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LEPTOSPIROSIS IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*) STRANDED ALONG THE CENTRAL CALIFORNIA COAST, 1981–1994

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ABSTRACT: Prevalence of leptospirosis was determined in California sea lions (*Zalophus californianus*) stranded live along the central California (USA) coast between January 1981 and December 1994. Clinical signs of renal disease were seen in 764 (33%) of 2338 animals examined; 545 (71%) of these 764 animals died, with similar gross lesions of nephritis. In silver impregnation stains of sections of formalin-fixed kidney, numerous loosely coiled spiral organisms were observed. *Leptospira pomona kennewicki* was cultured from four kidney samples in 1991. Epizootics of leptospirosis occurred in 1984, 1988, 1991, and 1994, and were more common in the autumn, typically affecting juvenile males. In 1991 and 1994, 47 animals sampled had antibody titers to *L. pomona* greater than 1:3200. In 1992, 20 animals sampled were seronegative, and in 1993 three of 20 animals sampled had low titers to *L. pomona*.

INTRODUCTION

Leptospirosis is a cosmopolitan disease of humans and a wide variety of both domestic and wild animals (Shotts, 1981). *Leptospira pomona* has caused both epizootic and enzootic disease in free-living California sea lions (*Zalophus californianus*). In 1970, unusually large numbers of sick and dead California sea lions were observed along the California and Oregon (USA) coast, and *Leptospira pomona* was isolated from the kidney and urine of affected animals (Vedros et al., 1971). A second epizootic was observed in central California and Oregon in 1984, and clinical progression of the disease was described (Dierauf et al., 1985). Observations of reproductive failure in California sea lions on the Channel Islands off the California coast and the isolation of *L. pomona* from premature pups in 1972 and 1973 was evidence that leptospirosis may be enzootic in California sea lions (Smith et al., 1974). The enzootic nature of the disease was also supported by the fact that renal disease was a consistently common finding in stranded sea lions between 1984 and 1990 (Gerber et al., 1993). In animals with renal disease, *L. pomona* was considered the most likely cause of disease, due to the presence of high titers of antibodies to *Leptospira* spp. and characteristic gross post mortem findings.

Our objectives were to document three further epizootics of leptospirosis in sea lions along the central California coast, and to review the pattern of disease over 14 yr, January 1981 to December 1994, as observed in sea lions admitted to a rehabilitation center.

MATERIALS AND METHODS

Stranded California sea lions appearing along the California coast (37°42′N, 123°05′W to 35°59′N, 121°30′W) between 1 January 1981 and 31 December 1994 were included in this study. Upon arrival at The Marine Mammal Center (TMMC), Sausalito, California, a rehabilitation facility, they were examined clinically, their sexes determined by examination of external genital morphology and their ages estimated utilizing standard body length, weight, sagittal crest development and pelage coloration (Mate, 1978). Animals 3 yr of age and under were classed as juveniles, 4 to 5 yr-olds as subadults, and animals older than 5 yr as adults. Blood samples were taken from the caudal gluteal vein using an 18 gauge × 38 mm needle (Bossart and Dierauf, 1990) and aliquots were placed into vacutainers containing either ethylene-diamine-tetra-acetic acid (EDTA) or serum separation gel and clot activator (Vacutain-

er, Becton Dickinson, Rutherford, New Jersey, USA). Complete blood counts were performed on EDTA samples using a Coulter Counter Model S-Plus IV (Coulter Electronic Inc., Hialeah, Florida, USA), differential cell counts were performed manually (Brown, 1980). Tubes containing serum were centrifuged at 3,000 × *G* within 2 hr of collection, the serum separated, and aliquots frozen at −20 C for future serological analysis. Serum biochemistry analyses were performed on a Vet test 8008 (Idexx Laboratories Inc, Westbrook, Maine, USA) and serum electrolytes were measured on a VetLyte Electrolyte Analyser (Idexx Laboratories Inc). Microagglutination tests using *Leptospira pomona* antigen (Galton et al., 1962) were performed at the California Veterinary Diagnostic Laboratory Service, Davis, California, on stored serum. Samples for serology were taken from 27 animals that died of renal disease in 1991 and 20 animals in 1994. Five of the latter were sampled twice, 14 days apart. Microagglutination tests were also performed on serum samples from 53 clinically normal juvenile animals that stranded in 1992 and 1993.

