

# RISK FACTORS FOR AN OUTBREAK OF LEPTOSPIROSIS IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*) IN CALIFORNIA, 2004

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**ABSTRACT:** Leptospirosis has been reported in California sea lions (*Zalophus californianus*) since 1970; however, the source of infection and mode of transmission remain unknown. To elucidate these features, demographic and environmental risk factors for leptospirosis were evaluated. California sea lion stranding records from northern California for 2004 were used to identify cases of leptospirosis ( $n=316$ ) and controls ( $n=143$ ). Demographic characteristics (age class, sex) and environmental factors, representing surrogates for exposure to dogs, cattle, rainfall, and freshwater sources, were compared between cases and controls with the use of a geographic information system (GIS) and logistic regression. Multivariate analyses revealed that summer and autumn seasons, juvenile age class, male sex, high dog-park density, and close proximity to dog parks were significantly associated with leptospirosis in sea lions, whereas county farmland cattle density, rainfall levels 30 days prior to stranding, human density, and proximity to freshwater sources were not associated. Thus, dogs and dog parks, or factors associated with them, might be further investigated to assess their relationship to leptospirosis in sea lions.

**Key words:** California sea lion, case-control study, epidemiology, geographic information system, leptospirosis, outbreak, *Zalophus californianus*.

## INTRODUCTION

Leptospirosis, caused by the spirochetal bacterium *Leptospira*, is an infectious disease that affects all mammals, including humans, and is currently considered re-emerging in dogs in the United States (Bolin, 1996; Levett, 1999; Prescott et al., 2002; Meites et al., 2004).

Leptospirosis has been documented in several species of marine mammals (Smith et al., 1977; Colegrove et al., 2005; Kik et al., 2006). Outbreaks of leptospirosis have been reported in California sea lions (*Zalophus californianus*) since 1970, *L. interrogans* var *pomona* being the most consistently recovered isolate, with cases occurring commonly in juvenile males in the autumn months (Vedros et al., 1971; Medway, 1980; Dierauf et al., 1985; Gulland et al., 1996).

The mode of transmission in California sea lions is unknown, but may involve

direct spread of urine among sea lions, or contact with stagnant sources of fresh water or river/stream outflow sites contaminated by domestic animals or wildlife species such as cattle, raccoons or rodents. In California, outbreaks in sea lions have occurred concurrently with increased incidence of leptospirosis in dogs statewide (Adin and Cowgill, 2000); however, whether or not there is transmission between terrestrial mammals and sea lions is unknown. The purpose of this study was to determine risk factors for leptospirosis in California sea lions in order to identify likely sources of infection.

## MATERIALS AND METHODS

### Study population

Study subjects were identified from the stranding records of The Marine Mammal Center (TMMC), a marine mammal rehabil-

itation center in Sausalito, California. Stranded sea lions were collected along the northern and central California coast in 2004, transported to TMMC and treated or euthanized based on prognosis.

A confirmed case was defined as a sea lion that stranded in 2004 in California between Mendocino and San Luis Obispo counties and displayed clinical signs compatible with leptospirosis, such as renal failure and abdominal pain (Gulland et al., 1996), abnormal serum chemistry (blood urea nitrogen >100 mg/dl, phosphate levels greater than calcium, and creatinine >1.0 mg/dl), and necropsy findings (e.g., marked swelling of kidneys, loss of differentiation between the renule medullae and cortices, pale-tan cortices and/or swollen, friable livers and severe gastric ulceration), and had a positive confirmatory laboratory test for leptospirosis such as: 1) a microscopic agglutination test (MAT) titer of  $\geq 800$  to serovars *L. pomona*, *bratislava*, *canicola*, *grippotyphosa*, *hardjo* or *icterohaemorrhagiae*; 2) positive polymerase chain reaction (PCR) (Cameron et al., in press); 3) isolation of the agent in culture from the kidney or urine (Zuermer et al., in press); or 4) histopathologic findings consistent with leptospirosis (Gulland et al., 1996; Colagross-Schouten et al., 2002).

A MAT titer cutoff of  $\geq 800$  was chosen because a single titer of this magnitude in symptomatic humans is generally indicative of clinical leptospirosis (Levett, 2001). Primers for *L. interrogans* serovars *bratislava*, *canicola*, *hardjo*, *icterohaemorrhagiae*, and *pomona*, and *L. kirschneri* serovar *grippotyphosa* were used for PCR testing (Cameron et al., in press).

