

ANTIBODIES TO MARINE CALICIVIRUSES IN THE STELLER SEA LION (EUMETOPIAS JUBATUS SCHREBER)

Authors: Barlough, Jeffrey E., Berry, Eugene S., Goodwin, Enid A., Brown, Robin F., DeLong, Robert L., et al.

Source: Journal of Wildlife Diseases, 23(1) : 34-44

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-23.1.34>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

ANTIBODIES TO MARINE CALCIVIRUSES IN THE STELLER SEA LION (*EUMETOPIAS JUBATUS* SCHREBER)

Jeffrey E. Barlough,^{1,2} Eugene S. Berry,¹ Enid A. Goodwin,³ Robin F. Brown,⁴ Robert L. DeLong,⁵ and Alvin W. Smith¹

ABSTRACT: Sera from 145 Steller sea lions (76 adults, three subadults, 37 pups, and 29 fetuses) were tested for neutralizing antibodies to nine marine calicivirus serotypes. Antibodies were found to San Miguel sea lion virus (SMSV) types 1, 5, 6, 7, 8, 10 and 13, and to Tillamook (bovine) calicivirus, but no antibodies were found to the walrus calicivirus. Titers (microtiter neutralization assay) ranged from 1:20 to 1:320, with many positive reactions at the higher dilutions ($\geq 1:80$). Antibodies to SMSV's 5 and 10 were most common among animals sampled in Alaskan waters, while antibodies to SMSV-6 were most common among pups from the southern Oregon coast. These data provide evidence that Steller sea lions, like their California sea lion (*Zalophus c. californianus* Lesson) counterparts, have experienced widespread exposure to multiple serotypes of marine caliciviruses.

INTRODUCTION

Reports published over the past 14 yr indicate that caliciviruses are circulating among a number of animal species of the Pacific Ocean basin, including pinnipeds, cetaceans and fish (Smith and Akers, 1976; Smith, 1981; Barlough et al., 1986a). These marine viruses are of considerable interest because they are physicochemically and morphologically indistinguishable from the exotic disease agent, vesicular exanthema of swine virus (VESV), and are capable of producing vesicular lesions in experimentally exposed domestic pigs (Bachrach and Hess, 1973; Schaffer and Soergel, 1973; Smith et al., 1973, 1980b; Breese and Dardiri, 1977; Smith et al., 1977; Wilder et al., 1977; Wilder and

Dardiri, 1978; Berry et al., unpubl. data). Such findings have served to support the hypothesis that the costly outbreaks of vesicular exanthema of swine (VES) that spread throughout California and eventually to the remainder of the United States between 1932 and 1956 originated from calicivirus reservoirs in the sea (Madin, 1973; Smith et al., 1973; Sawyer, 1976; Smith and Akers, 1976; Smith, 1981). The feeding of virus-contaminated fish scraps and marine mammal remains to domestic pigs has been proposed as the major means by which these "marine caliciviruses" gained access to terrestrial populations (Madin, 1973; Smith and Akers, 1976; Sawyer et al., 1978; Smith et al., 1980b; Smith, 1981).

Pinniped species from which marine caliciviruses have been isolated include the California sea lion, northern fur seal (*Calorhinus ursinus* Linné), northern elephant seal (*Mirounga angustirostris* Gill), and Pacific walrus (*Odobenus rosmarus divergens* Illiger) (see Barlough et al., 1986a, for review). Among these, caliciviruses have been recovered from two major pathologic conditions: from vesicular lesions on flippers of California sea lions and northern fur seals, and from cases of abortion and premature pupping among California sea lions. Seroepizootiologic

Received for publication 24 February 1986.

¹ Calicivirus Research Laboratory, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331, USA.

² Present address: Department of Veterinary Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA.

³ Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, Alaska 99502, USA.

⁴ Oregon Department of Fish and Wildlife, Marine Region, Marine Science Drive, Building 3, Newport, Oregon 97365, USA.

⁵ National Marine Mammal Laboratory, Northwest and Alaska Fisheries Center, National Marine Fisheries Service/NOAA, 7600 Sand Point Way, N.E., Seattle, Washington 98115, USA.

