INFECTIOUS DISEASE MONITORING OF THE ENDANGERED HAWAIIAN MONK SEAL

Authors: Aguirre, A. Alonso, Keefe, Thomas J., Reif, John S., Kashinsky, Lizabeth, Yochem, Pamela K., et al.

Source: Journal of Wildlife Diseases, 43(2) : 229-241

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.2.229

Journal of Wildlife Diseases, 43(2), 2007, pp. 229–241

INFECTIOUS DISEASE MONITORING OF THE ENDANGERED HAWAIIAN MONK SEAL

A. Alonso Aguirre,[1,9] Thomas J. Keefe,[2] John S. Reif,[2] Lizabeth Kashinsky,[3] Pamela K. Yochem,[4] Jeremiah T. Saliki,[5] Jeffrey L. Stott,[6] Tracey Goldstein,[6] J. P. Dubey,[7] Robert Braun,[8] and George Antonelis[8]

[1] Wildlife Trust, 460 West 34th Street, 17th Floor, New York, New York 10001, USA
[2] Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado 80523, USA
[3] Joint Institute for Marine and Atmospheric Research, University of Hawaii, 2570 Dole Street, Honolulu, Hawaii 96822-2396, USA
[4] Hubbs-Sea World Research Institute, 2595 Ingraham Street, San Diego, California 92109, USA
[5] Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Farm Road and Ridge, Stillwater, Oklahoma 74078, USA
[6] Department of Pathology, Microbiology, and Immunology, Laboratory for Marine Mammal Immunology, School of Veterinary Medicine, University of California, Davis, California 95616, USA
[7] USDA-ARS-ANRI-PBESL, Building 1001 BARC-EAST, 10300 Baltimore Avenue, Beltsville, Maryland 20705, USA
[8] Pacific Islands Fisheries Science Center, National Marine Fisheries Service, NOAA, 2570 Dole Street, Honolulu, Hawaii 96822, USA
[9] Corresponding author (email: aguirre@wildlifetrust.org)

ABSTRACT: As part of conservation efforts between 1997 and 2001, more than 25% (332 animals) of the endangered Hawaiian monk seal (*Monachus schauinslandi*) population was sampled in the northwestern Hawaiian Islands. Serum samples were tested for antibodies to viruses, bacteria, and parasites known to cause morbidity and mortality in other marine mammal species. Antibodies were found to phocine herpesvirus-1 by using an enzyme-linked immunosorbent assay, but seropositive results were not confirmed by virus neutralization test. Antibodies to *Leptospira bratislava*, *L. hardjo*, *L. icterohaemorrhagiae*, and *L. pomona* were detected in seals from several sites with the microagglutination test. Antibodies to *Brucella* spp. were detected using 10 conventional serologic tests, but because of inconsistencies in test results and laboratories used, and the lack of validation by culture, the *Brucella* serology should be interpreted with caution. Antibodies to *B. canis* were not detected by card test. *Chlamydophila abortus* antibodies were detected by complement fixation (CF) test, and prevalence increased significantly as a function of age; the low sensitivity and specificity associated with the CF make interpretation of results difficult. Antibodies to *Toxoplasma gondii* and *Dirofilaria immitis* were rarely found. There was no serologic evidence of exposure to four morbilliviruses, influenza A virus, canine adenovirus, caliciviruses, or other selected viruses. Continuous surveillance provides a means to detect the introduction or emergence of these or other infectious diseases, but it is dependent on the development or improvement of diagnostic tools. Continued and improved surveillance are both needed as part of future conservation efforts of Hawaiian monk seals.

Key words: *Brucella*, *Chlamydophila*, Hawaiian monk seal, *Leptospira bratislava*, *Monachus schauinslandi*, phocine herpesvirus, serology, *Toxoplasma gondii*.

INTRODUCTION

The Hawaiian monk seal (*Monachus schauinslandi*) is one of the most endangered marine mammals in the world. Populations of Hawaiian monk seals have declined in recent years and are under the threat of extinction (Ragen and Lavigne, 1999). Although infectious diseases and biotoxins have significantly affected other marine mammal populations, their potential impacts on Hawaiian monk seals are unknown. Ciguatoxin and mitotoxin have been suspected as causes of mortality in Hawaiian monk seals (Gilmartin et al., 1980), and sources of natural mortality have been described, including 1) mobbing (Hiruki et al., 1993); 2) starvation, primarily affecting juveniles (Banish and Gilmartin, 1992); 3) predation by sharks, particularly tiger sharks (*Galeocerdo cuvier*) and Galapagos sharks (*Carcharhynchus galapagoensis*) (Balazs and Whittow, 1979; Alcorn and Kam, 1986); 4) net

entanglement (Henderson, 2001); and 5) disease and trauma (Banish and Gilmartin, 1992). The importance of endoparasites as a cause of mortality is unknown, although practically all monk seals are infected (Dailey et al., 1988).

Because management efforts to enhance the recovery of the endangered monk seal have relied on captive care, translocation, or both, current and accurate information related to infectious diseases in this population is needed (Aguirre et al., 1999; Ragen and Lavigne, 1999; Aguirre, 2000). This study represents the first systematic effort to survey the Hawaiian monk seal population for evidence of infectious agents that have proven pathogenic to other marine mammals. We describe antibody prevalence and age-, sex- and site-specific risk factors associated with these prevalence estimates for Hawaiian monk seals representing all six breeding sites in the northwestern Hawaiian Islands (NWHI).

MATERIALS AND METHODS

Study sites and animals

Monk seals were sampled at six breeding sites in the NWHI (Fig. 1). During 1997–99, seals were sampled at three sites as part of a preliminary study to evaluate the health and disease status of the species. Sites included French Frigate Shoals (FFS; 23°45′N, 166°10′W), which has the largest breeding population but had a relatively low juvenile survival rate from 1985 to 2000; Midway Atoll (MID; 28°15′N, 177°23′W), which has the smallest population because of its severely depleted status but is showing signs of recovery; and Pearl and Hermes Reef (P&H; 27°50′N, 175°50′W). From 2000 to 2001, opportunistic sampling continued at these sites as well as Kure Atoll (KUR; 28°25′N, 178°10′W) where the population has increased at a rate of approximately 6% and 5%, respectively, from 1983 to 2000; and Laysan (LAY; 25°42′N, 171°44′W) and Lisianski (LIS; 26°02′N, 174°00′W) Islands, where seals remained relatively stable.

