AN INVESTIGATION INTO UNUSUAL MORTALITY IN THE HAWAIIAN MONK SEAL, MONACHUS SCHAUINSLANDI

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ABSTRACT

Increased mortality was reported in the endangered Hawaiian monk seal population at Laysan Island in the spring of 1978. An investigation of the possible causes of the mortality included sampling of healthy, sick, and dead individuals. Analyses comprised gross and microscopic pathology, hematology, serum chemistry, virology, bacteriology, parasitology, and toxicology. Gastric ulceration in varying degrees due to nematodes was a consistent finding. Evidence of caliciviruses (VESV and SMSV) and Salmonella was found in the population. Two of 18 seals tested had elevated total white blood cell counts. A few individuals differed significantly from mean serum chemistry values but no trend was apparent. Liver tissues of two seals tested for ciguatoxin and maitotoxin were positive.

Monachus schauinslandi
mortality
clinical pathology
ciguatera

INTRODUCTION

The Hawaiian monk seal, Monachus schauinslandi, is an endangered species which breeds only in the Northwestern Hawaiian Islands from

Necker Island west to Kure Atoll. Recent censuses indicate the total population has decreased by about 50% since 1958 (Johnson et al., in preparation).

In the spring of 1978, high mortality was observed in monk seals at Laysan Island (B.W. Johnson and P.A. Johnson, Aquatic Mammals Behavioral Research Company, Honolulu, Hawaii 96822, personal communication, 1978). Disease signs apparent in the monk seals were consistent. Animals come ashore emaciated or began to noticeably lose weight as they lay on the beach. The seals abandoned normal hauling out behavior--failing to move into the vegetation behind the beach crest at night. Within 2 to 3 weeks of beginning the weight loss, the animals became completely debilitated and then died in the splash zone or at the high tide line (B.W. Johnson and P.A. Johnson, personal communication, 1978).

This report discusses data collected on specimens taken from 19 dead and 18 live monk seals during April and May 1978 as part of an investigation into the reported mortality.

MATERIALS AND METHODS

Between 4 May and 13 May 1978 we collected specimens and data from a total of 24 Hawaiian monk seals at two locations in the Northwestern Hawaiian Islands to determine if there was any apparent disease process in the seals which might cause the mortality. Ten live yearlings or juvenile seals (MS-01-78 to MS-10-78) and one adult (MS-11-78) were sampled at Laysan Island. In addition, we received tissue sets in formalin[1] from 13 monk seals (collected by Brian W. and Patricia A. Johnson on Laysan Island, 1 March to 1 May 1978) which died at Laysan Island prior to our arrival. Samples were to be collected from a large number of sick as well as apparently healthy animals; however, a storm just prior to our arrival cleared the beaches at Laysan Island of most of the very sick animals with the disease signs mentioned earlier. Seal MS-11-78 was very emaciated and weak and died while being restrained for collection of the samples. At French Frigate Shoals six dead seals were found, only one of which was fresh enough to be necropsied, even through it had been dead at least a day and the tissues were badly autolysed. The other five were too decomposed to yield any information relative to cause of death.

The live animals were physically restrained and blood was collected from the intra-vertebral extradural vein. Packed red cell volumes and white blood cell counts were determined in the field. Serum and plasma for the other clinical bood tests and serological studies were frozen for later analysis. Clinical chemistry tests were performed using standard laboratory procedures (Bio-Science Laboratory, Van Nuys, California). Serum samples from all animals were tested for agglutinating

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

antibodies to Leptospira antigen pools nos. 1, 2, and 3.[2] They were also tested for serum neutralizing (SN) antibodies to 19 calicivirus types (vesicular exanthema of swine virus types A_{48}, C_{52}, D_{53}, F_{54}, G_{55}, I_{55}, J_{56}, K_{56}, San Miguel sea lion virus types 1, 2, 4, 5, and marine calicivirus isolates designated 427, 274, fluke, V86, 804T, and 913T) using previously described microtiter techniques (Monta and Bryon, 1974; Smith et al., 1976).

Leptospira isolation attempts were made on samples of liver, kidney, and cerebrospinal fluid taken from MS-11-78. The procedure has been previously described (Smith et al., 1974a; Smith et al., 1974b).

