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NATURALLY-OCCURRING LEPTOSPIROSIS IN NORTHERN FUR SEALS (*Callorhinus ursinus*)*

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Abstract: A 4-year study of Northern fur seal (*Callorhinus ursinus*) leptospirosis in the Bering Sea has shown that in newborn pups *Leptospira pomona* is associated with a multiple hemorrhage syndrome. Adults may develop an interstitial nephritis and shed organisms in the urine. The herd prevalence, based on microscopic slide agglutination tests, ranged between 7.0% and 15.4% for adult females and 3-4 year old bachelor bulls, whereas nursing pups averaging 4 months of age had a prevalence of 2%. These results are used to conclude that leptospirosis is not acquired primarily on the breeding rookeries but rather is more frequently acquired subsequent to the pups leaving the rookeries, presumably through the food chain during their first pelagic cycle.

INTRODUCTION

Bacteria of the genus *Leptospira* were first shown to infect Northern fur seals (*Callorhinus ursinus*) in 1972. At that time, *Leptospira pomona*^[4] was isolated from the liver of a dead fur seal pup showing lesions of multiple hemorrhage and from a debilitated 13-year-old fur seal bull.⁷ Two subsequent isolations have been made, one from the liver and the other from the kidney of two fur seal pups showing multiple hemorrhagic lesions. Because of this, we now associate the so-called "multiple hemorrhagic perinatal complex" with acute leptospirosis of the newborn. This same condition recently has been described in aborting California sea lions (*Zalophus californianus californianus*).⁸

Because the Northern fur seal herd is under intensive international management and because the annual harvest of fur seals is an important economic consideration for the Alaskan native populations of the Pribilof Islands, where the fur seals breed, efforts have been made to define leptospirosis within the fur seal populations. Such information is to be used for assessing the importance of this disease in overall seal survival and to determine if reproductive efficiency of fur seals could be enhanced using immunization programs. This paper describes the pathology of leptospirosis in newborn and adult fur seals, the prevalence of the disease over a 4-year period and discusses the possible sources of infection.

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[4] *Leptospira* serotyping was by courtesy of A. D. Alexander WHO/FAO Leptospira Reference Laboratory, Walter Reed Army Institute of Research, Washington, D.C.

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MATERIALS AND METHODS

Pathology

Since July, 1972, necropsies have been performed on 119 fur seal pups found dead on the St. Paul Island rookeries during the height of the breeding season. Selected tissue samples were taken from these animals. Kidney sections from 89 adult females and 60 normal pre-pelagic pups, preserved in 10% buffered formalin, were processed for histologic examination as previously described.⁶

Serology

For the last 4 summers about 200 serums have been collected each year from the bachelor bulls killed in the fur seal harvest on St. Paul Island. In addition, 89 serums were collected from adult female fur seals taken at sea during the Pelagic Research Studies conducted by the National Marine Fisheries Services.⁵ In November of 1974, 150 4-month old fur seal pups were bled prior to their first pelagic cycle.

Individual serums from all 1059 of these animals were combined into pools of 5 sera each and diluted with 0.85% NaCl to give individual serum dilutions of 1:10. These were screened for leptospiral antibody using a macroscopic slide agglutination test.^{4,8} The individual serums within each positive pool were titered for *Leptospira* agglutinating antibodies using the microscopic agglutination test.¹ Both commercial *Leptospira* antigens⁹ and a special *L. pomona* antigen prepared¹ from fur seal *Leptospira* isolates were used. Commercial *L. pomona* antiserum⁹ was used as a positive control and fetal bovine serum was used as a negative control.

RESULTS

The bull from which *L. pomona* was isolated⁷ was lethargic, dehydrated and moved with difficulty, presumably because of a badly traumatized and infec-

ted foreflipper. Microscopic examination revealed a multiple disseminated focal glossitis. Small granulomas with giant cells were seen in the mature collagen of the sclera. The liver showed individualization of hepatocytes, sclerosis of portal areas and occasional foci of chronic inflammatory cells. There was chronic interstitial nephritis with one subcapsular infarct containing epithelioid cells and histiocytes and a chronic mild enteritis involving the small intestine. Special stains (Gomori Methamine Silver, Gram's McCallum—Goodpasture and Acid Fast)² failed to reveal the presence of etiologic agents in the areas of chronic inflammation. Warthin-Starry³ silver stains of kidney sections revealed argyrophilic bent-wire forms presumed to be *Leptospira* located in the proximal convoluted tubules. The three newborn pups all showed multiple hemorrhagic lesions as follows: free blood was present in the peritoneal cavity, and there were subcapsular hemorrhages of the kidney and liver. The livers were friable and had rents, presumably the source of the free blood in the abdominal cavity. The anterior chamber of the eye frequently contained free blood. A consistent finding was subperiosteal hemorrhage either over the surface of the parietal bones or within the cranial cavity, usually associated with subdural hemorrhage and meningeal congestion. Two of these pups died within a few hours of birth, and the third was stillborn with the placenta attached.

Microscopic examination of the placenta revealed focal necrosis with scarring and polymorphonuclear infiltration. Silver positive forms resembling *Leptospira* were seen. In those specimens having hemorrhage in the anterior chamber of the eye, the vessels of the iris were greatly dilated and congested. Congestion was generalized, involving large and small intestine, uterus, thyroid, adrenal and thymus. Diffuse hemorrhage

⁵ Pelagic sampling accomplished through the courtesy of Dr. Clifford Fiscus, National Marine Fisheries Service, Seattle, Washington.

