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Source: Journal of Wildlife Diseases, 12(3) : 326-334

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-12.3.326

PREVALENCE AND DISTRIBUTION OF FOUR SEROTYPES OF SMSV SERUM NEUTRALIZING ANTIBODIES IN WILD ANIMAL POPULATIONS[1]

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Abstract: Serum neutralizing antibodies to four serotypes of San Miguel Sea Lion Virus (SMSV) were demonstrated in a variety of marine and terrestrial species. These results show a wide geographic distribution of SMS viruses in the marine environment and indicate that certain terrestrial mammals have been infected with these so-called marine viruses. Evidence is presented supporting the theory that unidentified submammalian marine species are a reservoir for SMSV.

INTRODUCTION

San Miguel Sea Lion Virus (SMSV) was first isolated from a California sea lion (*Zalophus californianus*) in Southern California in 1972. Shortly thereafter, a virus isolated from an emaciated northern fur seal (*Callorhinus ursinus*) pup dying of undetermined causes on the Pribilof Islands was shown to be of the same serotype, SMSV-1.[8] This finding was noteworthy because all of 120 northern fur seals (3-4 years old) examined in 1972, showed no neutralizing antibodies to SMSV-1, whereas 18 of 20 California sea lion cows were positive.[7,8] Apparently, a new SMSV serotype had been introduced into the fur seal herd, providing a unique opportunity to study the epizootiology of SMSV infection in a susceptible pinniped population.

Although SMSV-2 had been isolated from California sea lions but not from northern fur seals, 30% of the 3-4 year-old male fur seals tested in 1972 had neutralizing antibody titers to this particular serotype. We thus presumed that SMSV-2 was enzootic in the northern fur seal herd.[5]

Aside from the California sea lion and northern fur seal, serum neutralizing (SN) antibodies also were found in other marine species, including California gray whales (*Eschrichtius gibbosus*), northern elephant seals (*Mirounga angustirostris*) and Steller sea lions (*Eumetopius jubata*).[1] Other marine mammals such as sperm whales (*Physeter catodon*), Sei whales (*Balaenoptera borealis*), fin whales (*Balaenoptera physalus*), a white whale (*Delphinapterus leucas*), bearded seals (*Erignathus barbatus*), ringed seals (*Pusa hispada*), harbor seals (*Pagophilus greoulandicus*) and walruses (*Odobenus rosmarus*) had no antibodies against SMSV-1 and SMSV-2.[1] This is particularly interesting because the whales negative for SMSV antibodies are all deep water species, and the antibody-negative pinniped species are all native to the Bering Sea or Arctic Ocean and do not migrate

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[1] This work was sponsored by the Office of Naval Research and supported in part by the National Marine Fisheries Service.

Presented in part at the 1975 Annual Wildlife Disease Conference, 23 August 1975, Guelph, Ontario, Canada.

through Southern California waters. Perhaps more important, SMSV appeared to have bridged the land-sea barrier in that five feral swine and one feral sheep resident on the Santa Barbara Channel Islands were positive for SMSV-2 antibodies.[1,7]

Because of the wide geographic and varied host distribution of SMSV, we examined as many species as practical for SMSV antibodies, including those comprising some portion of the pinniped diet. The purpose of this paper is to report on the prevalence and spread of SMSV-1, SMSV-2 and two newly established SMSV serotypes (SMSV-4 and 5, Manuscript in preparation) in selected wild animal populations.

MATERIALS AND METHODS

Serum collection. Individual blood samples from 640 3-4 year-old fur seals were collected, during the annual harvest on St. Paul Island, over a 3-year period. Sea lion blood was obtained by cardiac puncture of 20 adult females.[2] Whale serums were collected from four species (prior to 1972) during processing at the Richmond California Whaling Station.[3] Feral swine, sheep and goats' blood was collected from major vessels and body cavities of the animals shot during the annual big game hunts on the Channel Islands.[4] All other pinniped serums were collected at various times and places as early as 1961.[5] Fish serums were obtained from a variety of California species taken near Monterey, San Miguel Island, the Farallon Islands and off Eureka on the Northern Californa Coast. All samples were frozen for transport and maintained in a —20 C freezer. Pools consisting of five serums were screened for antibodies against the four SMSV serotypes and the individual sera from positive pools were then titered.

Antibody titrations. Neutralizing antibody tests were employed using the microtiter assay system previously described[5] with some modification. Inactivated sera (56 C for 30 min) were initially diluted 1:10 and subsequently serial two-fold dilutions were made. Tests were run in quadruplicate (with appropriate virus and cell controls). One hundred $TCID_{50}$ of stock virus were added to each serum dilution and incubated for 1 hr at 37 C. Next, approximately 10,000 Vero (African Green Monkey kidney) cells were added to each microtiter well. All microtiter plates were incubated in 5% CO_2 and 95% air for 72 hrs before staining with crystal violet. Each well was graded positive or negative on the basis of monolayer destruction at serum dilutions of 1:10 or higher. Neutralizing antibody endpoints were expressed as the reciprocal of the lowest serum dilution that neutralized 50% of the infected wells.

