Leptospirosis Epizootic among California Sea Lions

Abstract. *A* Leptospira *species is suspected of being the etiological agent in a recent epizootic among California sea lions. The disease was confined to subadult males of the species* Zalophus c. californianus.

The North American California sea lion (*Zalophus c. californianus*) usually reaches population peaks off the central California coast in mid-May and late August (*1*). These increases in population reflect an influx of adult males. Present evidence suggests that many of these males come from the Channel Islands off the coast of southern California and possibly from northern Mexico after the breeding season (*2*).

From late August to early November 1970, the number of stranded California sea lions observed along the northern California coast was approximately four times higher than that usually seen during the migration period. There were 315 stranded animals documented, which included 125 dead animals. A similar epizootic in California sea lions was seen along the Oregon coast. In a single survey of Shell Island (Oregon coast) on 10 November 1970, 230 sick and 5 dead subadult males and two healthy adult males were observed. The disease on both California and Oregon coasts was confined to 2- to 8-year old subadult male *Zalophus c. californianus*.

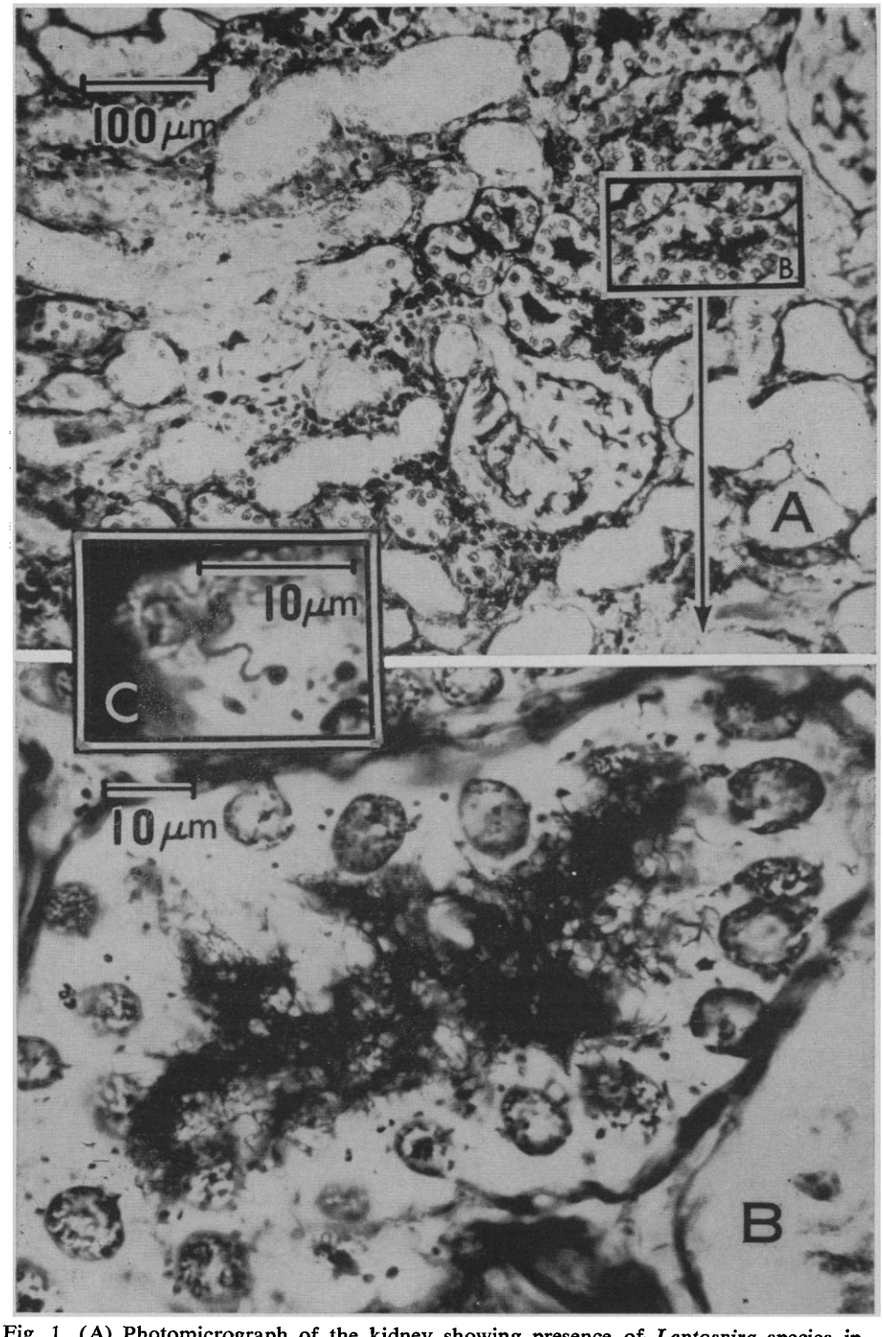

Fig. 1. (A) Photomicrograph of the kidney showing presence of *Leptospira* species in tubule lumina. (B) Enlargement of tubule shown in inset in A. (C) Individual *Leptospira* in a tubule.

The study reported here was confined to 40 stranded animals in the San Francisco Bay area. The sick animals were depressed and showed a disinclination to use the rear limbs and back. Their extreme thirst apparently caused the animals to seek sloughs or other sources of fresh water. When held in captivity, they also drank copious amounts of fresh water from hose or bucket. Fever was prominent in all animals, and one case of icterus of the oral mucosa was observed. Twenty-four animals were available for necropsy but only 15 for detailed examination of the tissues. We observed that at necropsy all animals had severe nephritis and thick, black bile in the gall bladder. Gastritis and a tenacious, mucoid pericardial fluid was seen in four animals; an enteritis was found in three animals.