Post mortem examinations were performed on all animals that died during rehabilitation. Following routine post mortem examination, samples of lung, heart, thyroid, stomach, ileum, colon, pancreas, spleen, liver, kidney, adrenal, gonad, urinary bladder, lymph node and brain were fixed by immersion in 10% neutral buffered formalin. Fixed tissues were embedded in TissuePrep (Fischer Scientific, Fairlawn, New Jersey, sectioned at 5 μm, and stained with hematoxylin and eosin. In 1991, slides of fixed kidney tissue from the 27 animals that were tested serologically for leptospirosis were stained with Warthin Starry silver stain (Luna, 1968). In 1994, slides of kidney from 10 animals that died with gross lesions of renal disease were stained with Steinart stain and Levaditis stain (Luna, 1968).

Samples of liver and lung from all animals examined at post mortem between January 1990 and December 1994 were cultured on blood agar with 5% added citrated sheep blood, and on MacConkey agar (PML, Tualatin, Oregon (USA)) incubated at 35 C, then examined at 24 and 48 hr (Carter, 1973). Bacteria were identified using the API 20E System (Sherwood Medical, Plainview, New York, USA) and colony and biochemical characteristics (MacFaddin, 1980). In 1991 and 1994, *Leptospira* spp. isolation was attempted from kidney and urine from 87 and 20 animals respectively. In 1991, 2 to 3 g samples of kidney from animals with gross lesions typical of leptospirosis were aseptically ground in 1% bovine serum albumin (BSA) and the tissue debris allowed to settle. The supernatant was filtered through a 0.22 μm millipore filter and was used to inoculate Fletchers and Ellinghausen. McCullough, Johnson and Harris (EMJH) media (Collins et al., 1995). Urine samples collected from 12 of the same 87 animals were centrifuged at 600 × *G* for 10 min., the supernatant removed and the pelleted material added to 1% BSA. Three serial tenfold dilutions (1:100, 1:1,000, 1:10,000) of the suspended material in 1% BSA were prepared and 1 ml of each dilution used to inoculate EMJH media. Inoculated tubes were incubated at 28 to 30 C and examined at weekly intervals using darkfield microscopy. The resulting four isolates were serotyped at the National Veterinary Diagnostic Laboratory (NVDL), Ames, Iowa (USA) (Faine, 1993). In 1994, twenty 2 to 3 g kidney samples were shipped to the Colorado Veterinary Diagnostic Laboratory, Fort Collins, Colorado (USA), in Fletcher's media and cultured as described.

In 1991, impression smears were made from kidneys of 104 sea lions with gross lesions typical of leptospirosis within 24 hr of death. Smears were also made from 52 of the 104 kidney specimens after they had been frozen at −20 C for greater than 4 wk. Smears were air-dried, fixed in acetone for 10 min, then examined for *Leptospira* spp. using a direct fluorescent antibody technique (Galton et al., 1962).

Hematology and serum biochemistry parameters were compared to normal ranges (Roletto, 1993) using a one sample *t*-test (Ott, 1993). Sex and age differences in prevalence of leptospirosis were compared using the chi-square test with Yate's correction factor (Ott, 1993). Statistical significance was determined at $P < 0.05$. A Mann-Whitney non-parametric rank analysis (Statview 4.01, Abacus Concepts, Berkeley, California) was used to compare the numbers of sea lions that died of leptospirosis, and the percent prevalence, in epizootic years to non-epizootic years.