Virus isolates. Stocks of the 4 SMSV serotypes were grown on Vero cells as previously described.[7]

RESULTS

Antibody titers in the various marine and terrestrial mammals are presented for SMSV serotypes 1, 2, 4 and 5 in Tables 1 to 4 respectively and a summary is presented in Table 5.

Antibodies to serotypes SMSV-1 and 4 were not found in any of the 3-4 year-old fur seals tested for 1972 and 1973 (Tables 1 and 3).

SMSV Type 5 from fur seals was first isolated in 1973. That year 2.5% of the 3-4 year-old fur seal bachelor bulls had antibody titers of 1:20 or greater, whereas 200 fur seal sera collected the previous year were uniformly negative (Table 4). In 1974, SMSV-5 was apparently epizootic as noted by a morbidity rate of 85%. The neutralizing antibody titers (in

[2] Samples supplied through the courtesy of William Gilmartin, Naval Undersea Center, San Diego, California 92132, USA.

[3] Samples furnished by H. M. S. Watkins, Naval Biomedical Research Laboratory.

[4] Samples furnished by T. Peace and D. G. Constantine, Naval Biomedical Research Laboratory.

[5] Provided through the courtesy of Professor V. M. Sarich, Department of Biochemistry, University of California, Berkeley, California 94720, USA.

TABLE 1. SMSV-1* serum neutralizing antibody titers of 10 mammalian species inhabiting the Pacific North American Continental Borderlands.**

Whales****		Pacific pinnipeds											Insular feral terrestrial mammals		
		Northern fur seals					California sea lions								
Neutralizing antibody titers	1968-70	Bachelor bulls 1972	Bachelor bulls 1973	Bachelor bulls 1974	Pelagic females 1974	Pups 1974	1970	1972	Bearded seal 1960-62	Harbor seal 1960-62	Steller sea lions*** 1961	Ringed seal 1960-62	Swine 1973	Goats 1973	Sheep 1973
Neg	34	120	200	220	87	150	9	2	7	4	1	22	52	13	11
1:10	0	0	0	0	1	0	2	7	0	0	0	0	1	0	0
1:20	0	0	0	0	1	0	1	5	0	0	0	0	0	0	0
1:40	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
1:80	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1:160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:1280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:2560	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total positive	0/34	0/120	0/200	0/220	2/89	0/150	3/12	18/20	0/7	0/4	0/1	0/22	1/53	0/13	0/11

* This virus was first isolated in 1972 from a California sea lion which had just delivered a premature pup.

** Some of these data were published previously (Akers, *et al.*[1] and Prato, *et al.*[7])

*** A pool of three animals reported as a single animal.

**** Seventeen of 34 were California gray whales.

TABLE 2. SMSV-2* serum neutralizing antibody titers of 10 mammalian sp ecies inhabiting the Pacific North American Continental Borderlands.****

Whales**		Pacific pinnipeds											Insular feral terrestrial mammals		
		Northern fur seals													
Neutralizing antibody titers	1968-70	Bachelor bulls 1972	Bachelor bulls 1973	Bachelor bulls 1974	Pelagic females 1974	Pups 1974	California sea lions 1970	California sea lions 1972	Bearded seal 1960-62	Harbor seal 1960-62	Steller sea lions*** 1961	Ringed seal 1960-62	Swine 1973	Goats 1973	Sheep 1973
Neg	29	80	145	219	75	149	0	0	7	4	0	22	47	13	10
1:10	0	20	34	1	5	1	1	1	0	0	1	0	0	0	0
1:20	3	8	10	0	6	0	0	2	0	0	0	0	2	0	0
1:40	1	10	11	0	3	0	4	5	0	0	0	0	1	0	1
1:80	1	2	0	0	0	0	2	7	0	0	0	0	2	0	0
1:160	0	0	0	0	0	0	5	4	0	0	0	0	1	0	0
1:320	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1:640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:1280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:2560	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total positive	5/34	40/120	55/200	1/220	14/89	1/150	12/12	20/20	0/7	0/4	1/1	0/22	6/53	0/13	1/11

* First isolated in 1972 from a California sea lion which had just delivered a premature pup.