Salmonella incidence in the seals was tested by collection of rectal swabs from all animals and placing them into transport medium in the field. These cultures were tested by one of the authors (Gilmartin) for salmonellae by beginning enrichment and isolation procedures previously described (Gilmartin et al., 1979) within 24 hours of collection of the sample but were also maintained in the holding medium for over 2 weeks, when another attempt was made at isolation of salmonellae (N.A. Vedros, Naval Biosciences Laboratory, Oakland, California 94625, personal communication, 1978).

Swabbings were taken from the nose, throat, and rectum of each animal for virus isolation. These and small slips of lung, liver, kidney, and tonsil from animal no. 11 were placed in ampules of phosphate-buffered glycerine, pH 7.2, and immediately frozen to -55°C. Tissues were thawed and ground up, then they and the swab samples were clarified by centrifugation at 3,000 rpm. Supernatant fluids were placed in a Vero monkey, Cercopithicus aethiops, kidney cells and porcine kidney cells (PK-15), incubated at 37° and 30°C, and passaged at least four times as previously described (Smith et al., 1974b).

Stool specimens were collected, as available, from the seals and frozen for later flotation and examination for ova.

Rectal temperatures were determined using an electronic thermistor with a flexible probe inserted at least 30 cm through the rectum.

Tissues for microscopic histopathologic studies were preserved in formalin and examined after hematoxylin and eosin staining.

One canine tooth was extracted from each of the dead seals for aging using a new technique developed for small cetaceans (Pierce and Kajimura, 1978).

Liver specimens from two seals (MS-11-78 and MS-12-78) were assayed for dioxan (2, 3, 7, 8 - tetrachlorodibenzo-p-dioxan) using gas chromatography and high resolution mass spectrometer techniques (M. Gross, University of Nebraska, Lincoln, Nebraska 68588, personal communication, 1978.)

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[2]Difco Laboratories, Detroit, Michigan. These pools contain Leptospira ballum, L. canicola, L. icterohemorrhagiae, L. bataviae, L. grippotyphosa, L. pyogenes, L. autumnalis, L. pomona, and L. wolffii.

Tests for tissue residues of ciguatoxin by radioimmunoassay procedures (Hokama et al., 1977) and ciguatoxin and maitotoxin by bioassay techniques were performed.[3]

Statistical analyses were performed on the clinical chemistry, hematology, and temperature data to test for individuals with values different than the mean. All the live seals sampled with one exception were young (yearlings to juveniles); therefore the adult (MS-11-78) was excluded from these statistical tests and the mean and standard deviation of the data from these animals were taken as a close approximation to parametric values since normals for the population were not known. The farthest-outlying variates within a given sample (i.e., sodium) were then tested to see if they statistically belonged within that sample using a one-tailed t-test of one variate against the assumed population mean (Sokal and Rohlf, 1969). Also, because of the great distance between the islands (approximately 320 nmi), the data for the young monk seals from Laysan Island (MS-01-78 through MS-10-78) were tested with a two-tailed Wilcoxon two-sample test (Sokal and Rohlf, 1969) against those from French Frigate Shoals (MS-13-78 through MS-19-78) for all categories to determine if there were differences between the island populations.

RESULTS AND DISCUSSION

Animals from two age groupings, the young and very old, were represented in the dead animals which were recovered by the Johnsons and the authors. Ten of 14 seals which died and were recovered at Laysan Island were between 1 and 5 years of age; the others were between 18 and 30 years. The net loss in monk seals at Laysan Island during the period from March to July 1978 is estimated to be at least 50 animals (Johnson and Johnson, 1980).

Of the 13 seals which had died prior to our arrival at Laysan Island and from which we received tissue sets, there were seven males and six females. Four of the six dead seals at French Frigate Shoals were females and sex could not be determined for the other two.

Twelve of the live young monk seals sampled were females, five were males and the single adult at Laysan Island (MS-11-78) was a male. Four of the seals sampled at Laysan Island (MS-01, MS-04, MS-07, and MS-10) became emaciated and disappeared by mid-June 1978. The seals sampled at French Frigate Shoals were not similarly monitored.

Statistical tests were performed on the clinical data to identify animals with test results significantly different ($P \leq 0.05$) from the mean of all monk seals sampled. The tests were done to aid in recognizing ill animals in a species for which these clinical parameters had not been determined.

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[3]Radioimmunoassay for ciguatoxin and bioassay for ciguatoxin and maitotoxin were performed by Dr. Y. Hokama and Dr. J. Miyahara, respectively, at the University of Hawaii, John A. Burns School of Medicine, Pathology Department, Honolulu, Hawaii 96822.