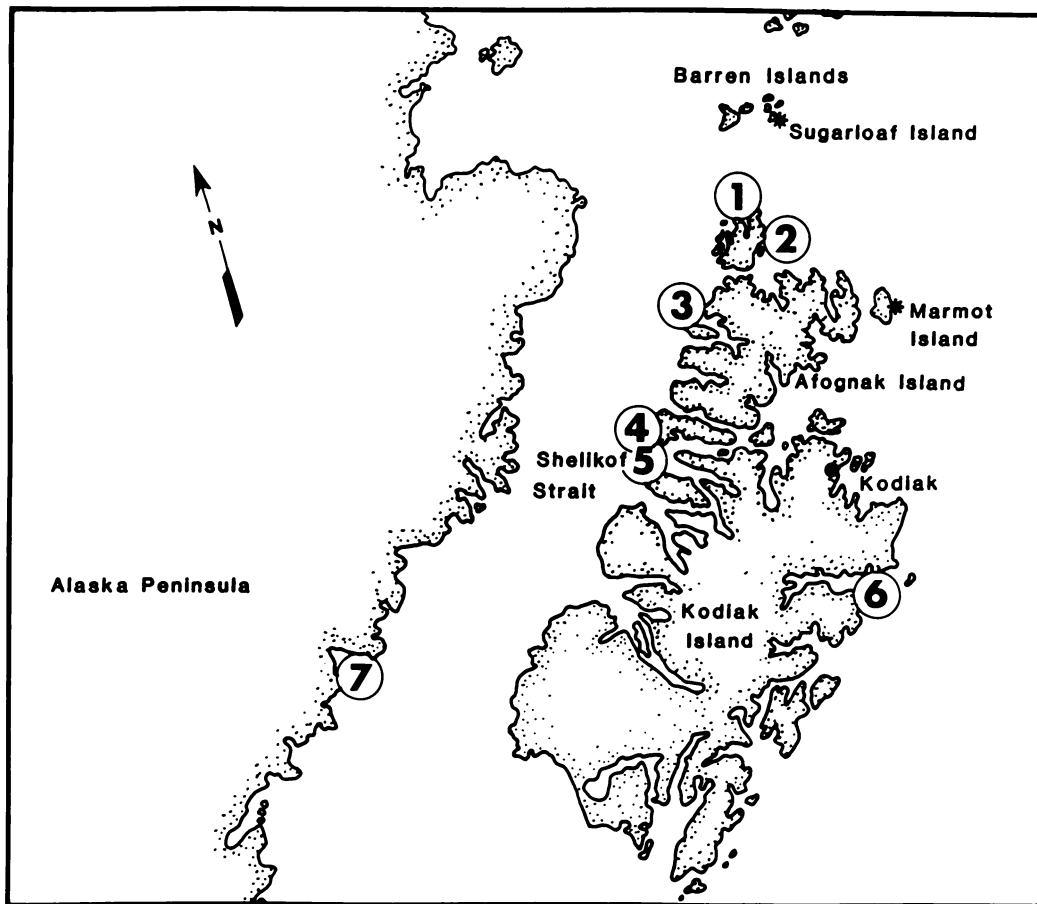


FIGURE 1. Collection sites of 74 adult/subadult Steller sea lions in the vicinity of Kodiak Island, Alaska, in the spring of 1985 (n = number of sea lions collected at each site). 1. Latax Rocks (n = 21), 2. Sea Otter Island (n = 22), 3. Black Cape (n = 2), 4. Raspberry Cape (n = 1), 5. Driver Bay (n = 1), 6. Gull Point (n = 1), and 7. Puale Bay (n = 26). Asterisks (Sugarloaf Island, Marmot Island) indicate locations of major sea lion rookeries.

studies indicate that caliciviruses are ubiquitous among populations of California sea lions, with antibodies recorded not only to recent calicivirus isolates—the San Miguel sea lion virus (SMSV) serotypes—but also to many VESV serotypes (Smith et al., 1973, 1976, 1979; Akers et al., 1974; Prato et al., 1974; Smith and Latham, 1978; Berry et al., unpubl. data). Virologic and serologic studies both have produced evidence for calicivirus epizootics among northern fur seals on the Bering Sea breeding rookeries (Prato et al., 1974; Smith et al., 1974, 1976; Smith et al., 1977);

however, involvement of this species in enzootic calicivirus transmission cycles appears to be less significant than that of California sea lions (Prato et al., 1974; Smith et al., 1976, 1979; Smith and Latham, 1978). Calicivirus antibodies in Pacific walruses are of sporadic occurrence and generally of low titer (Smith et al., 1983b; Barlough et al., 1986b), while antibodies in northern elephant seals appear to be quite rare (Akers et al., 1974; Smith and Latham, 1978; Smith et al., 1979; Barlough et al., unpubl. data).