** Seventeen of 34 whales tested were California gray whales. All 5 animals with antibody titers were gray whales.

*** Serum pooled from 3 animals reported as a single animal.

**** Some of these data were published previously (Akers, *et al.*[1] and Prato, *et al.*[7]).

TABLE 3. SMSV-4* serum neutralizing antibody titers of 10 mammalian species inhabiting the Pacific North American Continental Borderlands.

Whales***		Pacific pinnipeds											Insular feral terrestrial mammals		
		Northern fur seals													
		Bachelor bulls			Pelagic females	Pups	California sea lions		Bearded seal	Harbor seal	Steller sea lion**	Ringed seal			
Neutralizing antibody titers	1968-70	1972	1973	1974	1974	1974	1970	1972	1960-62	1960-62	1961	1960-62	Swine 1973	Goats 1973	Sheep 1973
Neg	34	200	200	220	89	150	10	19	7	4	1	5	53	13	11
1:10	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0
1:20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:1280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:2560	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total positive	0/34	0/200	0/200	0/220	0/89	0/150	2/12	1/20	0/7	0/4	0/1	0/5	0/53	0/13	0/11

* This virus was first isolated in 1973 from an aborted California sea lion fetus.

** A pool of three animals reported as a single animal.

*** Seventeen of 34 were California gray whales.

TABLE 4. SMSV-5* serum neutralizing antibody titers of 10 mammalian species inhabiting the Pacific North American Continental Borderlands.

California gray whales***		Pacific pinnipeds											Insular feral terrestrial mammals		
		Northern fur seals													
Neutralizing antibody titers	1968-70	Bachelor bulls 1972	Bachelor bulls 1973	Bachelor bulls 1974	Pelagic females 1974	Pups 1974	California sea lions 1970	California sea lions 1972	Bearded seal 1960-62	Harbor seal 1960-62	Steller sea lion** 1961	Ringed seal 1960-62	Swine 1973	Goats 1973	Sheep 1973
Neg	13	200	195	41	69	110	9	6	7	4	0	5	51	13	11
1:10	3	0	0	3	18	5	6	9	0	0	1	0	2	0	0
1:20	0	0	1	5	1	0	0	4	0	0	0	0	0	0	0
1:40	0	0	1	7	1	2	1	1	0	0	0	0	0	0	0
1:80	0	0	1	16	0	13	0	0	0	0	0	0	0	0	0
1:160	0	0	2	27	0	9	0	0	0	0	0	0	0	0	0
1:320	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0
1:640	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0
1:1280	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0
1:2560	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Total positive	3/16	0/200	5/200	179/220	20/89	29/139	7/12	14/20	0/7	0/4	1/1	0/5	2/53	0/13	0/11

* SMSV virus was first isolated in 1973 from vesicles on the flippers of a northern fur seal.

** A pool of three animals reported as a single animal.

*** Only California gray whales were examined.

TABLE 5. Summary of test results for SMSV neutralizing antibodies in 11 mammalian species using 100 $TCID_{50}$ of virus.*

Species	Antigenic types of SMSV			
	SMSV-1**	SMSV-2**	SMSV-4	SMSV-5
Gray whales	—	+	—	+
Whales — Sperm, Sei, Fin and White	—	—	—	—
California sea lion	+	+	+	+
Northern fur seal	+	+	—	+
Northern elephant seal**	—	+	NT***	NT***
Bearded seal	—	—	—	—
Harbor seal	—	—	—	—
Steller sea lion	—	+	—	+
Ringed seal	—	—	—	—
Walrus	—	—	—	—
Feral sheep	—	+	—	—
Feral goat	—	—	—	—
Feral swine	+	+	—	+

* Results of Tables 1, 2, 3 and 4 are summarized.
** Summary of previously published data (Akers *et al.*[1])
*** Not tested. (NT)

TABLE 6. Fish common to the California coast tested for neutralizing antibodies to SMSV serotypes 1, 2, 4 and 5.*

Species**	Number tested
Black cod***	5
Ling Cod *Ophiodon elongatus*	5
Sable Rock Cod***	5
Widow Rock Fish *Sebastes entomelas*	3
Kelp Rock Fish *Sebastes atrovirens*	1
Yellow and Orange Rock Fish *Sebastes* spp.	1
Black Tail Rock Fish *Sebastes* spp.	4
Red Rock Fish *Sebastes* spp.	1
Yellow Tail Rock Fish *Sebastes flavidus*	10
Black Rock Fish *Sebastes melanops*	8
Striped Perch *Embiotoca lateralis*	1
Surf Perch***	2
Red Snapper***	1
Red Sea Bass***	1
Dover Sole *Microstomus pacificus*	7
Cabazon *Scorpaenichthys marmoratus*	4
Spanish Mackeral *Scomberomorus concolor*	10
Hake *Merluccius productus*	67
Wolf Eeel *Anarrhichthys ocellatus*	1

* All serum samples were negative for neutralizing antibodies when tested at the 1:10 dilution.
** The Wolf Eel was collected in the Bering Sea. All other collections were made in California with the Hake coming from Eureka and the other species coming from either the Farallon Islands, San Miguel Island or Monterey Bay.
*** Unable to determine the scientific names.

a few cases reaching as high as 1:2560) were the highest seen to date in any naturally-occurring SMSV infections. SMSV-5 antibodies were found in 70% of the sea lion sera collected in 1972 (Table 4), two years before the prevalence reached that proportion in the northern fur seal bachelor bulls.