All animals were captured and handled in accordance with the National Marine Fisheries Service guidelines for the capture and handling of Hawaiian monk seals to minimize potential adverse impacts on the animals. Careful handling techniques and conservative selection procedures have no deleterious effects on monk seals (Baker and Johanos, 2002).

Specimen collection

Seals were captured while hauled out on the beach. Mature seals (subadults and adults, >4 yr) were captured with a hoop net. Immature seals (juveniles, >1 to 3 yr) were captured with a stretcher. Weaned pups (<1 yr) were captured by hand. Diazepam (Steris Laboratories Inc., Phoenix, Arizona, USA) was given intravenously, and after induction, cardiac rate, respiratory rate, and rectal temperature (digital thermometer, Fisher Scientific, Pittsburgh, Pennsylvania, USA) were recorded. Physical examinations of seals were performed by a veterinarian and were limited to visual identification of abnormalities, determination of body condition and hydration, sex, size class, and evidence of trauma. Seals were flipper-tagged and passive integrated transponder-tagged, scars/marks were recorded for identification purposes, and axillary girth and standard length were recorded. Age, sex, and size classes were determined by reviewing previous identification cards on file or by visual examination at the time of capture. After all biomedical and morphometric procedures were completed, seals were released and monitored post-release for 10 to 60 min or until normal behavior was observed. Beaches were surveyed days or weeks later to monitor post-handling condition.

Blood (35–60 ml) was collected from the bilaterally divided extradural veins by inserting an 18-gauge 3.5-in spinal needle between the dorsal spinous processes of the third, forth, or fifth lumbar vertebrae (Geraci and Lounsbury, 1993). Blood specimens were immediately transferred into SST (25-ml) Vacutainer® tubes (BD Biosciences, Rutherford, New Jersey, USA). Tubes were kept in the shade at ambient temperature for 30–60 min to allow normal coagulation process and then transferred into a cooler with plastic freezer packs. Serum specimens were separated by centrifugation at 2,000 rpm for 10 min, pipetted into 1-ml aliquots in cryogenic vials (Nalgene, Rochester, New York, USA), placed in liquid nitrogen (−176 C) in the field, and transferred to a −86 C ultracold freezer upon arrival in Honolulu before serologic analysis.

Laboratory methods

Aliquots containing 1–2 ml of frozen serum were sent to various veterinary diagnostic laboratories for antibody testing as follows.

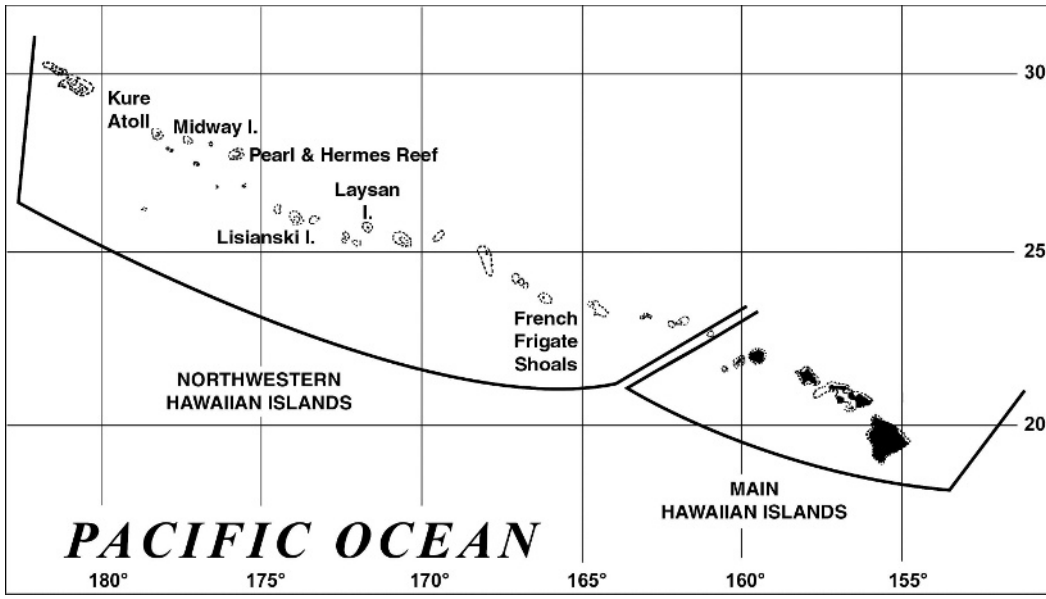

FIGURE 1. Primary breeding sites of the Hawaiian monk seal (*Monachus schauinslandi*) population in the northwestern Hawaiian Islands, including French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes reef, Midway Atoll, and Kure Atoll.

Morbilliviruses: Serum specimens were tested at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL, Stillwater, Oklahoma, USA) for the presence of antibodies to four morbilliviruses, including canine distemper virus, phocine distemper virus, dolphin morbillivirus, and porpoise morbillivirus by using the microplate virus neutralization test (VNT) as described previously (Saliki and Lehenbauer, 2001). A subset of serum samples also was tested at the USDA Foreign Animal Disease Diagnostic Laboratory (Plum Island, New York, USA). The protocol used by both laboratories for morbillivirus serologic testing was identical except for minor adjustments reported previously (O'Hara et al., 1998). Threshold titers of ≥8 were considered positive.

Phocine herpesvirus-1: An indirect enzyme-linked immunosorbent assay (ELISA) was used at the Laboratory for Marine Mammal Immunology (Davis, California, USA) to measure group-specific antibodies to herpesvirus, by using phocine herpesvirus-1 (PHV-1) as antigen (King et al., 2001). At OADDL, the microtiter VNT also was used to test for evidence of exposure to phocine herpesvirus-1 by using the same technique as described for morbilliviruses.

Influenza A virus: Sera were tested for antibodies to influenza A virus at the USDA National Veterinary Services Laboratory (NVSL, Ames, Iowa, USA) by using the agar gel immunodiffusion test (Office International Des Epizooties, 2000).

Canine adenovirus: The VNT was used at the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington, USA) to determine antibody titers against canine adenovirus-1 (CAV-1). Threshold titers were considered positive at ≥8 (Appel and Robson, 1973).