A probable case was defined as clinical illness with one or more of the following findings: abnormal serum chemistry or gross necropsy findings as described above for confirmed cases. Controls were defined as a sea lion stranded in 2004 with a MAT titer of <800 for any *Leptospira* serovar and negative for any other diagnostic or clinical findings as described for cases. Among controls, 33% (47/143) were tested for leptospirosis with the MAT; however, further diagnostics were not performed.

#### Definition of risk factors

**Demographic:** Sea lions were grouped into age categories (Greig et al., 2005), and included adults (>5 yr), subadults (4–5 yr for males and 2–5 yr for females), juveniles (2–3 yr for males and 2–5 yr for females), yearlings (1–2 yr) and pups (<1 yr). Juveniles and subadult females were combined into the same category due to the difficulty of distinguishing animals in these

two age classes. Pups ( $n=4$ ) were collapsed into the yearling group because of small numbers. Stranded sea lions for which sex was undetermined or unknown were excluded from the analysis.

**Environmental:** Environmental risk factors were assessed with the use of a geographic information system (GIS) (ArcGIS ESRI, Redlands, California, USA). Environmental data were overlaid onto the stranding-location data to determine exposure to these factors (Nicholson and Mather, 1996). For stranding location, the latitude and longitude of every sea lion stranding was obtained with the use of commercially available software programs (Topozone, Maps a la carte, Inc., North Chelmsford, Massachusetts, USA; www.topozone.com; Google Earth, Google, Inc., California, USA; www.earth.google.com) and loaded into the GIS.

Strandings were grouped into the following seasons based on the stranding month: winter (January–March), spring (April–May), summer (June–August), or autumn (September–December). As cattle may be a reservoir of *L. interrogans* var *pomona* (Miller et al., 1991), county cattle density was calculated from the total county cattle population divided by county farmland area, as primary data on cattle densities were unavailable. Cattle counts were obtained from the National Agricultural Statistics Service (United States Department of Agriculture, 2006). County human population density (surrogate for exposure to domestic dogs and urban rats) was evaluated as a risk factor by compiling data for human population density from the 2000 United States census (United States Census Bureau, 2006). The measure of population density was refined by calculating the density per square kilometer of county subdivision rather than at the county level. Dog-park locations, a surrogate for exposure to leptospires shed by dogs, were identified by using dog-park directories (Eco-Choices, Inc., 2006) and deriving the geographic coordinates for each of 171 parks. Additionally, dog-park density per hydrologic unit (watershed) was also included as a risk factor.

Watershed data from the National Hydrography Dataset (United States Geological Survey, 2006) was used to determine locations of freshwater bodies, from which distance to a stranding was derived, to use as a surrogate for exposure to leptospires shed by wild and domestic animals. Information on mean precipitation 1 mo prior to the stranding in the hydrologic unit (watershed) of the stranding was obtained with the use of California

watershed digital data files from the California Watershed Portal (California State Water Resources Control Board, 2007). Precipitation measurements from the California Data Exchange Center (California Department of Water Resources, 2007), and from an annual summary of climatologic data for 2004 available from the National Climatic Data Center (National Oceanic and Atmospheric Administration, 2004) were also used. A lag period of 1 mo was selected to account for survival of leptospire for 2–3 wk in water-soaked soil (Smith and Self, 1955) and an average incubation period for leptospirosis of 10–14 days in human outbreaks (Heymann, 2004). A separate GIS extension was used to perform distance analyses from each stranding (ArcGIS, SpatialEcology.com). County sea lion census data for 2004 was obtained from aerial surveys conducted in July by the National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, California (NMFS, unpubl. data) and totaled by county. Environmental factors for the strandings were identified by overlaying the locations onto the digital environmental data.