SMSV-2 has been isolated only once and this was in 1972 from an adult female California sea lion which had recently aborted. However, antibodies to this virus occur in more mammalian species than any of the other three SMSV types tested (Table 5). In addition, SMSV-2 antibodies were present in Steller sea lions' serums collected in 1961 (Table 2) showing this virus to have been active 13 years previously. In addition, SMSV-2 antibodies were present in 33% of the 3-4 year-old fur seals tested in 1972, 25% in 1973. The prevalence dropped to less than 1% in the animals sampled in 1974. All fish serums were uniformly negative for antibodies to all four SMSV serotypes (Table 6).

DISCUSSION

California sea lions have greater involvement with SMSV than any other species examined. Whether this is by virtue of species susceptibility or greater exposure is unknown. Certain factors tend, however, to support the latter possibility: fur seals have proven highly susceptible to SMSV-5, and yet 70% of the California sea lions tested had SN antibodies two years before the prevalence approached this epizootic level in fur seals. SMSV-4 was first isolated from sea lions in 1973, and two individuals had SN antibodies in 1970; yet all northern fur seals tested for antibodies in 1972, 1973 and 1974 were negative. The decline of SMSV-2 in fur seal herds would indicate that epizootics of SMSV-2 are not reoccurring on a regular basis. SMSV-1 was isolated from a single fur seal in 1972; yet this agent has been shown to have infected only 2 of 779 fur seals tested. Twenty-five percent of the serums from California sea lions sampled in 1970 were positive for SMSV-1 antibodies, and 90% of those sampled two years later had antibodies.

If we conclude that California sea lions have a higher degree of experience with SMSV, this might be due to (a) their geographic location, (b) their specific feeding habits, or (c) because the disease is primarily a disease of sea lions, and the sea lion itself is the reservoir host with infection of other marine species occurring incidentally or secondarily. In examining these three possibilities, the sea lion as the primary reservoir is unlikely because the relatively small total animal population (100,000 or less) (Robert DeLong, personal communication, 1973) appears too small to provide enough susceptible individuals for continuous propagation of all SMSV serotypes now known to exist while at the same time exerting selective pressures, presumably immunologic, for the formation of new serotypes. Moreover, the concept that immunologic pressure might induce mutagenesis conflicts with the fact that VESV produces solid serospecific immunity in swine.[2,4,6]

Possibly therefore, the geographic location and the sea lions' habits, presumably dietary, may constitute the primary source of SMSV. Certain dietary components could be a common factor unique to Southern California and to marine mammals in the area as well as those passing through during some portion of their annual migration. From this, we conjecture that some lower marine form occurring in the Southern California waters is the primary reservoir for SMSV. Therefore, several species of fish which, at times, may be eaten by sea lions, have been examined for SMSV antibodies. Although none were detected for the serotypes tested, there are several dozen species of fish native only to the waters south of San Francisco Bay. Many of these are known to be prey for sea lions, and only a few have been examined for SMSV antibodies.

Continuing serologic studies should be made on those species of fish found primarily from San Francisco Bay southward, with particular attention given to

the opaleye (*Girella nigricans*), the opalescent squid (*Lolago opalescence*), anchovy (*Anchova* spp.) and perhaps the cyclic sardine (*Sardinops sagax caeruleas*). Also, certain other long-lived carnivorous fish species such as rock fish (*Sabastes* spp.) and cabazone (*Scorpaenichthys marmoratus*) resident near the sea lion habitat should also be sampled. Supplemental to this broad approach would be the initiation of studies to determine the susceptibility of fish to infection. The opalescent squid has been one species suggested (Robert DeLong, personal communication 1974), and the opaleye would be another. This latter species is an intermediate host in the life cycle of *Parafilariodes decorus*[3] the lung worm of California sea lions. This last aspect is attractive in that it offers a possible teleost, nematode, marine mammal cycle for the SMSV.

Acknowledgments

The authors wish to thank Mr. Douglas Skilling and Mr. Arthur Latham for their fine technical assistance and Drs. Stewart H. Madin, Neylan A. Vedros and Mark Keyes for their helpful discussions.

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Received for publication 10 October 1975