Caliciviruses and other selected viruses: The Laboratory for Calicivirus Studies (LCS, Corvallis, Oregon, USA) used the VNT to test for evidence of group-specific antibody against San Miguel sea lion virus (SMSV) serotypes 1 to 17, walrus calicivirus 7420, feline calicivirus F-9, W-6 calicivirus, vesicular exanthema of swine virus (VESV) A48, primate calicivirus, mink calicivirus strain MV 20-3, cetacean calicivirus strain 041, bovine calicivirus BCV Bos-1, mystery pig disease calicivirus strain P42BN, canine calicivirus strain 731, *Oryctolagus* calicivirus, Hawaiian (temporary designation) calicivirus, McAll human calicivirus, cheetah calicivirus, and reptile calicivirus strain 002. The VNT also was used to test for antibodies to walrus adenovirus-1, human herpesvirus-2, fur seal herpes virus, walrus retrovirus, walrus enterovirus, and pinniped

TABLE 1. Distribution of Hawaiian monk seals (*Monachus schauinslandi*) by breeding site, sex, and age group in the northwestern Hawaiian Islands, 1997–2001.

Age class	Location[a]						Total
	FFS	KUR	LAY	LIS	MID	P&H	
Weanling							
Male	24	1	5	4	4	0	38
Female	13	4	5	4	4	0	30
Unknown	1	0	0	0	2	0	3
Immature							
Male	17	7	6	8	6	10	54
Female	24	4	6	2	7	5	48
Unknown	0	1	11	1	2	7	22
Adult							
Male	29	4	15	5	6	14	73
Female	19	4	6	5	11	14	59
Unknown	4	0	0	0	0	1	5
Total	131	25	54	29	42	51	332

[a] FFS = French Frigate Shoals, KUR = Kure Atoll, LAY = Laysan Island, LIS = Lisianski Island, MID = Midway Atoll, P&H = Pearl & Hermes Reef.

rotavirus. Threshold titers were considered positive at ≥8 (Smith et al., 1977).

A subset of serum samples also were tested using VNT at the FAADL for evidence of exposure to the following viruses: VESV A48, B51, C52, D53, E54, F55, G55, H54, I55, J56, K54, and 1934B; and SMSV serotypes 1 to 2 and 4 to 13. All viruses were from the FAADL repository except for SMS viruses that were provided by A. Smith (LCS).

Brucella spp.: Serum specimens collected before August 1999 were screened for *Brucella canis* and *B. abortus* antibodies by using the standard card agglutination test (SCA) at OADDL. Positive samples to *B. abortus* then were referred to the Federal Brucellosis Laboratory (Oklahoma City, Oklahoma, USA). These samples were retested using SCA, particle concentration fluorescence immunoassay (PCFIA), *Brucella* buffered antigen standard plate agglutination test (BAPA), complement fixation (CF) test, standard plate test (SPT), and Rivanol test, as described previously (MacMillan, 1992). Positive threshold titers for each test are described in Table 3.

Serum specimens collected after August 1999 were tested at NVSL for the presence of antibodies to *B. abortus* by using the rapid automated presumptive test, standard tube agglutination test, fluorescence polarization assay (FPA), Rivanol test, BAPA, PCFIA, CF, and SPT (Office International Des Epizooties, 2000). There are no official interpretation guidelines for classifying specimens in marine mammals by using serologic tests developed for cattle.

Leptospira spp.: Testing of samples collected before August 1999 was performed at the LCS by using the microscopic agglutination test (MAT) for group antibodies against *L. pomona* antigen at a serum dilution of 1:10. Each sample was diluted with 0.85% NaCl and titrated to an end point (dilution showing 50% agglutination) by using a series of doubling dilutions at 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200. *Leptospira pomona* antigen was used to demonstrate evidence of exposure to serovars *pomona* (Pomona), *icterohaemorrhagiae*, *grippotyphosa* (Moskva V), *autumnalis*, *ballum*, and *serjoe*. Specimens collected after August 1999 were tested at the California Veterinary Diagnostic Laboratory Service (Davis, California, USA) with MAT by using the *L. pomona* antigen as reported previously (Gulland et al., 1996). Threshold titers were considered positive at ≥100 for all serovars.

Chlamydophila abortus: Serum samples were tested for antibody to *C. abortus* (formerly *Chlamydia psittaci*) by micro- and macrocomplement fixation (CF) at NVSL. Sera demonstrating a CF titer of IgG antibodies ≥20 were considered as evidence of prior natural exposure (Office International Des Epizooties, 2000).

Toxoplasma gondii: Specimens were tested at the USDA Parasite Biology, Epidemiology and Systematics Laboratory (Beltsville, Maryland,

TABLE 2. Threshold titers and antibody prevalences of selected infectious agents in Hawaiian monk seals (*Monachus schauinslandi*) in the northwestern Hawaiian Islands, 1997–2001.

Infectious agent	Test[a] (threshold titer)	Location[b]					
		FFS	KUR	LAY	LIS	MID	P&H
Canine distemper virus	VNT (>8)	0/131	0/25	0/54	0/29	0/42	0/51
Phocine distemper virus	VNT (>8)	0/131	0/25	0/54	0/29	0/42	0/51
Dolphin morbillivirus	VNT (>8)	0/131	0/25	0/54	0/29	0/42	0/51
Porpoise morbillivirus	VNT (>8)	0/131	0/25	0/54	0/29	0/42	0/51
Phocine herpesvirus-1	VNT (>8)	0/108	NT	NT	NT	0/10	0/43
Phocine herpesvirus-1	ELISA (>1 U/ml)	12/68 (18%)	1/2 (50%)	0/27	0/23	0/19	0/3
Fur seal herpes virus	VNT (>8)	2/59 (3%)	NT	NT	NT	0/10	0/33
Human herpesvirus-2	VNT (>8)	3/59 (5%)	NT	NT	NT	0/10	0/51
Seal influenza	AGID (qualitative)	0/48	NT	NT	NT	0/9	0/36
Canine adenovirus	VNT (>8)	0/39	NT	NT	NT	0/20	0/3
Walrus adenovirus-1	VNT (>8)	0/53	NT	NT	NT	0/10	4/48 (8%)
Caliciviruses (32 serotypes)	VNT (>8)	0/59	NT	NT	NT	0/10	0/48
Vesicular exanthema of swine (12 serotypes)	VNT (>8)	0/48	NT	NT	NT	0/9	0/36
San Miguel sea lion virus (17 serotypes)	VNT (>8)	0/48	NT	NT	NT	0/9	0/36
Walrus retrovirus	VNT (>8)	0/53	NT	NT	NT	0/10	0/48
Walrus enterovirus	VNT (>8)	0/53	NT	NT	NT	0/10	0/48
Pinniped rotavirus	VNT (>8)	0/53	NT	NT	NT	0/10	0/48
Brucella canis	SCA (qualitative)	0/111	NT	NT	NT	0/29	0/51
Leptospira spp.	MAT (100)	0/53	NT	NT	NT	0/9	0/48
Leptospira canicola	MAT (100)	0/65	0/25	0/54	0/29	0/18	0/8
Leptospira bratislava	MAT (100)	2/65 (3%)	3/25 (13%)	10/54 (10%)	0/29	0/18	0/8
Leptospira grippotyphosa (Moskva V)	MAT (100)	0/65	0/25	0/54	0/29	0/18	0/8
Leptospira hardjo	MAT (100)	3/65 (5%)	0/25	0/54	0/29	0/18	0/8
Leptospira icterohaemorrhagiae	MAT (100)	0/65	1/25 (4%)	0/54	0/29	0/18	0/8
Leptospira pomona	MAT (100)	0/65	0/25	0/54	3/29 (10%)	1/18 (6%)	0/8
Chlamydophila abortus	CF (20)	36/94 (38%)	0/2 (0%)	18/26 (70%)	11/29 (38%)	16/27 (60%)	12/32 (37%)
Toxoplasma gondii	MAT (>125)	1/57 (2%)	NT	NT	NT	1/10 (10%)	0/50
Dirofilaria immitis	ELISA (>0.200)	2/51 (4%)	NT	NT	NT	0/10	0/50