⁶ Difco *Leptospira* Antigen, Difco Laboratories, Detroit, Michigan.

was an inconsistent finding in the liver, lung and kidney. Warthin-Starry stains occasionally revealed leptospiral forms in the liver and kidney.

A summary of the overall prevalence and titers of *Leptospira* agglutinating serum antibodies among Northern fur seals is given in Table 1. The highest

antibody titer (1/640) was seen in the 13-year-old bull shedding leptospirae in his urine. The prevalence of *Leptospira* antibodies in the pelagic females was 10.2% or approximately the same as that seen in the bachelor bulls, whereas the prevalence in pre-pelagic 4-month-old pups was only 2.0%.

TABLE 1. *Leptospira pomona** agglutinating serum antibody titers for northern fur seals sampled in the Bering Sea from 1972-1975.

	Number of animals yielding serum of indicated titer						
	Bachelor bulls: 3-4 years old				Adult females: mixed ages	Pre-pelagic pups 4 months old	
	1972	1973	1974	1975	1974	1974	1975
1:1280	0	0	0	0	0	0	0
1:640	1***	0	0	0	0	0	0
1:320	1	0	0	0	0	0	0
1:160	1	1	0	0	0	0	0
1:80	10	10	5	8	2	0	0
1:40	9	11	9	5	4	0	0
1:20	7	3	6	1	3	1	1
1:10	2** (2)	0	5	0	0	2	2
negative	170	176	195	186	80	147	147
positive	31/201	24/200	25/220	14/200	9/89	3/150	3/150

* All serums were tested for antibodies using commercial pools #1 (containing *L. ballum*, *L. canicola*, and *L. icterohaemorrhagiae*), #2 (containing *L. bataviae*, *L. grippityphosa* and *L. pyrogens*) and #3 (containing *L. autumnalis*, *L. wolffi* and *L. pomona*). Nearly all were negative to pools #1 and #2. Those positive to pool #3 were tested against the individual commercial antigens and the *L. pomona* pinniped isolate antigen.

**() Total of animals positive for *Leptospira autumnalis*.

*** This animal was a 13-year-old idle bull shedding *Leptospira pomona* in the urine.

DISCUSSION

Evidence continues to support previous reports that acute leptospirosis of newborn pinnipeds causes a multiple hemorrhage syndrome.^{6,7} We have examined and cultured a total of 30 newborn pups for evidence of leptospirae. Twenty-one of these exhibited multiple hemorrhages and *L. pomona* was isolated only in those cases where lesions of multiple hemorrhage occurred. Although these

same animals were examined for evidence of other microbial pathogens, none were found.

One perplexing problem has been the inconsistency with which leptospirae can be isolated from newborn fur seals presumably dying of leptospirosis. This may be a simple reflection of inadequate methods, but on the other hand, the *L. pomona* isolated from pinnipeds is known to produce cytotoxins (manuscript

in preparation) and therefore one may speculate that cytotoxins are responsible for loss of vascular integrity resulting in multiple and frequently massive hemorrhage. The presence of toxins within the tissues of an *in utero* or newborn pup may not require that the pup tissues themselves be infected with leptospires. In partial support of this concept, leptospiral induced abortion in cattle is difficult to confirm because of the inconsistency with which leptospires can be isolated from the fetus. One explanation has been that the abortion is caused by toxic materials rather than by bacterial invasion of the fetus itself.³

One surprising aspect of this study has been the low titers and prevalence of *Leptospira* serum antibodies in the pre-pelagic pups. Female fur seals first breed at age five or six. Gestation lasts 12 months and pups are weaned when 4 months old. At that time, they begin their first pelagic cycle and most of them do not return to the hauling grounds until they are 2 years old. It has been presumed that large numbers of pups were infected with *Leptospira* on the rookeries during the 4-month nursing period, thus beginning their first pelagic cycle, infected with leptospires, and that the 3-4 year-old bachelor bulls showing leptospiral antibodies were the survivors of this sequence.

It now appears that the pre-pelagic pups have had less exposure to leptospirosis than the general population. This is based on the low number of

individuals having serum antibodies and on the generally low antibody titers compared to the general population. These observations support our conclusion that although transmission of leptospirosis may certainly occur on the rookeries, the most important sources of infection are to be found in the ocean environments inhabited by fur seals.

This idea is further reinforced if one presumes that the low antibody titers of the pups are passive or maternal antibodies and are not the result of active immunity. The prevalence of *Leptospira* antibodies is approximately the same between 3-4 year-old bachelor bulls and mixed age breeding females, indicating that the initial exposure to *Leptospira* may occur prior to 3 or 4 years of age. Should antibody titers drop rapidly subsequent to infection then we would have no basis for concluding that the major exposure to leptospires occurs prior to age 3 or 4 and instead, the general population prevalence of approximately 7-15% might simply reflect just that portion of the population which had been recently exposed to *L. pomona*. If that were so, the exposure would still be presumed to have occurred at sea.

On the basis of our findings, we now suggest that the fur seal pups, just prior to their first pelagic cycle, make up a population that is generally susceptible to leptospirosis. If an effective *Leptospira* vaccine were administered to this group, it may provide the maximum protective effect for fur seals.

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