#### Data analysis

Differences in covariate distribution among cases and controls were determined by chi-square test for categorical variables and *t*-test for continuous variables. Tests were considered statistically significant at a two-sided *P*-value < 0.05. Spearman rank correlation was used to detect any collinearity between predictors. Logistic regression was used to display univariate analyses (i.e., crude odds ratios [OR]) and 95% confidence intervals (CI) for each predictor and the Wilcoxon rank-sum test for trend was used for ordinal categorical predictors. Model building was performed by considering variables that were thought to be important a priori, or that were statistically significant (*P* < 0.20) in the univariate analyses, and including them in the preliminary multivariate logistic regression model (Hosmer and Lemeshow, 2000). Their contribution to the model was assessed by partial *F* tests (Hosmer and Lemeshow, 2000). Once a preliminary main effects model was obtained, variables were retained in the model if they were significant (*P*-value < 0.05). Adjusted odds ratios and 95% confidence intervals were calculated with the use of the final logistic regression model and included age and sex as a priori confounders. Overall fit of the final logistic model was assessed with the use of Hosmer–Lemeshow goodness-of-fit statistics (Hosmer and Lemeshow, 2000). Continuous

variables that were not normally distributed were log-transformed. Analyses were performed with Stata 9.0 for Windows (STATA Corp., College Station, Texas, USA).

## RESULTS

A total of 485 marine mammals stranded in northern and central California in 2004, and were admitted to The Marine Mammal Center. After removing duplicates (*n* = 10), fetuses (*n* = 6), and strandings outside the study area (*n* = 4) and study period (*n* = 6), 459 animals remained for analysis. Fetuses (*n* = 2), animals from outside the study area (*n* = 2), and multiple stranding reports for a single animal (*n* = 6) were excluded from the study. A total of 316 animals that stranded with a diagnosis of leptospirosis were identified, of which 194 died during treatment at the rehabilitation center, 36 were euthanized, and 86 were released. Confirmed cases (*n* = 143) were identified either by positive MAT titers alone (*n* = 64); histopathology alone (*n* = 51); or a combination of positive histopathology and MAT titers (*n* = 23), positive histopathology, MAT and PCR (*n* = 1), or positive MAT and PCR (*n* = 4). Of the 96 “confirmed” cases that were not MAT tested, one was positive for leptospirosis by PCR, and 95 were examined histologically and had lesions consistent with leptospirosis. The highest serologic response was to *L. interrogans* var *pomona*, although lower titers to *L. interrogans* var *bratislava* and *icterohaemorrhagiae* were also detected. Of the 173 probable cases of leptospirosis, 119 were diagnosed by clinical signs and gross necropsy findings, and 54 by clinical signs and serum biochemistry. The remaining 143 sea lions served as controls. Conditions diagnosed in the controls included domoic acid toxicity (*n* = 47), trauma (*n* = 23), malnutrition/emaciation (*n* = 17), unknown causes (*n* = 11), entanglement in fishing gear (*n* = 9), abscess (*n* = 7), and other miscellaneous conditions or combinations of diseases (*n* = 29). Forty-seven (33%) controls were tested for leptospiro-

TABLE 1. Prevalence of leptospirosis in stranded California sea lions by county, California, 2004.

County	No. of cases	County sea lion population	Prevalence (%)
Mendocino	14	0	—
Sonoma	23	5	—
Marin	41	0	—
Contra Costa	4	0	—
San Francisco	30	3,260	0.9
Alameda	6	0	—
San Mateo	29	2,301	1.3
Santa Cruz	58	3	—
Monterey	93	452	20.6
San Luis Obispo	18	6,376	0.3

sis by MAT and were negative, and 96 controls were not tested.

The apparent focus of the outbreak appeared to be Monterey County with a prevalence of 21% (Table 1). Of the 316 cases, 93 (29%) stranded in Monterey County, and 151 (48%) stranded in Monterey and Santa Cruz counties. The outbreak began in mid-July, peaked during the first weeks of September and ended in November (Fig. 1). The overall prevalence of leptospirosis was 69% (316/459). The highest prevalence was 87% (219/251) in juvenile males, followed by 68% (21/31) in subadult males. The lowest

prevalence was in juvenile females (0%; no cases reported). Univariate analyses revealed that sea lions with leptospirosis were significantly younger than controls ( $P < 0.0001$ ), with yearlings (16%) and juveniles (69%) more commonly represented. Cases also had a higher proportion of males ( $P < 0.0001$ ); summer and autumn strandings ( $P < 0.0001$ ; Fisher's exact test); and strandings in Monterey, Santa Cruz, and Marin counties ( $P < 0.0001$ ; Fisher's exact test). Cases were located closer to dog parks and freshwater sources ( $t = 3.95$ ,  $P = 0.0001$  and  $t = 2.95$ ,  $P = 0.003$ , respectively) than controls, and stranded in counties with greater human population density ( $t = -2.69$ ,  $P = 0.0075$ ) and in watersheds with higher precipitation ( $t = 2.13$ ,  $P = 0.03$ ). Collinearity was found between the covariates human density and dog-park density per hydrologic unit and between number of cattle per county farmland and number per hydrologic unit.