RESULTS

Between 1 January 1981 and 31 December 1994, 2,338 California sea lions stranded live along the central California coast and were admitted to TMMC. Of these, 764 (33%) had similar clinical signs including depression, anorexia, polydipsia, dehydration, reluctance to use the hindflippers, and, in extreme cases, vomiting, muscular tremors, and abdominal pain; abdominal pain was determined by adoption of a hunched position and holding the

TABLE 1. Mean (± SD) serum biochemical values in California sea lions (*Zalophus californianus*) with leptospirosis confirmed by observation of spirochete-like organisms in silver-stained sections of kidney, and in animals with clinical signs resembling leptospirosis.

Parameter	Leptospirosis confirmed (n = 20)	Leptospirosis suspect (n = 357)	Reference value[a] (n = 80)
Sodium (mEq/l)	157 ± 16	158 ± 8	149 ± 2
Potassium (mEq/l)	4.14 ± 1.3	4.2 ± 2	4.6 ± 0.4
Calcium (mg/dl)	9.3 ± 1.6	9.2 ± 0.8	9.4 ± 0.5
Phosphorous (mg/dl)	14.2 ± 5.4	13.8 ± 2.3	5.9 ± 1.06[b]
Blood Urea Nitrogen (mg/dl)	176 ± 57	182 ± 31	44 ± 20
Creatinine (mg/dl)	7.6 ± 4.6	8.1 ± 1.5	0.7 ± 0.2

[a] From Roletto (1993).

[b] These values are from 15 clinically healthy animals with other blood parameters within the normal range examined at The Marine Mammal Center in 1994.

hindflippers over the abdomen. No significant hematologic changes were detected in blood samples collected from 367 of these 764 animals. Statistically significant serum biochemical changes included elevated blood urea nitrogen, phosphorus, sodium and creatinine levels (Table 1).

Five hundred and forty-five (71%) of these 764 animals died, and had similar gross lesions on post mortem examination. These were marked swelling of the kidneys, loss of differentiation between renule medullae and cortices, pale tan colored cortices and occasionally subcapsular hemorrhages and hemorrhage at the corticomedullary junction (Fig. 1). In addition to renal lesions, approximately one-third of the animals had swollen, friable livers with thick, tenacious bile in the gall bladder and severe gastric ulceration. On histological examination of tissues from 127 animals, we observed subacute to chronic, multifocal, severe lymphoplasmocytic interstitial nephritis (Fig. 2). This was characterized by mild thickening of the basement membranes of glomerular capillaries, necrosis

FIGURE 1. Kidney from a California sea lion with leptospirosis. Note severe loss of renule definition. Bar = 2 cm.

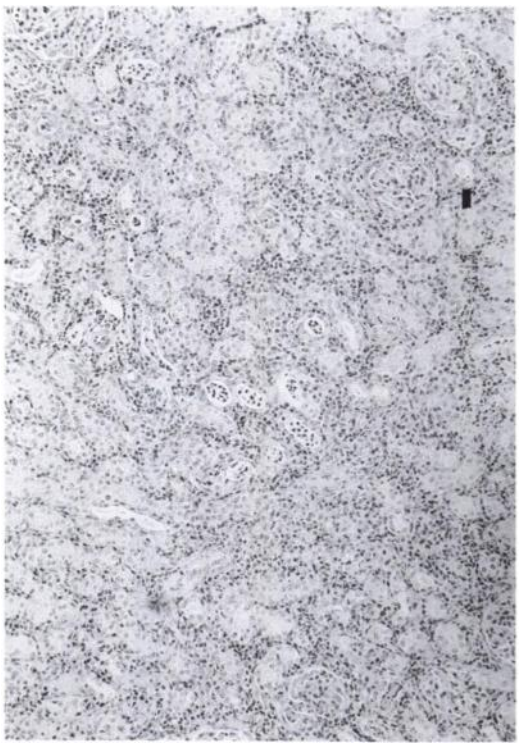

FIGURE 2. California sea lion kidney with lymphoplasmocytic interstitial nephritis. Hematoxylin and eosin stain. Bar = 100 μm

