Our results showed that this strain belonged to *L. interrogans* serogroup Pomona, serovar Pomona.

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The transmission pathways through which a dolphin acquires *Leptospira* infection are currently unknown.

Surveys conducted on marine mammals have shown that these animals can harbor a wide variety of microorganisms, including zoonotic agents (Waltzek et al. 2012). To date, the only isolation from cetaceans was reported in a study conducted in Argentina (Loffler et al. 2015). In the case of pinnipeds, one possible source of the spread of the pathogen into the sea lion population is contact with fresh water contaminated by the urine of domestic animals and rodents. Our study highlights the presence of a pathogenic *Leptospira* strain in a dolphin stranded from the Mediterranean Sea. The isolation of a potentially zoonotic serovar from a dolphin could represent the risk of human disease acquisition during recreational and occupational activities. These findings highlight the need to conduct deeper longitudinal investigations, including other animal species that live in this ecosystem.

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