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Source: Journal of Wildlife Diseases, 35(1) : 108-111

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-35.1.108

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Journal of Wildlife Diseases, 35(1), 1999, pp. 108–111
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Antibodies against *Leptospira interrogans* in California Sea Lion Pups from Gulf of California

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ABSTRACT: One hundred and twenty-five serum samples from California sea lion (*Zalophus californianus californianus*) pups, and one from an adult female from eight reproductive rookeries located in seven islands in the Gulf of California (Mexico), were collected during the 1994–96 reproductive seasons. These were tested for antibodies to 19 serovars of *Leptospira interrogans* using a Microscopic Agglutination Test (MAT). Forty-one samples (32%) had antibody levels from 1:20 to 1:320 to one or more serovars. The most frequently detected serotypes were *Leptospira interrogans hardjo* (n = 13), *cynopteri* (8), *ballum* (6), and *szwajizak* (5). Serovars with the highest prevalence were *Leptospira interrogans hardjo* and *serjoe* (1:320), *ballum* (1:160), and *cynopteri*, *girppotyphosa*, and *tarassovi* (1:80). Based on these results, exposure of sea lions to *L. interrogans* serovar *hardjo* seems to be relatively common among colonies located in the islands of the Gulf of California in contrast with those located on the Pacific coast, where the most frequently detected serovar is *L. interrogans* serovar *pomona*.

Key words: *Leptospira interrogans* serovar *hardjo*, pinnipeds, sea lion, serology, survey, *Zalophus californianus*.

Leptospirosis is a worldwide disease that affects domestic and wild animals as well as humans. The disease is characterized by fever, petechial hemorrhages, hepatic and renal failure, and abortion (Desmarchelier, 1987). *Leptospira interrogans* serovar *pomona* has been detected as a cause of abortions and death among California sea lions (*Zalophus californianus californianus*) along the California and Oregon coasts (USA) since 1970 (Vedros et al., 1971). Antibody levels against *L. interrogans* serovar *pomona* had been detected from 1:100 to 1:6,400 by a Microscopic Agglutination Test (MAT) (Smith et al., 1977). The potential of sea lion to human transmission has not been explored, nevertheless leptospirosis in humans has been correlated to contact with animals, mainly cattle, and *Leptospira interrogans* serovar *hardjo* is one of the causal agents of disease and abortion in humans (Desmarchelier, 1987; Prescott and Zuerner, 1993). Our objective was to determine the serologic prevalence of different leptospira serological profiles among sea lion inhabitants in colonies of the Gulf of California.

One hundred and twenty-five blood samples from manually-restrained California sea lion pups were taken from the caudal gluteal vein using a 18 gauge × 38 mm needle, and placed into sterile vacutainers (Vacutainer, Becton Dickinson, Rutherford, New Jersey, USA) for serum separation. All pups sampled were neonates (0- to 160-days-old) and sampling was done during the breeding seasons (June and July) 1994–96 from seven different rookeries of six islands in the north-central part of the Gulf of California (Mexico) (Fig. 1). The adult female sampled was seen days after she gave birth to a stillborn pup and was chemically restrained with Telazol® (A. H. Robins Co., Richmond, Virginia, USA) and sampled at Los Islotes rookery in the southern region of the Gulf of California on February 1995 (Table 1). In all instances, sera were separated by centrifugation and stored at −4 C until the microagglutination test (MAT) was performed, as previously described by Myers (1985). Nineteen serovars of *L. interro-*