The changes in the liver were slight and varied. Hyperplasia of the Kupffer's cells, erythrophagocytosis, and hemosiderosis were prominent findings. In addition, some animals had dilated bile ducts containing inspissated bile, minimal fatty changes, and necrosis of an occasional hepatocyte. Dissociation of the hepatocytes in the cords was not observed.

The kidney was the only organ with significant histopathologic lesions. The most conspicuous finding was the diffuse interstitial nephritis characterized by accumulations of mononuclear leukocytes, predominantly lymphocytes with a few plasma cells, in both the cortex and the medulla but not in the pelvis. The glomeruli were essentially unaffected, but atrophy of the convoluted tubules was noted in some areas. An occasional tubule had undergone degeneration and necrosis. Collections of degenerated polymorphonuclear neutrophils were commonly found in the tubular lumina. Large numbers of spirochetes were observed in silver-stained (*3*) kidney sections of all animals (Fig. 1). The lumina of many tubules were almost occluded by these bacteria. The microorganisms in the

kidney tissues were identified by the direct fluorescent antibody technique. Positive reactions were obtained with the conjugated antiserums to serotypes *canicola, icterohaemorrhagiae, autumnalis* and *pomona* (*4*) in the only kidney section submitted for testing (animal No. 4). Scar tissue was not apparent. Nephritic lesions of this nature suggest a subacute to chronic interstitial nephritis with recurrent attacks by the causative agent. The absence of inflammation in the pelvis indicated a hematogenous mode of infection. The purulent exudate in the tubules was secondary and probably resulted from terminal bacteremia.