[a] VNT = virus neutralization test, ELISA = enzyme-linked immunosorbent assay, AGID = agar gel immunodiffusion test, SCA = standard card agglutination test, MAT = microscopic agglutination test, NT = not tested.

[b] FFS = French Frigate Shoals, KUR = Kure Atoll, LAY = Laysan Island, LIS = Lisianski Island, MID = Midway Atoll, P&H = Pearl & Hermes Reef.

USA) by using the modified MAT as described previously (Dubey and Desmonts, 1987). Serum specimens were originally tested at 1:25, 1:50, and 1:500 dilutions by using mercaptoethanol incorporated in formalin-fixed whole tachyzoites. Sera with antibodies were titrated in twofold dilutions. No information on specificity and sensitivity of this

TABLE 3. Threshold titers and antibody prevalences to *Brucella abortus* in Hawaiian monk seals (*Monachus schauinslandi*) by using different serologic tests and laboratories in the northwestern Hawaiian Islands, 1997–2001.

			Location[c]					
Test[a]	Lab[b]	Threshold titer	FFS	KUR	LAY	LIS	MID	P&H
SCA	OADDL	Qualitative	6/109 (5%)	NT	NT	NT	3/10 (30%)	9/51 (18%)
SCA	FADDL	Qualitative	3/46 (6.5%)	NT	NT	NT	3/9 (33%)	5/36 (14%)
PCFIA	FBL	>0.68	5/109 (5%)	NT	NT	NT	3/10 (30%)	0/51
PCFIA	NVSL	>0.68	4/109 (4%)	0/25	3/54 (5%)	1/29 (3%)	8/42 (19%)	1/48 (2%)
BAPA	NVSL	Qualitative	3/109 (3%)	0/25	5/54 (9%)	2/29 (7%)	12/42 (28%)	5/51 (10%)
CF	FBL	<1:10	4/109 (4%)	NT	NT	NT	3/10 (30%)	0/51
CF	NVSL	<1:10	0/109	0/25	0/27	0/29	0/18	NT
Rivanol	FBL	<1:25	3/109 (3%)	NT	NT	NT	2/10 (20%)	NT
Rivanol	NVSL	<1:25	0/60	0/25	0/27	0/29	0/42	NT
SPT	FBL	<1:25	2/109 (2%)	NT	NT	NT	3/10 (30%)	9/51 (18%)
SPT	NVSL	<1:25	2/109 (2%)	0/25	5/54 (9%)	3/29 (10%)	9/42 (21%)	3/48 (6%)
RAP	NVSL	>5%	2/109 (2%)	0/25	8/54 (15%)	0/29	9/18 (50%)	5/51 (10%)
STT	NVSL	<1:25	1/109 (1%)	0/25	4/54 (7%)	0/29	5/42 (12%)	4/48 (8%)
FPA	NVSL	≥86	4/53 (7%)	0/25	3/29 (10%)	0/29	7/42 (17%)	14/48 (29%)

[a] SCA = standard card test, PCFIA = particle concentration fluorescence immunoassay, BAPA = buffered antigens standard agglutination test, CF = complement fixation test, SPT = standard plate test, RAP = rapid automated presumptive test, STT = standard tube agglutination test, FPA = fluorescence polarization test.

[b] OAADL = Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma, USA; FAADL = USDA Foreign Animal Disease Diagnostic Laboratory, Plum Island, New York, USA; FBL = Federal Brucellosis Laboratory, Stillwater, Oklahoma, USA; NVSL = National Veterinary Services Laboratory, Ames, Iowa, USA.

[c] FFS = French Frigate Shoals, KUR = Kure Atoll, LAY = Laysan Island, LIS = Lisianski Island, MID = Midway Atoll, P&H = Pearl & Hermes Reef, NT = not tested.

test for the diagnosis of *Toxoplasma* infection in monk seals is available. Based on previous validation studies in pigs (Dubey, 1997) and a previous survey in harbor seals (Lambourn et al., 2001), an MAT titer of ≥25 was considered indicative of previous exposure.

Dirofilaria immitis: Serum specimens were tested at IDDEX Laboratories (Davis, California, USA) for the presence of antibodies to the canine heartworm, *Dirofilaria immitis*, by using an ELISA *D. immitis* antigen test (DiroCHECK, Synbiotics Corporation, San Diego, California, USA). Threshold titers were considered positive at optic readings of ≥0.200.

Statistical analysis

The sample size for FFS, MID, and P&H samplings before August 1999 was determined by 1) assuming that the detection of a disease process is a binomial process yielding positive or negative results; 2) setting desired power of the laboratory analyses at 80%; and 3) assuming that an endemic disease found in a subpopulation would be present in at least 20% of the seals (Thrusfield, 1995; Aguirre et al., 1999). Several seals were captured multiple times, and for the purpose of this study, data were reduced to one record per seal if seals were recaptured within 2 wk of the first handling. Monk seals were stratified by gender and grouped into three age groups.