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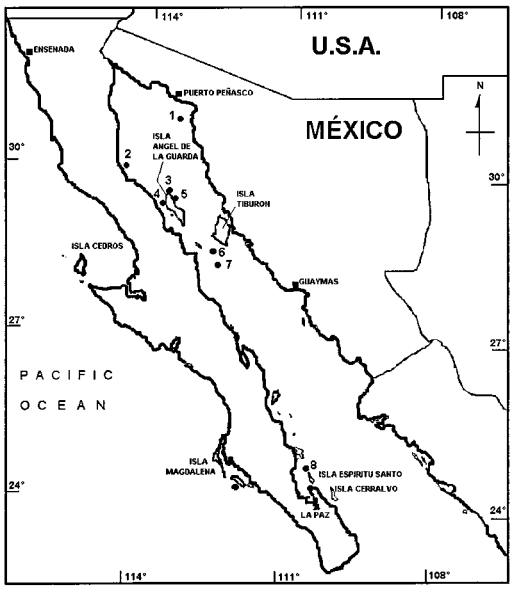


FIGURE 1. The Islands with California sea lion rookeries included in this study in the Gulf of California are (1) San Jorge, (2) El Coloradito, (3) Granito, (4) Angel de la Guarda (Los Machos), (5) Angel de la Guarda (Los Cantiles), (6) San Esteban, (7) San Pedro Mártir, and (8) Los Islotes.

gans were used as antigens. Those serovars are part of the collection of the Departamento de Microbiología e Inmunología (Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Distrito Federal, Mexico). The collection was previously obtained from the former Zoonoses Pan-American Center (OPS/OMS; Buenos Aires, Argentina). For the MAT, 4 to 7 day Stuart media live cultures of *L. interrogans* serovars and two control sera (positive and negative) were inactivated at 56 C for 30 min. A 1:25 sera dilution in 96-well microtiter plates (Nunc, Gaitheresburg, Maryland USA) was used as initial dilution and observed under dark field microscopy (0.7/0.85, Carl Zeiss, West Germany). Levels as low as 1:20 were considered positive and dilutions up to 1:640 were done with the positive reactors.

Forty-one of 126 sera (32%) were positive for *L. interrogans* serovars with levels ≥ 1:20 in the MAT. From those, 11 sera were positive to more than one serovar.

TABLE 1. Number of blood samples from California sea lions/island (rookery)/year in the Gulf of California (Mexico)

Island (rookery)	1994	1995	1996
San Jorge	0	10	1
El Coloradito	0	5	1
Granito	0	0	2
Angel De La Guarda (Los Machos)	0	0	3
Angel De La Guarda (Los Cantiles)	77	5	3
San Esteban	0	9	5
San Pedro Martir	0	0	4
Los Islotes	0	1[a]	0
Total	77	30	19

[a] Adult female.

Thirty, seven, two and two sera reacted against one, two, three or more than three serovars, respectively. Antibody seroprevalence to different serovars were: *L. interrogans* serovar *hardjo* 13 (22%); *cynopteri* 8 (14%); *ballum* 6 (10%); *szwajizak* 5 (9%); *grippotyphosa* and *serjoe* 4 (7%); *autumnalis*, *canicola* and *pyrogenes* 2 (5%); *bataviae*, *pomona* and *tarassovi* 2 (3%); and *australis*, *icterohaemorrhagiae*, and *wolffi* 1 (2%) (Table 2). There was no reaction against *L. interrogans* serovars *bratislava*, *celledoni*, *hebdomadis* nor *paidjan*. The highest levels were found against *L. interrogans* serovars *hardjo* and *serjoe* (1:320); *ballum* (1:160) and *cynopteri*, *girppotyphosa*, and *tarassovi* (1:80). Antibody levels against *L. interrogans* serovar *autumnalis*, *bataviae*, *canicola*, and *szwajizak* were 1:40; and against *L. interrogans* serovar *australis*, *icterohaemorrhagiae*, *pomona*, and *pyrogenes* were as low as 1:20. The adult female sera showed titers of 1:40 against *L. interrogans* serovar *hardjo*.

Several authors have found *L. interrogans* serovar *pomona* to be the main serovar associated with leptospirosis in California sea lions (Dierauf et al., 1985; Hodder et al., 1992; McIlatan et al., 1971; Smith et al., 1974). Such studies were carried out at rookeries on the Pacific Coast of California. In contrast, the present study revealed *L. interrogans* serovar *po-*

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TABLE 2. Leptospiral serologic results in 126 blood samples of California sea lion pups (and one adult female) from islands of the Gulf of California.