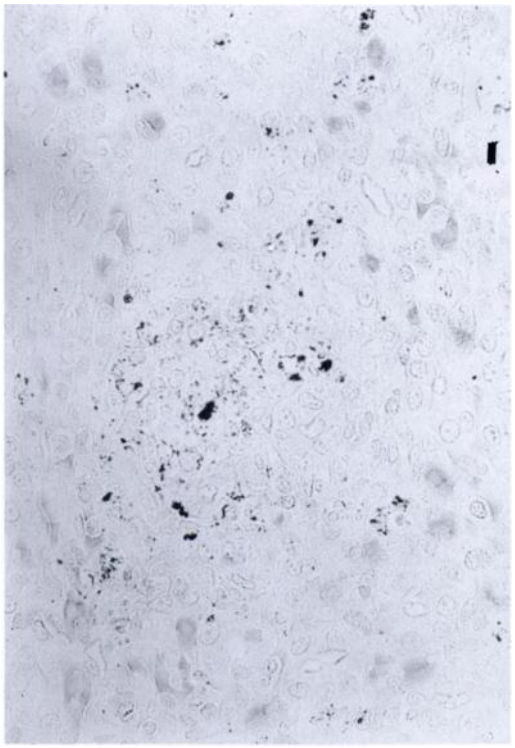

FIGURE 3. Silver stained material in Levaditi's stained section of kidney from a California sea lion. Bar = 100 μm.

of convoluted tubular epithelium, accumulation of cellular debris, and bacteria within convoluted tubules and collecting ducts and a moderate accumulation of plasma cells and lymphocytes within interstial tissues. In silver impregnation stains on kidney sections from 37 animals, there were thin, loosely coiled spiral organisms compatible with spirochetes in all kidney samples examined (Fig. 3).

Of the 87 kidney and 12 urine samples cultured in 1991, spirochete organisms resembling *Leptospira* spp. were seen on 12 of the kidney and three of the urine samples on darkfield microscopic examination. Organisms were isolated from four kidney samples, and identified as *Leptospira interrogans,* serovar *pomona,* strain *kenniwicki* by NVDL. No spirochetes were isolated from 20 kidney samples cultured in 1994. Additional bacteria cultured from kidney samples in all years included *Escherichia coli, Enterobacter* sp., *Klebsiella* sp., *Salmonella* sp., *Proteus mirabilis,* and *Pseudomonas* sp.

TABLE 2. Antibody prevalence and titers to *Leptospira pomona* in California sea lions (*Zalophus californianus*) between 1991 and 1994.

Year	Number sampled	Positive (%)	Antibody titer 1:3,200	1:800	1:400	1:100
1991	27	100	27	0	0	0
1992	33	15	3	0	1	1
1993	20	15	0	1	0	2
1994	25	100	25	0	0	0

Eighty-nine of the 104 fluorescent antibody tests on fresh kidneys in 1991 were positive, all tests on frozen kidneys were negative. Slides from frozen samples were difficult to read due to excessive background fluorescence and ruptured cells.

All 52 serum samples from animals with clinical signs of renal disease in 1991 and 1994 had antibody titers to *L. pomona* greater than 1:3,200 (Table 2). The five animals sampled twice at 14 day intervals had titers greater than 1:3,200 on both occasions. Fifteen animals from 1994 had titers to *L. gryppo,* greater than 1:3,200, 12 had titers to *L. icterohemorrhagiae* greater than 1:3,200, and 18 had similar titers against *L. bratislava.* Samples from five (15%) of 33 animals in 1992 and three of 20 animals in 1993 had antibodies to *Leptospira* spp. but in 1993, titers were lower (Table 2).