When each individual seal's hematology and clinical chemistry test results were compared to the mean of the group, many had at least one test value different from the mean (Table 1). There were only three cases where two animals differed from the mean ($P \leq 0.05$) in the same direction on the same test: MS-05 and MS-07, elevated total white cell count; MS-06 and MS-18, high cholesterol; and MS-08 and MS-15, high alpha-1 globulin.

Monk seals MS-05 and MS-07, with the high white cell counts, are noteworthy because MS-07 is one of four animals which disappeared and presumably died later in the season. MS-07 had the highest white cell count (18,700) of all seals tested and was one of three young seals sampled which appeared underweight and lethargic. Neither of these two with the elevated total white cell counts had any other outstanding clinical data values. Of the other two monk seals which appeared underweight at sampling, one (MS-06) had only a significantly elevated cholesterol and glucose level, which may indicate a fasting animal, and the other (MS-01) had no clinical blood tests different from the mean of the group.

The three other animals which disappeared in an emaciated condition during the summer, MS-01, MS-04, and MS-10, did not exhibit any remarkable findings except for a high lactic dehydrogenase (LDH) in MS-10.

The only other animals with any noteworthy abnormal clinical pathology were MS-09 with a high total protein and beta globulin and MS-16 with a very low packed red cell volume and a high serum glutamic pyruvic transaminase (SGPT). Salmonella sieburg was isolated from a rectal swab taken from MS-09 and it is the only seal from which salmonellae were recovered (N.A. Vedros, personal communication, 1978). Although Salmonella are common isolates in some pinnipeds (Gilmartin et al., 1979), the high beta globulin and total protein in this animal are probably not related to a chronic infectious bout with this organism as no serum antibody could be detected (N.A. Vedros, personal communication, 1978).

The high SGPT of MS-16 would indicate some liver pathology. The low hematocrit may be due to hemorrhage associated with severe gastric ulceration due to nematode infestation which will be discussed below. Despite the frequency and apparent severity of these parasitic ulcerations observed in dead animals, MS-16 was the only living seal tested which had a low packed cell volume.

The rectal temperature statistics in Table 1 show that all animals tested were within a range of 1.5°C. All of these animals were asleep and dry when initially approached so there had probably been little or no physical activity prior to our restraining them. Thus, these temperatures (with a mean of 36.3°C) reflect resting status, and are very close to that previously reported for young Hawaiian monk seals. Several monk seals were monitored throughout the restraint period, and no change in the temperature reading was noted. Temperatures taken by various means in some other phocids are reported between 36.0°C and 37.0°C.

TABLE 1. SERUM CHEMISTRY, BLOOD COUNTS, AND RECTAL TEMPERATURE STATISTICS FROM 17 YOUNG HAWAIIAN MONK SEALS, 1978

Test	Mean	Standard Deviation	Range	Animals Significantly Different at $P \leq 0.05$	
Sodium (meq/liter)	152.7	8.1	134-167	MS-08 (134)	MS-18 (167)
Potassium (meq/liter)	5.84	0.63	4.6-7.0	MS-03 (7.0)	MS-08 (4.6)
Chloride (meq/liter)	108.7	4.8	96-119	MS-08 (96)	MS-13 (119)
Calcium, total (meq/liter)	5.54	0.37	5.0-6.1		
Inorganic phosphorus (mg/100 ml)	7.49	1.49	5.3-9.6		
Cholesterol (mg/100 ml)	206.7	54.5	121-314	MS-06 (314)	MS-18 (311)
Urea nitrogen (mg/100 ml)	37.1	12.3	21-63	MS-17 (63)	
Uric acid (mg/100 ml)	2.74	0.51	1.7-3.4	MS-02 (1.7)	
Bilirubin, total (mg/100 ml)	0.38	0.28	0.2-1.2		
Alkaline phosphatase (units)	222.0	131.2	74-580		
LDH (units)	758.9	454.7	62-1,640		
SGPT (units)	137.8	57.6	76-290	MS-16 (290)	
SGOT (units)	146.9	45.7	72-220		
Glucose (mg/100 ml)	91.1	24.6	49-141	MS-06 (141)	
Total protein (g/100 ml)	7.32	1.01	4.9-9.5	MS-09 (9.5)	
Albumin (g/100 ml)	2.82	0.36	34		
Alpha-1 globulin (g/100 ml)	0.32	0.33	0.08-1.2	MS-08 (1.2)	MS-15 (0.9)
Alpha-2 globulin (g/100 ml)	1.09	0.53	0.4-1.96		
Beta globulin (g/100 ml)	0.80	0.25	0.4-1.3	MS-09 (1.3)	
Gamma globulin (g/100 ml)	2.30	0.57	1.3-3.4	MS-06 (3.4)	MS-08 (1.3)
Albumin/globulin ratio	0.64	0.09	0.5-0.8		
Packed red cell volume (5)	57.1	4.9	46.0-62.5	MS-16 (46.0)	
White cell count, total (cells/mm^3)	9,745	3,178	5,170-18.700	MS-05 (15,400)	MS-07 (18,700)
Rectal temperature (°C)	36.3	0.54	35.5-37.0		