Leptospira interrogens serovar	1:20	1:40	1:80	1:160	1:320	Total (%)
australis	1	—[a]	—	—	—	1 (2)
autumnalis	2	1	—	—	—	3 (5)
ballum	2	2	1	1	—	6 (10)
bataviae	1	1	—	—	—	2 (3)
bratislava	—	—	—	—	—	0
canicola	2	1	—	—	—	3 (5)
calledoni	—	—	—	—	—	0
cynopteri	6	1	1	—	—	8 (14)
grippotyphosa	3	—	1	—	—	4 (7)
hardjo	1	4[b]	3	4	1	13 (22)
hebdomadis	—	—	—	—	—	0
icterohaemorrhagiae	1	—	—	—	—	1 (2)
paidjan	—	—	—	—	—	0
pomona	2	—	—	—	—	2 (3)
pyrogenes	3	—	—	—	—	3 (5)
serjoe	3	—	—	—	1	4 (7)
szwajizak	1	4	—	—	—	5 (9)
wolffi	1	—	—	—	—	1 (2)
tarassovi	1	—	1	—	—	2 (3)
Total	30	14	7	5	2	58

[a] —, None found.
[b] Including adult female.

mona in only two serum samples at titer of 1:20. *L. interrogans* serovar *hardjo* has been recognized as one of the most widely distributed pathogens among cattle worldwide, including Mexico (Ellis et al., 1986; Caballero et al., 1989). In humans, serodiagnosis has detected *L. interrogans* serovar *australis*, *canicola*, *hardjo*, *hebdomadis*, *icterohaemorrhagiae*, *pomona*, and *pyrogenes* in farmers, abattoir workers, and rural inhabitants with a previous history of animal contact (Desmarchelier, 1987).

There were no clinical signs of leptospirosis among the sea lion pups sampled, even those with titers of 1:320. The estimated average age was 1-mo-old, ranging from pups of approximately 8 days to 2-mo-old. There are no previous studies of pathogenicity of *L. interrogans* serovar *hardjo* for pinnipeds and detection of titers in population of sea lions opens the question about the possible source of infection with this serovar. Other potential mammalian reservoirs which inhabit some of the islands like San Esteban, Angel de la Guarda, and Granito are rodents (*Peromyscus* spp. and introduced *Rattus rattus*, *Rattus norvegicus*, and *Mus musculus*) and introduced feral house cats (Bourillón et al., 1988). Infection from contaminated water sources should also be considered. Those islands also are temporarily occupied by fisherman. Contact of humans, especially fishermen, with the same infection source is not uncommon and may result in indirect transmission.

Since all sampled pups were newborn, we propose that large numbers of pups were infected during the first few days of the nursing period, or acquired passive or maternal antibodies.

This work was done with the support of the Universidad Nacional Autonoma de México (UNAM), grant: IN503995; the CONACyT grants: 1122PB and to project number 400302-5-3371-N, and one donation from Africam Safari Zoo. We thank the Facultad de Ciencias (UNAM), Centro de Investigación Científica y de Educación

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Superior de Ensenada (CICESE), Centro Interdisciplinario de Ciencias Marinas (CICIMAR) of the Instituto Politécnico Nacional, and Fullerton College staff and students colaboration, whom helped with the field work. We especially thank A. Zavala, A. Lazo de la Vega, MC. García Rivas, D. Brousset, C. Heath, M. Forzán, I. Ramírez and A. Camacho. We also thank the Secretaría de Marina and the Instituto Nacional de Ecología, Dirección General de Vida Silvestre, for the research permits DOO-700- (2)01104 and DOO-700 (2)-1917.

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Received for publication 4 December 1997.

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