After adjusting for age class and sex in the final logistic regression model, summer and autumn season remained significantly associated with leptospirosis, as did the density of dog parks per hydrologic unit of the stranding location (Table 2). Increasing distance to dog parks was associated with a significantly decreased risk of leptospirosis. Human and county

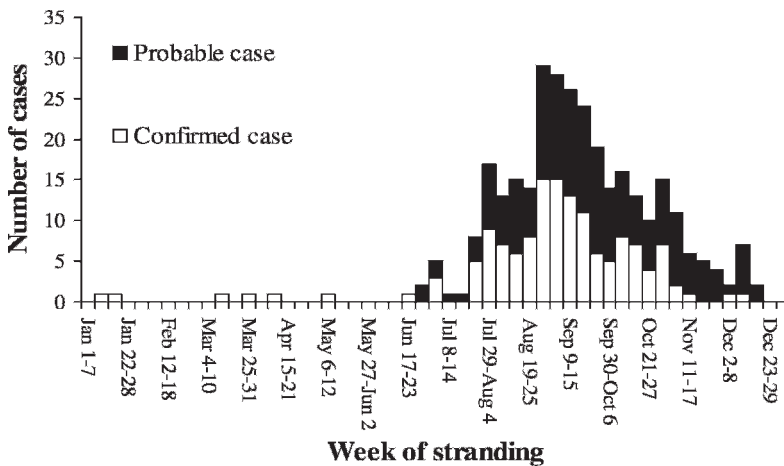


FIGURE 1. Epidemic curve of an outbreak of leptospirosis in California sea lions, California, 2004.

TABLE 2. Logistic regression analysis of risk factors for leptospirosis in stranded California sea lions, California, 2004.

Risk factor <sup>a</sup>	Odds ratio	95% confidence interval	<i>p</i>
Season			
Winter	1.00	0.00	—
Spring	0.43	0.08–2.31	0.329
Summer	16.74	4.32–64.90	0.000
Autumn	11.61	2.95–45.71	0.000
Dog-park density per stranding site hydrologic unit	1.95	1.18–3.22	0.009
Distance to dog park (km)	0.75	0.60–0.93	0.009

<sup>a</sup> Adjusted for age class and sex.

farmland cattle density, rainfall level 30 days prior to stranding, and proximity to fresh water were not associated with risk of leptospirosis. The logistic regression model for leptospirosis exhibited a fair overall fit (Hosmer and Lemeshow chi-square 368.91,  $P=0.143$ ). Analysis using only confirmed cases gave similar results (Norman et al., 2007).

## DISCUSSION

The strong associations between sex (males), stranding season, and an increased risk of leptospirosis confirm findings in previous studies (Gulland et al., 1996; Colagross-Schouten et al., 2002; Greig et al., 2005). The occurrence of this outbreak in 2004 follows the observation of cyclic large-scale leptospirosis epizootics recognized since the early 1970s, with a distinct 3–4-yr periodicity is separated by enzootic maintenance of the disease (Vedros et al., 1971; Dierauf et al., 1985; Gerber et al., 1993; Gulland et al., 1996). This study, evaluating an outbreak year, confirms observations by Greig et al. (2005), which evaluated both endemic and outbreak years, in demonstrating the association of age, sex, and season with leptospirosis and provides new information on the potential risk of proximity to and density of dog parks. The seasonal distribution of cases may reflect the temporal and spatial distribution of sea lions, as after the breeding season, most of the adult and subadult males leave the

rookeries and move northward, while females remain nearer the rookeries (King, 1983). An increased number of cases in summer and autumn also have been found in dogs (Ward, 2002), cattle (Miller et al., 1991), horses (Barwick et al., 1997), and humans (Ferguson, 1993; Katz et al., 2002), during and immediately following periods of heavy rainfall. However, these studies, which were either not conducted in California, or focused on the entire country, showed no association of rainfall with leptospirosis in California.