Cases of renal disease typical of leptospirosis were observed at post mortem examination in all years between 1981 and 1994 other than 1982. Significantly ($P < 0.01$) higher prevalences were observed in 1984, 1988, 1991 and 1994 (Fig. 3). The 1984 epizootic has been previously documented (Dierauf et al., 1985). In each year, cases were seen between July and December (Fig. 4), the season when highest numbers of sea lions strand in central California. Peak numbers were seen in September in 1984, 1988 and 1991, and in October in 1994. Cases were most com-

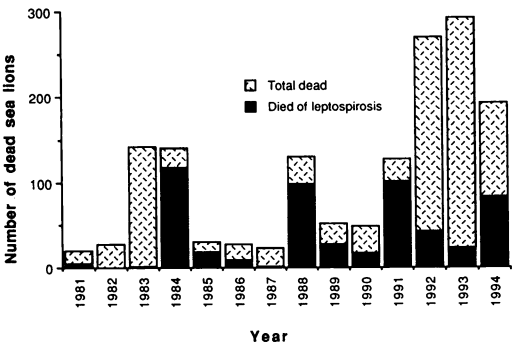

FIGURE 4. Number of California sea lions examined post mortem at The Marine Mammal Center with typical lesions of leptospirosis, January 1981 to December 1994.

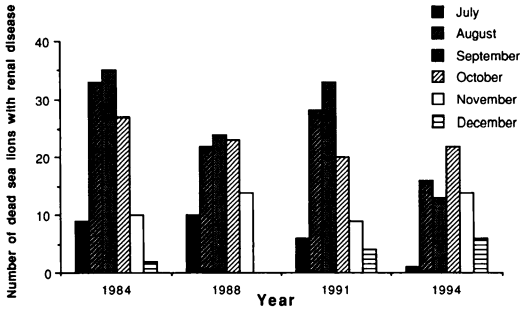

FIGURE 5. Number of California sea lions per month dying from renal disease in four successive leptospirosis epizootics.

mon in juvenile male animals ($P < 0.01$ for both sex and age class) (Fig. 5).

DISCUSSION

The clinical signs, gross lesions and histological changes observed were similar to those described previously in California sea lions considered to have died from leptospirosis (Dierauf et al., 1985).

Despite the availability of fresh tissues, *Leptospira pomona* was cultured from only four animals. This probably reflects the difficulties involved in culturing this organism, and isolation of leptospires may not be a suitable method for routine diagnosis of infection. The principal difficulty encountered was the contamination of culture with other bacteria, a problem that was overcome in 1991 by the use of bacteriologic filters. In contrast, on using silver stains on formalin-fixed tissues, presence of organisms was established in all cases examined. Thus, silver stains may be a more reliable, as well as less expensive, method for routine diagnosis. Using the fluorescent antibody technique, we detected organisms more effectively than with culture. However, slides were often difficult to read due to excessive background fluorescence, and results were susceptible to variation in reader experience.

Antibody titers were high (1:3,200) in all animals tested at the time of clinical disease and for 2 wk during treatment. Reaction to *L. gryppo, L. bratislava* and *L. icterohaemorrhagiae* probably was a result of cross-reactivity to these serotypes, although it could have resulted from exposure to leptospires other than *L. pomona.* These high titers, combined with the serum biochemical changes of hyperphosphatemia, high blood urea nitrogen, and high creatinine, were useful in presumptive diagnosis of infection in the live animal.

The seasonal distribution of cases may be a consequence of the migratory behavior of California sea lions. Animals breed on islands off the southern California coast in May and June each year, migrating north after the breeding season to feed off central and northern California, and sometimes as far north as British Columbia, Canada (Riedman, 1990). Males tend to be more migratory than females, and adult animals more so than juveniles (Riedman, 1990). Animals are thus only present within the study area in significant numbers from July onwards in any year. Seasonality in host presence may explain seasonality in case incidence. The incidence of leptospirosis in other areas of the California sea lion range is unclear. Leptospirosis has been detected in southern California, but was considered rare in 680 sea lions stranded between 1970 and 1981 (Howard et al., 1983). More detailed information on geographical distribution of cases is required to determine whether or not sea-