Antibody prevalence estimates based on sufficient numbers of positive samples (at least 20), and potential effects of study site, age, and sex were evaluated by means of stepwise multiple logistic regression (MLR) analysis. Age was included both as a categorical and as a continuous variable in separate MLR analyses. Statistical differences among means were detected by multiple comparison procedures including a multifactorial analysis of variance (ANOVA) with unequal sample sizes by using the regression approach. The ANOVA model was used to compare serologic results and interactions of sex, size, and season. Antibody prevalence estimates were determined by study site (FFS, KUR, LAY, LIS, MID, and P&H), age group (weanling, immature, and adult), and sex (female, male, and unknown). Statistical differences among the means with insufficient numbers of positive samples (<20) were detected by multiple comparison procedures including a multifactorial ANOVA with unequal sample sizes by using the regression approach. The ANOVA model was used to compare serologic results and interactions of sex, size, and season. Results were considered statistically significant at $P \geq 0.05$. Analyses were performed using SAS 6.12 software (SAS Institute, 1988).

RESULTS

Seals

Between 1997 and 2001, 332 seals were captured and sampled (Table 1). At FFS, specimens from all three age groups were collected from 9 to 21 March 1997, from 30 June to 23 August 1997, from 25 May to 29 June 1998, from 7 July to 2 September 1998, and from 13 January to 4 February 1999. Recently weaned seals were sampled from 28 May to 26 September 1999 and from 21 January to 11 February 2000.

Pearl and Hermes Reef seals were sampled from 25 October to 2 November 1997 and from 16 to 22 February 1998. Seals at MID were sampled from 25 to 29 June 1998, from 29 August to 8 September 1999, and from 31 December 2000 to 15 January 2001. The LAY samples were collected from 3 to 20 March 2000, from 18 March to 27 April 2001, from 5 to 17 October 2001, and from 30 October to 12 November 2001. Samples were collected at LIS from 13 to 22 October 2000 and on 17 April 2001. Seals at KUR were sampled 18 May 2001 and from 30 October to 12 November 2001.

Serologic testing

Viruses: There was no serologic evidence of exposure to any of the four morbilliviruses in any of the seal samples. Eight seals (seven adult females and a juvenile male) from FFS had high titers (250–500) to PHV-1 when using the group-specific herpesvirus ELISA test. Also, a young seal of unknown sex captured at KUR had a high titer (6,400). However, all of these samples tested negative when using the VNT for PHV-1. Two specimens were positive to fur seal herpesvirus 206 and human herpes II at FFS. Four serum

samples collected from P&H were positive to walrus adenovirus. Also, one sample from P&H was positive to a walrus retroviruslike agent. All specimens tested negative for A virus, CAV 1, fur seal adenovirus, caliciviruses (32 serotypes), VESV (12 serotypes), California sea lion rotavirus, and walrus enterovirus 7 and 19 (Table 2).

Brucella spp.: Antibody prevalences for *Brucella abortus* ranged from 5% to 33% at three sites (FFS, MID, and P&H) by using SCA as the standard test by two laboratories (Table 3). Significant differences in prevalence were identified when the three sites where compared using SCA. Tests results varied by test and location, and prevalence increased with age (Table 3). All samples collected at KUR were negative to all tests. All specimens were negative to *B. canis*.

Leptospira spp.: Specimens tested at the LCS were negative for antibodies to all tests.

Leptospira serovars: Positive titers (100–200) were detected at University of California-Davis Veterinary Diagnostic Laboratory to *L. bratislava* (11 seals at FFS, KUR, and LAY), *L. hardjo* (three seals at FFS), *L. icterohaemorrhagiae* (one seal at KUR), and *L. pomona* (four seals at LIS and MID) in sera collected from seals after August 1999 (Table 2).

Chlamydophila abortus: Prevalence of antibodies to *C. abortus* varied significantly among age groups ($P<0.001$), almost entirely due to the much higher prevalence in adults (84%) compared with weanlings and juveniles (47% and 51%, respectively). Prevalence of *Chlamydophila* also varied significantly among the six sites ($P<0.001$). Most of the variation can be attributed to the much higher prevalence at P&H and MID (84% and 74%, respectively) than at the remaining four study sites: FFS, 58%; LAY, 58%; KUR, 50%; and LIS, 40%.

Toxoplasma gondii and D. immitis: Antibodies (titers of 25 and 500) to *T. gondii* were identified in two adult females at FFS and MID, respectively, by using MAT. In addition, two of 51 seals from FFS tested seropositive with the ELISA *D. immitis* antigen test (Table 2).

DISCUSSION

Antibodies to potential pathogens in Hawaiian monk seals were detected against phocine herpesvirus-1, fur seal herpesvirus, human herpesvirus, walrus adenovirus-1, *Brucella* spp., *L. bratislava*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. pomona*, *C. abortus*, *Toxoplasma gondii*, and *D. immitis*.

Although antibodies were detected against three herpesviruses, results are difficult to interpret. The failure to confirm seropositive PH-1 ELISA results by VNT, and the low prevalence of antibodies detected against fur seal herpesvirus and human herpesvirus-2, suggest problems with test specificity and may indicate the presence of an additional or unique herpesvirus in Hawaiian monk seals. DNA was obtained from nasal swab samples from 95 of the 122 monk seals and positive PCR products were obtained from 20% (19/95) of the seals. These included six of the nine seals in captivity and 13 of the 86 free ranging animals (Goldstein et al., 2006). Additional studies are necessary to link this novel virus to disease. Continuous surveillance paired with virus isolation attempts may provide a more complete understanding of these and other infectious agents in this endangered population (Aguirre et al., 2002).

Caliciviruses frequently have been identified in pinnipeds by serological screening as well as isolations from lesions. Calicivirus infection in marine mammals of coastal California seems to be endemic (Smith and Boyt, 1990). The lack of group antibodies to all caliciviruses in the specimens tested is inconsistent

with previous findings in monk seals. Sera from 10 FFS monk seal females sent to Oahu for rehabilitation were positive for calicivirus by using an immunoblot procedure (NMFS, unpubl. data [from 1998]). Similarly, immunoblot preparations for calicivirus group antigens were positive by using a monoclonal antibody probe and direct electron microscopy, but virus could not be cultured from rectal swabs in most of 19 seals captured on FFS during April 1992 (Poet et al., 1993).

The results from this study indicated that antibodies to a *B. abortus*-like organism are present in the population. Specimens were tested at four reference laboratories by using at least one of nine different serologic tests. Serious discrepancies were apparent between individual laboratories and test results (Table 3), which may be related to the use of tests designed for the detection of *B. abortus*.

In a recent survey of 144 Hawaiian monk seals for *Brucella* spp. antibodies, 11.6% of seals tested seropositive by both competitive ELISA (cELSIA) and FPA; results suggested that both tests would be appropriate for the species, but further work to validate test results is needed (Nielsen et al., 2005). Retamal et al. (2000) concluded that isolation and characterization of the organism coupled with highly specific serologic tests such as cELISA are needed to confirm infection in Antarctic fur seals (*Arctocephalus gazella*).