The high prevalence of cases of leptospirosis in Monterey county may partially be due to movements of infected sea lions and season; however, this contrasts to the finding in Greig et al. (2005), which also included nonoutbreak years and revealed that sea lions that stranded north of Santa Cruz to the Oregon border were more likely to be infected with leptospirosis than outside this region. However, Greig et al. (2005) analyzed cases of leptospirosis over 10 yr, whereas the present study focused on one outbreak year. The value in locating the focus of outbreaks is that this may aid in establishing the source of the agent, which is more difficult to establish with enzootic cases.

The concentration of strandings and cases around metropolitan areas could be a function of increased reporting, with greater numbers of sea lion strandings reported in areas of high human population density. Higher concentrations of dog parks also tend to be located in the vicinity

of metropolitan areas. In Adin and Cowgill (2000), 28/36 (77%) of leptospirosis cases detected originated from coastal communities surrounding San Francisco. In the present study, the significantly increased risk of leptospirosis with increasing dog-park density suggests that the density of dogs along the coast may play a role in exposure of sea lions to leptospires.

The significance of male sex in the epidemiology of the disease is uncertain, but most likely represents a behavioral component contributing to the transmission of the organism. The association between sex and leptospirosis may also be a consequence of sea lion migration, as males are more migratory than females. Similarly, male dogs were found to be at higher risk for leptospirosis than females (Ward et al., 2004), a finding that may be attributable to their roaming behavior. The higher prevalence of antibodies detected in older sea lions may indicate previous exposure to leptospires, but not necessarily current infection or reservoir status, which is problematic when one is trying to assess the role of sea lions in the transmission and maintenance of leptospirosis. The lack of significance with other environmental factors evaluated may have been due to confounding from other unknown environmental factors or from other factors that were not investigated, but may indirectly serve as a risk factor for leptospirosis such as sea lion migration and movements.

There were several limitations in this study. In the primary analysis, leptospirosis included confirmed and probable cases. Probable cases may have included sea lions with diseases similar to leptospirosis, therefore underestimating the true association between a risk factor and stranding due to leptospirosis. However, analysis using only confirmed cases yielded similar results. If sea lions that have leptospirosis are more likely to strand and be reported than those that strand due to other causes, this might bias the results away from the null. It is assumed that

stranding rates are unrelated to the cause of stranding. However, in this study population, a large proportion of the controls were sea lions with domoic acid intoxication, which display neurologic deficits and often strand in inappropriate locations; such animals may be more likely to be reported stranded, which could falsely decrease the odds ratio associated with risk of leptospirosis.

The use of proxy measures of exposure to cattle, dogs, and humans were not measures of individual-level exposure. These indicators may not have been representative of environmental conditions at the time sea lions were exposed to leptospires, resulting in misclassification of exposures. In addition, unavoidable misclassification of environmental data might have occurred because of wide-ranging movements and migration, particularly of males (Weise et al., 2006). Because males are not constrained by dependent young at a rookery and do not provide parental care (King, 1983), they can extend their foraging range, placing them at greater risk of acquiring and spreading leptospirosis. Due to their migratory behavior, males may serve to expose conspecifics not only at rookeries, but also at haul-out sites on the mainland. The source of infection and stranding site are often not the same location, complicating efforts to determine where the agent was acquired. To gain sensitivity in describing the risks associated with environmental exposures, additional analyses that use more detailed information on animal movements to ascertain environmentally related exposures are needed.

Future research on leptospirosis should focus on refining environmental exposures to examine adequately the possibility of an environmental causality for leptospirosis in sea lions and investigate the possible role of terrestrial wildlife in the transmission of leptospires to sea lions. In addition, dogs and dog parks, or factors associated with them, might be further investigated to assess their relationship to leptospirosis

in sea lions. Although these factors may be useful in elucidating the spread of leptospirosis to sea lions, further research should focus on defining *Leptospira* species and strains involved in outbreaks and their source and survival in the environment, including surveillance of potential reservoir hosts, and evaluation of exposure at rookeries.

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