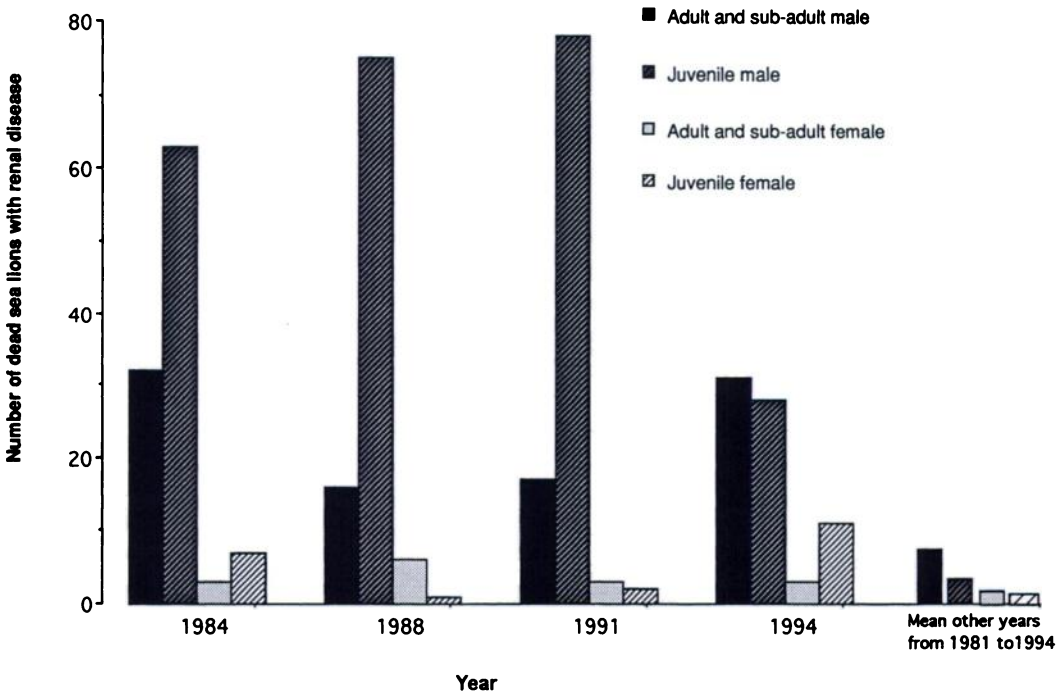

FIGURE 6. Number of California sea lions of different age and sex classes dying from renal disease in four successive leptospirosis epizootics.

sonality of cases is simply a reflection of sea lion migratory behavior.

There may also be seasonal variation in availability of leptospires in the environment. In cattle, leptospirosis is more common in late summer and autumn, and prevalence in different regions within the USA is correlated with mean air temperatures (Miller et al., 1991).

There is a periodicity in the number of cases observed per year, with a significantly higher prevalence occurring every 3 to 4 yr. Although these data only represent cases observed at a single rehabilitation center in central California and not annual mortality in the free-living population, a similar periodicity has been observed in mortality of California sea lions on the south east Farallon Island, 25 miles off the California coast from San Francisco (P. Pyle, pers. comm.). Larger numbers of dead sea lions than usual were observed on this island from September to December in 1988, 1991 and 1994. The cause of death of most animals was not determined, but in 1994, kidneys from one animal were grossly swollen with severe loss of renule definition, and severe interstitial nephritis was observed histologically in sections of formalin-fixed kidney.

Disease epizootics may result from enhanced survival, reproduction or transmission of the pathogen, or increased susceptibility of the host population (Grenfell and Dobson, 1995). *Leptospira* spp. survive well under warm, moist, alkaline conditions and are killed at salinity greater than 1% (Faine, 1993). The source of leptospires infecting sea lions is unknown, but by analogy with other species, stagnant pools may be important (Faine, 1993). Environmental survival of leptospires in stagnant pools could be affected by climatic conditions altering sea temperature and rainfall.