Rectal swab cultures from more than half of the animals yielded Edwardsiella tarda which is of dubious significance as an intestinal tract pathogen.

Neither viruses nor leptospires were isolated from any sample; however, animal MS-05 did carry SN antibodies against VESV I_{55} at the 1:40 dilution and animals MS-13 and MS-19 carried SN titers of 1:10 against SMSV-1. All other tests for virus and Leptospira antibodies were negative; however, the finding of calicivirus antibodies (VESV and SMSV) in 3 of 18 animals certainly suggests occasional contact with these agents and may be some indication that virus reservoirs exist along the Northwestern Hawaiian Islands chain. Alternatively, northern elephant seals, Mirounga augustirostris, have been reported as far west as Midway Islands (M.J. Rauzon, National Fish and Wildlife Laboratory, Anchorage, Alaska 99503, personal communication, 1978), the western limit of the monk seal range, and caliciviruses have been isolated repeatedly from nursing and weaned elephant seals along the southern California coast (A.W. Smith, Naval Biosciences Laboratory, Oakland, California 94625, personal communication, 1978, 1979). Although there is no evidence to suggest that the recent die-off was in any way related to the presence of caliciviruses, it should be remembered that these agents have been associated with a vesicular disease and reproductive failure in California sea lions, Zalophus californianus, northern fur seals, Callorhinus ursinus, and domestic swine and cats.

Parasite ova found in the stool of the 10 young live seals at Laysan Island are described in Table 2. The following flatworm ova were recovered from the gastrointestinal tract of the adult (MS-11-78) which died at Laysan Island: Corynosoma rauschi, Contracaecum turgidum, Diphyllobothrium cameroni, D. elegans, and D. hians. Contracaecum turgidum, Corynosoma rauschi and D. hians were found in the stomach and intestines of MS-12-78 at French Frigate Shoals. These same parasite species were represented in many of the 13 animals which died at Laysan Island between 1 March and 1 May 1978.

All of the animals from which the tissue sets were collected, including the two examined by the authors, were cachetic and severely emaciated. Common findings in these 15 animals included: heart, lack of adipose tissue on the epicardium surface; liver, centralobular congestion, with foci of centralobular necrosis; lungs, congestion and alveolar hemorrhage in about half of the seals; spleen and lymph nodes, little or no evidence of lymphopoietic activity; testes, no evidence of spermatogenesis in males estimated to be subadult to adult; and, gastrointestinal tract, numerous foci of ulceration (many were actively hemorrhaging) with nematodes embedded deep into the stomach wall in all animals and many had additional intestinal lesions from cestodes, similarly embedded in the mucosa.

It is important to note that in December 1978, two additional monk seals were found dead at Laysan Island (B.W. Johnson and P.A. Johnson, personal communication, 1978) in an emaciated condition resembling that seen in April and May; however, these seals, on examination, had very light gastric nematode infestations and only minor ulceration at the phylorus.

TABLE 2. PARASITE OVA IN STOOL OF YOUNG LAYSAN MONK SEALS, 1978

Monk Seal	Cestode Ova*	Capillorid Type Ova*
MS-01	M	--
MS-02	H	L
MS-03	H	--
MS-04	M	L
MS-05	N.D.	N.D.
MS-06	N.D.	N.D.
MS-07	N.D.	N.D.
MS-08	H	--
MS-09	H	--
MS-10	H	--

*Number of ova in 400 power microscope field: L (light) = <25, M (moderate) = 25 to 75, H (heavy) = >75, N.D. = not determined

The extensive pathology caused by parasites, even though common to all of the monk seals which were necropsied during the period of high mortality in the spring, may be the result of seasonal fluctuations in gastric nematode parasite load and not, necessarily, a major factor in the spring 1978 mortality. Gastric nematode infestations, many with associated ulcerations, are relatively common in pinnipeds and since gastrointestinal tracts of only emaciated animals were examined, it is not possible to know the associated parasite pathology in the "normal" population. Table 2, however, indicates many of the apparently normal seals were carrying heavy cestode loads.