Biologically and genetically unique strains of *Brucella* have been identified in marine mammals since the mid-1990s in Europe and North America (House et al., 2002). *Brucella* antibodies have been detected in many species of pinnipeds and cetaceans in the Northern and Southern hemispheres, suggesting a common source of infection, such as fish in the marine food web (Godfroid, 2002). The strains do not seem to be members of known *Brucella* species, and a new species (*B. maris*) has been proposed for some isolates (Jahans et al., 1997). A *Brucella* spp., later identified as *B. delphini*, was isolated from aborted fetuses of bottlenose dolphins along the California coast (Miller et al., 1999). The question remains if *Brucella* can cause disease in monk seals and the potential reproductive impact to the population is unknown; there have been no isolates to date. With a few exceptions, the majority of marine brucellae have been isolated from subcutaneous tissues or organs with no obvious signs of pathology (Maratea et al., 2003). Based on the inconsistencies among multiple tests and laboratories demonstrated in this study and the lack of validation by isolation of organism or relevant clinical signs, the seropositive *Brucella* findings in monk seals should be interpreted with caution. We recommend that the highly sensitive cELISA and FPA should be implemented and complemented with confirmation of infection by culture or molecular-based techniques (Aguirre et al., 2002; Nielsen et al., 2005).

Leptospira antibodies have been previously reported in two juvenile monk seals tested during translocation efforts at FFS in 1992 (Poet et al., 1993). The significance of these low antibody titers (100) was considered questionable, because of an absence of clinical signs or pathologic evidence of disease and because antibody titers detected during epizootics of leptospirosis among California sea lions were much higher (Vedros et al., 1971). Antibody prevalences ranging from 3 to 13% at three sites (FFS, KUR, and LAY) were detected against *L. bratislava*. To the best of our knowledge, this is the first serologic evidence of this serotype in marine mammals. Molecular and histopathologic studies hold the greatest promise for fast, sensitive, and specific diagnostic tests in the future and may provide insight on the epidemiology of this potential pathogen in Hawaiian monk seals.

The family Chlamydiaceae has recently been revised based on phylogenetic analyses and reclassified into two genera and nine species (Everett et al., 1999). The CF test used in monk seals was developed to diagnose infections with *C. abortus*, formerly *C. psittaci*. The high prevalence of antibodies to *C. abortus*, ranging from 36% to 70% in all populations except KUR, may indicate that *C. abortus* or a similar pathogen may be endemic to Hawaiian monk seals. However, the CF test is not a reliable indicator of individual animal infection and has low sensitivity and specificity, making assessment of results difficult (Booth and Blanshard, 1999; Buendra et al., 2001). This information is particularly important to the interpretation of our results, because the test has not been validated for monk seals. Previous reports of chlamydial infections in the marine environment include *C. psittaci* avian antigen in green turtles (*Chelonia mydas*) in the Hawaiian Islands (Aguirre et al., 1994) and an isolate of *C. abortus* in a brown skua (*Catharacta antarctica*) in the South Georgian archipelago (Herrmann et al., 2000). Further testing of monk seals should incorporate molecular techniques to detect and identify this agent from blood, rectal, vaginal, and penile urethral swabs (Hartley et al., 2001; Helps et al., 2001; Poppert et al., 2002).

Evidence of a *T. gondii* in the marine ecosystem and infection of sea otters (*Enhydra lutris*) has been recently documented; land-based surface runoff has been implicated as the source (Miller et al., 2002a). *Toxoplasma gondii* is known to be endemic in rodents on small Pacific Atolls that support feral cat populations (Wallace et al., 1972), and monk seals could be infected through contact with dead rodents containing tissue cysts or through effluents contaminated cat feces. In Oahu, a captive adult monk seal presented active ocular disease with high antibody titers ($\geq$1,200) to *T. gondii* (NMFS, unpubl. data [from 1999]). More recently, *T. gondii* was identified as the cause of mortality in an adult monk seal with visceral and cerebral lesions in Otzuka beach, Island of Kawai. Immunohistochemical staining and detection of the *T. gondii* DNA confirmed the diagnosis (Honnold et al., 2005). The indirect fluorescent antibody test (Miller et al., 2002b) may present new diagnostic capabilities to identify infection in Hawaiian monk seals, especially as the population continues to grow in the main Hawaiian Islands. However, in the absence of clinical signs or high antibody prevalences, there is no reason to think that *T. gondii* currently represents a health problem for wild Hawaiian monk seals (Aguirre, 2000).

This is the first epidemiologic study of wild Hawaiian monk seals providing important information on infectious diseases that potentially can affect the health and management of this endangered species. Understanding the significance of seropositive animals in the absence of signs of disease or confirmatory isolation of an infectious agent is a common difficulty in wildlife studies, and the role of infectious diseases in the monk seal population decline remains an open question. Serologic testing and surveillance are extremely valuable tools to avoid the introduction of emerging infectious diseases and endemic pathogens. Given the use of translocation as a management tool in the past, and its possible consideration in the future, our findings support the need for continuous monitoring and evaluation of health in future conservation efforts of Hawaiian monk seals. Monk seal populations seem to be relatively naïve and highly susceptible to mammalian viruses such as morbillivirus (Lu et al., 2003), and special attention should be given to minimize the spread of disease among subpopulations should one be detected. More specific and sensitive diagnostic and screening techniques to understand the microbiologic and immunologic status of the species are needed and may be

achieved through the development of susceptible cell lines (Lu et al., 1998, 2000, 2003), molecular probes, and specific immunoglobulins for monk seal serologic testing.

ACKNOWLEDGMENTS

The authors thank Jason Baker, Mitch Craig, Mary Donohue, Melissa Shaw, Bradley Ryon, and Brent Stewart for valuable assistance during the field effort. This research was sponsored in part under contract 40JJNF900155 from the NMFS Southwest Fisheries Science Center, Honolulu Laboratory, Hawaii, USA. The logistic and technical support of field personnel from National Marine Fisheries Service Protected Species Investigation, US Fish and Wildlife Service, Hawaii Wildlife Fund, and Hubbs-SeaWorld Research Institute is gratefully acknowledged.

LITERATURE CITED

AGUIRRE, A. A. 2000. Health assessment and disease status studies of the Hawaiian monk seal. US Department of Commerce, NOAA NMFS Administrative Report H-00-01, Honolulu, Hawaii, 44 pp.