The Steller (or northern) sea lion (*Eu-*

TABLE 1. Plaque-purified marine calicivirus serotypes used in serum neutralization assays.

Virus serotype	Isolate no.	Origin of isolate		Year	Reference
		Host species	Location		
SMSV-1 ^a	1233	Northern fur seal	St. Paul Island, Alaska	1972	Smith et al. (1974)
SMSV-5	207-73	Northern fur seal	St. Paul Island, Alaska	1973	Smith, unpubl. data ^b
SMSV-6	57-T	Northern fur seal	San Miguel Island, California	1975	Smith et al. (1980b)
SMSV-7	Gn-26	Opaleye fish	San Nicolas Island, California	1976	Smith et al. (1980b)
SMSV-8	274	Northern fur seal	St. Paul Island, Alaska	1976	Smith et al. (1981a)
SMSV-10	V-87-77	Northern fur seal	St. Paul Island, Alaska	1977	Smith et al. (1981a)
SMSV-13	CSL-461	California sea lion	Fort Cronkhite, California	1984	Berry et al., unpubl. data
Walrus calicivirus	7420	Pacific walrus	South-central Chukchi Sea	1977	Smith et al. (1983b)
Tillamook calicivirus	217-T	Domestic cattle	Clowerdale, Oregon ^c	1981	Smith et al. (1983a)

^a SMSV = San Miguel sea lion virus.^b Isolate 207-73 is serologically indistinguishable from SMSV-5 prototype isolate 205-73 (Smith et al., 1977).^c Tillamook County, Oregon.

metopias jubatus Schreber) is the largest and most abundant sea lion species in the world, with a wide distribution along the northern Pacific rim from Japan to southern California (Kenyon and Rice, 1961; Braham et al., 1980; Schusterman, 1981). The center of abundance ranges from the Gulf of Alaska to the western limits of the Aleutian Island chain (Braham et al., 1980), overlapping considerably with northern fur seals around the Aleutians and on the Pribilof Islands, and with California sea lions along the western coast of the lower continental United States (Fiscus and Baines, 1966; Jameson and Kenyon, 1977; Braham et al., 1980; King, 1983). To date, however, caliciviruses have not been isolated from Steller sea lions, although preliminary serologic evidence of exposure to at least two SMSV serotypes and to at least four VESV serotypes does exist (Akers et al., 1974; Smith et al., 1976; see Discussion). Recently, evidence of a general decline in the eastern Aleutian population of Steller sea lions (Braham et al., 1980) and of diminished pup production (Calkins, 1985) has been presented. As part of an expansive investigation into this problem, directed by the National Marine Fisheries Service/NOAA, we tested Steller sea lion sera from several sources for neutralizing antibodies to a range of marine calicivirus serotypes. This work was performed not only for the purposes of this specific investigation, but also to document the prevalence and distribution of antibodies to marine caliciviruses in this serologically neglected pinniped species.

MATERIALS AND METHODS

Serum samples

Adult (71 animals) and subadult (three animals) Steller sea lions were collected in April and May 1985, from seven haul-out sites in the vicinity of Kodiak Island, Alaska (Fig. 1). Animals were killed by rifle-shot, and blood was removed either from bullet wounds, dorsal venous sinuses, or body cavities. Thirteen males

TABLE 2. Neutralizing antibodies to marine caliciviruses in Steller sea lions.