No dioxan was detected in the liver samples tested. Ciguatoxin and maitotoxin bioassay analyses of liver tissues from the adult which died at Laysan Island (MS-11-78) and the juvenile at French Frigate Shoals (MS-12-78) were positive. Estimated levels were 30 to 50 times that found in the liver of a control monk seal which had been maintained in captivity for 15 years. Radioimmunoassay for ciguatoxin in the same tissues revealed the liver of MS-11-78 to be about 25% above the control liver, while MS-12-78 was 9% below the control. Subsequent studies, the results of which will be published elsewhere, have shown that eels (known to be a part of the monk seal diet), collected near the islands on which the monk seals haul out, can debilitate and kill northern elephant seals after consumption of as little as 1.7% of the animal's body weight (DeLong and Gilmartin, in preparation).

The parasite associated pathology and the presence of ciguatoxin in the animals were the major findings which might account for this die-off of monk seals. Lack of any pathology in any organ systems (other than gastrointestinal) may discount any infectious disease processes of viral or bacterial origin.

Further study is needed to assess the impact of heavy gastrointestinal parasitism on pinnipeds relative to their general health and ability to feed and otherwise function normally. The signs displayed by the dying monk seals observed at Laysan Island are not inconsistent with what might be expected if the parasites were responsible, but they also could have been caused by the ciguatera syndrome. Ciguatera, which will kill a phocid seal, is known to be present in tropical reef environments and is present in the island chain in at least one of the monk seals' food fish. Continued disease monitoring of the seal population and experimental work in parasitology and ciguatera toxicology will be necessary to resolve the impact of these on the Hawaiian monk seal.

REFERENCES

DeLong, R.L., and W.G. Gilmartin. Ciguatoxin feeding experiments with a model phocid seal. National Marine Mammal Laboratory, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, Seattle, Washington 98115. In preparation.

Gilmartin, W.G., P.M. Vainik, and V.M. Neill. 1979. Salmonellae in feral pinnipeds off the southern California coast. Journal of Wildlife Diseases 15(4):511-514.

Hokama, Y., A.H. Banner, and D.B. Boylan. 1977. A radioimmunoassay for the detection of ciguatoxin. Toxicon 15:317-325.

Johnson, A.M., R.L. DeLong, C.H. Fiscus, and K.W. Kenyon. Status of the Hawaiian monk seal population, 1978. National Marine Fish and Wildlife Laboratory, Anchorage, Alaska 99503. In preparation.

Johnson, B.W., and P.A. Johnson. 1980. The Hawaiian monk seal on Laysan Island, 1978. Draft report to the Marine Mammal Commission, Washington, D.C. 20006, 39 pp.

Monta, A.S., and E.R. Bryon. 1974. Micro-neutralization test for detection of rhinovirus antibodies. Proceedings of the Society for Experimental Biology and Medicine 145:690-694.

Pierce, K.V., and H. Kajimura. 1978. Acid etching and highlighting for defining growth layers in cetacean teeth. Proceedings of the International Conference on Determining Age of Odontocete Cetacean. La Jolla, California, 5-7 September 1978.

Smith, A.W., R.J. Brown, D.E. Skilling, and R.L. DeLong. 1974a. Leptospira pomona and reproductive failure in California sea lions. American Journal of Veterinary Medical Association 165:996-998.

Smith, A.W., C.M. Prato, W.G. Gilmartin, R.J. Brown, and M.C. Keyes. 1974b. A preliminary report on potentially pathogenic microbiological agents recently isolated from pinnipeds. Journal of Wildlife Diseases 10(1):54-59.

Smith, A.W., T.G. Akers, C.M. Prato, and H. Bray. 1976. Prevalence and distribution of four serotypes of SMSV serum neutralizing antibodies in wild animal populations. Journal of Wildlife Diseases 12:326-334.

Sokal, R.R., and F. J. Rohlf. 1969. Biometry: The Principles and Practice of Statistics in Biological Research. San Francisco: W.H. Freeman and Company, 776 pp.