A variety of tissues was examined for isolation of microbial agents; a report on microorganisms recovered will be published elsewhere. By dark-field examination, leptospiras were seen in kidney suspensions of all 15 sick animals and in the urine of one animal successfully treated with penicillin, streptomycin, and vitamin B complex and given free access to water. For isolation, 1 ml (undiluted and tenfold serial dilutions to 10^{-6}) of saline suspensions of kidney and liver were inoculated directly into liquid medium (*5, 6*, p. 46) and intraperitoneally into guinea pigs [one guinea pig (300 to 400 g) for each dilution]. A similar procedure was used with dilutions of blood and urine. The liquid cultures were incubated at 30°C. The guinea pigs were bled by heart puncture 14 days after injection. The guinea pig blood was then inoculated into liquid medium and incubated at 30°C. The cultures were incubated for 6 weeks, during which they were examined by dark-field microscopy. Small numbers of *Leptospira* and other contaminating bacteria were observed in all cultures from kidney, urine, and blood, but not in those from liver. We attempted to remove contaminating bacteria by injecting 1 ml of each culture intraperitoneally into a guinea pig and bleeding the guinea pig by heart puncture within 15 minutes (*6*, p. 45). The guinea pig blood was then inoculated into liquid medium and incubated at 30°C for 2 to 3 weeks. All cultures contained *Leptospira* in addition to contaminating bacteria. Pure cultures of *Leptospira* were finally obtained by a modification of the filter membrane technique (*7*). Samples of each culture were placed on membrane filters (0.22 μm, Millipore) previously set aseptically on the surface of solid Fletcher's medium. After incubation for 24 hours at 30°C the filters were removed and samples of the agar directly below the membrane were inoculated into Fletcher's medium. The *Leptospira* grew rapidly and in pure culture within 48 hours at 30°C. The morphology of the *Leptospira* was typical.

Table 1. Antibody titers to *Leptospira* species in animals with the acute stage of the disease and in convalescent and normal California sea lions. Results are reported as the highest dilution in which 50 percent or more of the cells agglutinated in the microscopic agglutination test. Tests on serums from sick and convalescent animals were performed through the courtesy of the National Animal Diseases Laboratory, Ames, Iowa. Serum obtained 2 weeks after treatment of animal No. 9 is termed convalescent. Results with individual serums from five healthy sea lions held in captivity are termed normal.

Animal No.	Antibody titers to *Leptospira* species			
	pomona	*icterohaemorrhagiae*	*grippotyphosa*	*autumnalis*
1	10,000	1,000	1,000	1,000
2	100,000	1,000	1,000	10,000
3	10,000	1,000	1,000	10,000
4	10,000	1,000	0	10,000
5	1,000	1,000	1,000	1,000
6	10,000	0	0	1,000
7	10,000	1,000	1,000	10,000
8	100,000	1,000	1,000	10,000
9	10,000	1,000	1,000	1,000
Convalescent	10,000	1,000	1,000	1,000
Normal	0	0	0	0

The serums of ten sick animals, one convalescent animal, and five healthy animals were analyzed for agglutinating antibodies to various *Leptospira* (Table 1). Antibody titers to serotypes *pomona* and *autumnalis* were generally higher than those to other serotypes tested. The antibody titer in the one convalescent animal bled 2 weeks after initiation of treatment was the same as that in the sick animals. This may be due to the difficulty in documenting the time of onset of illness in this epizootic. Healthy, subadult male California sea lions were not available for necropsy during the epizootic. However, the serums of five healthy animals of a similar age and species previously held in captivity were examined for agglutinating antibodies to *Leptospira*. All serums had negative titers.

These clinical and laboratory studies strongly suggest that the etiological agent of the epizootic in California sea lions is a *Leptospira* species. The syndrome was compatible with clinical leptospirosis, and this diagnosis was supported by the recent observations on San Nicholas Island that the 1970 abortion rate increased threefold over the 1969 rate. Abortions associated with leptospirosis have been well documented in domestic animals (*8*).

The only other documented episode of increased mortality of these animals occurred in 1947. In that year, the increased numbers of stranded animals was tentatively diagnosed as being due to bacterial pneumonia. Until definitive epidemiological studies are carried out, the role of biological or chemical agents acting as predisposing factors to the observed leptospirosis cannot be ruled out. The fact that the disease was confined to subadults suggests that leptospirosis may be enzootic in California sea lions and that some degree of herd immunity may exist. Conceivably, the increased rate of disease during 1970 reflected changes in population; spread of the infection might be facilitated by increased population density.

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