Climatic and oceanographic conditions along the California coast are periodically dramatically altered by a combination of wind and oceanic current changes known as El Niño. During El Niño events, sea

current and wind changes result in an increase in thermocline depth and warmer surface waters in the eastern Pacific, with water temperatures off California increasing by 2 to 3 C (Cane, 1983). These conditions lead to nutrient deficient waters along the shores of North and South America. This deficiency causes changes in food supply up the food chain, and can affect species at higher trophic levels, including pinnipeds (Trillmich and Ono, 1991). Recent El Nino events occurred in 1982 to 1983, 1986 to 1987 and 1991 to 1993 (Duxbury and Duxbury, 1994). Epizootics of leptospirosis in 1984, 1988, and 1994 thus occurred in the autumn following an El Niño event. This temporal relationship could be a consequence of climatic changes altering survival of leptospires. However, during El Niño years, greater numbers of sea lion pups are abandoned by their mothers, and pups have a slower growth rate and higher mortality in their first 2 mo of life (Trillmich and Ono, 1991). These changes may alter host population susceptibility, and epizootics of disease could occur as a consequence of changes in host susceptibility rather than pathogen availability.

Typically, there are host-adapted and host-unadapted strains of leptospires (Heath and Johnson, 1994). Host-adapted strains usually cause mild disease and abortions in their host, cause a high percentage of seropositive hosts in the population and are shed in urine for long periods (Heath and Johnson, 1994). Unadapted strains cause sporadic severe disease in the host, low prevalence of seropositive hosts in the population and are usually only shed for short periods by host individuals. Based on the severity of leptospirosis observed in sea lions, the organism is not adapted to sea lions, and may therefore survive in reservoir hosts of other species. Other potential sources of infection are carrier animals of other species, such as small rodents and wild pigs living on the Channel Islands (Shotts, 1981). However, the high number of seropositive animals following an epizootic is evidence that the organism is also behaving in a host-adapted fashion. It is possible that recovered sea lions may continue to shed leptospires in their urine, and thus act as sources of infection for other sea lions.

Changes in transmission of infection may also result in epizootics of disease. Although the mode of transmission of leptospirosis between California sea lions is unknown, in other mammalian species transmission involves urine of carrier animals, either directly or indirectly (Shotts, 1981). However, vectors may also be involved in transmission. The role of fish as potential sources or vectors of infection has been ignored, with the exception of experimental infection of goldfish (*Carassius auratus*) (Maestrone and Benjaminson, 1962). These fish could be experimentally infected, but did not show signs of disease. Based on studies on seroprevalence of leptospirosis in northern fur seals (*Callorhinus ursinus*) infection of this host species may occur at sea; thus fish may play a role (Smith et al., 1977).

Increase in transmission of infectious disease commonly results from an increase in host population density (Agaev, 1990). Epizootics may result from an absolute increase in host population density, or from an increased proportion of non-immune animals within the population (Grenfell and Dobson, 1995). In China, an 8 yr epidemic cycle of leptospirosis in humans occurs (Faine, 1993). Epidemics are correlated with high rainfall at rice harvest time, but require high mouse population densities (mice are the reservoir species in this case) and a decline in human antibody prevalence to baseline levels to occur. Raised immunity in the human population correlates with declines in the epidemics.

The high number of leptospirosis cases in juvenile animals, combined with the changes in seroprevalence between 1992 and 1994, is evidence that epizootics of leptospirosis are occurring when sufficient numbers of susceptible, non-immune sea lions are born into the population. More

detailed knowledge of the age structure of the California sea lion population and more extensive age-structured seroprevalence surveys, combined with mathematical modeling of the disease dynamics, are required to determine whether changes in host population immune status could generate periodicity in incidence of leptospirosis in sea lions.

Leptospirosis is thus a common, easily diagnosed zoonotic disease of California sea lions stranding in central California, with regular epizootics occuring in the autumn months. It affects survival of sea lions, and may therefore have important consequences on the population dynamics of this species (Grenfell and Dobson, 1995). Further studies are required to elucidate the source of infection and mode of transmission to sea lions, and to determine the nature and duration of the immune response in sea lions following infection.

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