———, G. H. BALAZS, B. ZIMMERMAN, AND T. R. SPRAKER. 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. Journal of Wildlife Diseases 30: 8–15.

———, T. M. O'HARA, T. R. SPRAKER, AND D. A. JESSUP. 2002. Monitoring the health and conservation of marine mammals and sea turtles and their ecosystems. *In* Conservation medicine: Ecological health in practice, A. A. Aguirre, R. S. Ostfeld, G. M. Tabor, C. A. House and M. C. Pearl (eds.). Oxford University Press, New York, pp. 79–84.

———, J. REIF, AND G. ANTONELIS. 1999. Hawaiian monk seal epidemiology plan: Health and disease status studies. US Department of Commerce, NOAA-TM-NMFS-SWFSC-280, 63 pp.

ALCORN, D. J., AND A. K. H. KAM. 1986. Fatal shark attack on a Hawaiian monk seal (*Monachus schauinslandi*). Marine Mammal Science 3: 313–315.

APPEL, M. J., AND D. S. ROBSON. 1973. A microneutralization test for canine distemper virus. American Journal of Veterinary Research 34: 1459–1463.

BALAZS, G. H., AND G. C. WHITTOW. 1979. First record of a tiger shark observed feeding on a Hawaiian monk seal. Elepaio 39: 107–109.

BANISH, L. D., AND W. G. GILMARTIN. 1992. Pathological findings in the Hawaiian monk seal. Journal of Wildlife Diseases 28: 428–434.

BAKER, J. D., AND T. C. JOHANOS. 2002. Effects of research handling on the endangered Hawaiian monk seal. Marine Mammal Science 18: 500–512.

BOOTH, R. J., AND W. H. BLANSHARD. 1999. Diseases of koalas. *In* Zoo and wild animal medicine: Current therapy 4, M. E. Fowler and R. E. Miller (eds.). W. B. Saunders, Philadelphia, Pennsylvania, pp. 321–333.

BUENDRA, A. J., F. CUELLO, L. DEL RIO, M. C. GALLEGO, M. R. CARO, AND J. SALINAS. 2001. Field evaluation of a new commercially available ELISA based on a recombinant antigen for diagnosing *Chlamydophila abortus* (*Chalmydia psittaci* serotype 1) infection. Veterinary Microbiology 78: 229–239.

DAILEY, M. D., R. V. SANTANGELO, AND W. G. GILMARTIN. 1988. A coprological survey of helminth parasites of the Hawaiian monk seal from the northwestern Hawaiian Islands. Marine Mammal Science 4: 125–131.

DUBEY, J. P. 1997. Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. Veterinary Parasitology 71: 307–310.

———, AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal 19: 337–339.

EVERETT, K. D. E., R. M. BUSH, AND A. A. ANDERSEN. 1999. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five species, and standards for the identification of organisms. International Journal of Systematic Bacteriology 49: 415–440.

GERACI, J. R., AND V. J. LOUNSBURY. 1993. Marine mammals ashore: A field guide for strandings. Texas A&M University Sea Grant College Program Publication TAMU-SG-93-601, College Station, Texas, 305 pp.

GILMARTIN, W. G., R. L. DELONG, A. W. SMITH, L. A. GRINER, AND M. D. DAILEY. 1980. An investigation into unusual mortality in the Hawaiian monk seal, *Monachus schauinslandi*. *In* R. W. Grigg and R. T. Pfund (eds.). Proceedings on status of resource investigation in the northwestern Hawaiian Islands, University of Hawaii, Honolulu, UNIHI-SEAGRANT-MR-80-04, pp. 32–41.

GODFROID, J. 2002. Brucellosis in wildlife. Revue Scientifique et Techique Office International des Epizooties 21: 277–286.

GOLDSTEIN, T., F. M. D. GULLAND, R. C. BRAUN, G. A. ANTONELIS, L. KASHINSKY, T. K. ROWLES, J. A. K. MAZET, L. M. DALTON, B. M. ALDRIDGE, AND J. L. STOTT. 2006. Molecular identification of a novel gamma herpesvirus in the endangered

Hawaiian monk seal (*Monachus schauinslandi*). Marine Mammal Science 22: 465–471.

GULLAND, F. M. D., M. KOSKI, L. J. LOWENSTINE, A. COLAGROSS, L. MORGAN, AND T. SPRAKER. 1996. Leptospirosis in California sea lions (*Zalophus californianus*) stranded along the central California coast, 1981–1994. Journal of Wildlife Diseases 32: 572–580.

HARTLEY, J. C., S. KAYE, S. STEVENSON, J. BENNETT, AND G. RIDGWAY. 2001. PCR detection and molecular identification of *Chlamydiaceae* species. Journal of Clinical Microbiology 39: 3072–3079.

HELPS, C., N. REEVES, S. SÉVERINE TASKER, AND DAVE HARBOUR. 2001. Use of real-time quantitative PCR to detect *Chlamydophila felis* infection. Journal of Clinical Microbiology 39: 2675–2676.

HENDERSON, J. R. 2001. A pre- and post-MARPOL Annex V summary of Hawaiian monk seal entanglements and marine debris accumulation in the northwestern Hawaiian Islands, 1982–1998. Marine Pollution Bulletin 42: 584–589.

HERRMANN, B., R. RAHMAN, S. BERGSTRÖM, J. BONNEDAHL, AND B. OLSEN. 2000. *Chlamydophila abortus* in a brown skua (*Catharacta antarctica lonnbergi*) from a subantarctic island. Applied and Environmental Microbiology 66: 3654–3656.

HIRUKI, L. M., I. STIRLING, W. G. GILMARTIN, T. C. JOHANOS, AND B. L. BECKER. 1993. Significance of wounding to female reproductive success in Hawaiian monk seals (*Monachus schauinslandi*) at Laysan Island. Canadian Journal of Zoology 71: 469–474.

HONNOLD, S. P., R. BRAUN, D. P. SCOTT, C. SREEKUMAR, AND J. P. DUBEY. 2005. Toxoplasmosis in a Hawaiian monk seal (*Monachus schauinslandi*). Journal of Parasitology 91: 695–697.

HOUSE, C., A. A. AGUIRRE, AND J. A. HOUSE. 2002. Emergence of infectious diseases in marine mammals. *In* Conservation medicine: Ecological health in practice, A. A. Aguirre, R. S. Ostfeld, G. M. Tabor, C. A. House and M. C. Pearl (eds.). Oxford University Press, New York, pp. 104–117.

JAHANS, K. L., G. FOSTER, AND E. S. BROUGHTON. 1997. The characterization of *Brucella* strains isolated from marine mammals. Veterinary Microbiology 57: 373–382.

KING, D. P., A. R. LIE, T. GOLDSTEIN, B. M. ALDRIDGE, F. M. D. GULLAND, M. HAULENA, M. A. ADKISON, L. J. LOWENSTINE, AND J. L. STOTT. 2001. Humoral immune responses to phocine herpesvirus-1 in Pacific harbor seals (*Phoca vitulina richardsii*) during an outbreak of clinical disease. Veterinary Microbiology 80: 1–8.

LAMBOURN, D. M., S. J. JEFFRIES, AND J. P. DUBEY. 2001. Seroprevalence of *Toxoplasma gondii* in harbor seals (*Phoca vitulina*) in southern Puget Sound, Washington. Journal of Parasitology 87: 1196–1197.

LU, Y., A. A. AGUIRRE, R. C. BRAUN, AND P. LOH. 1998. Establishment of monk seal cell lines. In Vitro Cell and Developmental Biology 34: 367–369.

———, ———, C. HAMM, Y. WANG, Q. YU, P. C. LOH, AND R. YANAGIHARA. 2000. Establishment, cryopreservation, and growth of 11 cell lines prepared from a juvenile Hawaiian monk seal, *Monachus schauinslandi*. Methods in Cell Science 22: 115–124.

———, ———, Y. WANG, L. ZENG, P. C. LOH, AND R. YANAGIHARA. 2003. Viral susceptibility of newly established cell lines from the Hawaiian monk seal, *Monachus schauinslandi*. Diseases of Aquatic Organisms 57: 183–191.

MACMILLAN, A. 1992. Conventional serological tests. *In* Animal brucellosis, K. Nielsen and J. R. Duncan (eds.). CRC Press, Boca Raton, Florida, pp. 153–197.

MARATEA, J., D. R. EWALT, S. FRASCA, JR., J. L. DUNN, S. DE GUISE, L. SZKUDLAREK, D. J. ST. AUBIN, AND R. A. FRENCH. 2003. Evidence of *Brucella* sp. infection in marine mammals stranded along the coast of southern New England. Journal of Zoo and Wildlife Medicine 34: 256–261.

MILLER, M. A., I. A. GARDNER, C. KREUDER, D. M. PARADIES, K. R. WORCESTER, D. A. JESSUP, E. DODD, M. D. HARRIS, J. A. AMES, A. E. PACKHAM, AND P. A. CONRAD. 2002a. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). International Journal for Parasitology 32: 997–1006.

———, ———, A. PACKHAM, J. K. MAZET, K. D. HANNI, D. JESSUP, J. ESTES, R. JAMESON, E. DODD, B. C. BARR, L. J. LOWENSTINE, F. M. GULLAND, AND P. A. CONRAD. 2002b. Evaluation of an indirect fluorescent antibody test (IFAT) for demonstration of antibodies to *Toxoplasma gondii* in the sea otter (*Enhydra lutris*). Journal of Parasitology 88: 594–599.

MILLER, W. G., L. G. ADAMS, T. A. FITCH, N. F. CHEVILLE, J. P. PAYEUR, D. R. HARLEY, C. HOUSE, AND S. H. RIDGWAY. 1999. *Brucella*-induced abortions and infection in bottlenose dolphins (*Tursiops truncatus*). Journal of Zoo and Wildlife Medicine 30: 100–110.

NIELSEN, O., K. NIELSEN, R. BRAUN, AND L. KELLY. 2005. A comparison of four serologic assays in screening for *Brucella* exposure in Hawaiian monk seals. Journal of Wildlife Diseases 41: 126–133.

OFFICE INTERNATIONAL DES EPIZOOTIES. 2000. Manual of standards for diagnostic tests and vaccines. World Organization for Animal Health, Paris, France, 957 pp.

O'HARA, T. M., C. HOUSE, J. A. HOUSE, R. S. SUYDAM, AND J. C. GEORGE. 1998. Viral serologic survey of

bowhead whales in Alaska. Journal of Wildlife Diseases 34: 39–46.

POET, S. E., W. GILMARTIN, D. E. SKILLING, M. P. CRAIG, AND A. W. SMITH. 1993. Detection of a non-cultivatable monk seal calicivirus using a cDNA hybridization probe. IAAAM Proceedings 24: 85–89.

POPPERT, S., A. ESSIG, R. MARRE, M. WAGNER, AND M. HORN. 2002. Detection and differentiation of Chlamydiae by fluorescence in situ hybridization. Applied and Environmental Microbiology 68: 4081–4089.

RAGEN, T. J., AND D. M. LAVIGNE. 1999. The Hawaiian monk seal: Biology of an endangered species. *In* Conservation and management of marine mammals, J. R. Twiss, Jr. and R. R. Reeves (eds.). Smithsonian Institution Press, Washington, D.C., pp. 224–245.

RETAMAL, P., O. BLANK, P. ABALOS, AND D. TORRES. 2000. Detection of anti-*Brucella* antibodies in pinnipeds from the Antarctic territory. Veterinary Record 146: 166–167.

SALIKI, J. T., AND T. W. LEHENBAUER. 2001. Monoclonal antibody-based competitive enzyme-linked immunosorbent assay for detection of morbillivirus antibody in marine mammal sera. Journal of Clinical Microbiology 39: 1877–1881.

SAS INSTITUTE. 1988. SAS/STAT user's guide. SAS Institute, Cary, North Carolina, 1028 pp.

SMITH, A. W., AND P. M. BOYT. 1990. Caliciviruses of ocean origin: A review. Journal of Zoo and Wildlife Medicine 21: 3–23.

———, C. M. PRATO, AND D. E. SKILLING. 1977. Characterization of two new serotypes of San Miguel sea lion virus. Intervirology 8: 30–36.

THRUSFIELD, M. 1995. Veterinary Epidemiology, 2nd Edition. Blackwell Science, Cambridge, Massachusetts, 479 pp.

VEDROS, N. A., A. W. SMITH, J. SCHOENWALD, G. MIGAKI, AND R. C. HUBBARD. 1971. Leptospirosis epizootic among California sea lions. Science 172: 1250–1251.

WALLACE, G. D., L. MARSHALL, AND M. MARSHALL. 1972. Cats, rats and toxoplasmosis on a small Pacific island. American Journal of Epidemiology 95: 475–482.

Received for publication 28 April 2004.