Titer	Virus*								TCV
	SMSV-1	SMSV-5	SMSV-6	SMSV-7	SMSV-8	SMSV-10	SMSV-13	WCV	
Bering Sea adults (1976)									
Neg	4	2	4	5	5	4	4	5	4
1:20	1	1	0	0	0	0	0	0	0
1:40	0	0	0	0	0	1	1	0	1
1:80	0	1	1	0	0	0	0	0	0
1:160	0	1	0	0	0	0	0	0	0
Fraction positive ^b	1/5	3/5	1/5	0/5	0/5	1/5	1/5	0/5	1/5
Kodiak Island adults/subadults (1985)									
Neg	69	64	69	72	72	51	65	74	72
1:20	0	2	4	0	1	3	3	0	1
1:40	1	2	0	1	1	5	2	0	0
1:80	3	1	0	0	0	10	3	0	1
1:160	0	1	1	0	0	3	1	0	0
1:320	1	4	0	1	0	2	0	0	0
Fraction positive	5/74	10/74	5/74	2/74	2/74	23/74	9/74	0/74	2/74
Kodiak Island fetuses (1985)									
Neg	29	26	29	28	29	26	28	29	29
1:20	0	1	0	1	0	2	1	0	0
1:40	0	2	0	0	0	1	0	0	0
Fraction positive	0/29	3/29	0/29	1/29	0/29	3/29	1/29	0/29	0/29
Rogue Reef pups (1985)									
Neg	36	34	28	36	37	32	33	37	36
1:20	0	1	2	0	0	0	2	0	1
1:40	0	1	1	0	0	1	0	0	0
1:80	0	0	4	1	0	2	2	0	0
1:160	1	0	2	0	0	1	0	0	0
1:320	0	1	0	0	0	1	0	0	0
Fraction positive	1/37	3/37	9/37	1/37	0/37	5/37	4/37	0/37	1/37

^a SMSV = San Miguel sea lion virus; WCV = walrus calicivirus; TCV = Tillamook (bovine) calicivirus.

^b No. positive/no. sampled. Positive = neutralizing antibody titers $\geq 1:20$.

and 61 females were sampled. Twenty-eight of the females (45.9%) contained fetuses within their uteri from which blood was also obtained. Twins were discovered in one uterus, so that a total of 29 fetuses (18 males, 11 females) were sampled.

Thirty-seven pups (≤ 6 wk of age) were sampled in June 1985, during tagging operations on Rogue Reef, near the mouth of the Rogue River, Gold Beach, Oregon. Blood was collected from the hind flippers by drainage from checkmark sites (cartilaginous extensions of the flipper digits). Eighteen male pups and 19 female pups were sampled.

Also available were samples from five adult Steller sea lions (two males, three females) obtained in March and April 1976, in the eastern

Bering Sea. These samples were collected aboard the NOAA research vessel *Surveyor*, and were kindly provided to us by Mr. L. M. Shults, formerly of the Institute of Marine Science, University of Alaska, Fairbanks.

Blood cells were removed from clotted samples by low-speed centrifugation, and the sera were then heat-inactivated at 57 C for 30 min in a water bath in preparation for serum neutralization (SN) testing. All serum samples were stored at -20 C.

Viruses

Plaque-purified stocks of nine marine calicivirus serotypes (Table 1) were propagated in Vero cell cultures (CCL 81, American Type Culture Collection, Rockville, Maryland) grown

TABLE 3. Calicivirus antibody profiles of seropositive Steller sea lions (excluding fetuses).

Animal no.	Virus*								TCV
	SMSV-1	SMSV-5	SMSV-6	SMSV-7	SMSV-8	SMSV-10	SMSV-13	WCV	
Bering Sea adults (1976)									
SUV-01-76	— ^b	—	1:80	—	—	1:40	1:40	—	—
SUV-04-76	1:20	1:20	—	—	—	—	—	—	—
SUV-05-76	—	1:160	—	—	—	—	—	—	—
SUV-06-76	—	1:80	—	—	—	—	—	—	—
SUV-25-76	—	—	—	—	—	—	—	—	1:40
Kodiak Island adults/subadults (1985)									
401	—	1:40	—	—	—	—	—	—	—
406	1:80	—	—	—	—	1:80	—	—	—
408	—	—	—	—	1:20	—	—	—	—
411	—	—	—	—	—	1:80	—	—	—
412	1:320	1:40	1:20	—	—	—	—	—	1:40
413	—	1:320	—	—	—	1:80	—	—	—
416	—	—	—	—	—	—	1:20	—	—
417	—	—	—	1:40	—	1:320	1:40	—	—
418	—	—	—	—	—	1:20	—	—	—
422	—	—	—	—	—	1:80	—	—	—
423	—	—	—	—	—	1:160	1:80	—	—
424	—	1:20	—	—	—	—	—	—	—
425	—	—	—	—	—	1:80	—	—	—
426	1:80	—	—	—	—	—	1:40	—	—
427	1:80	—	—	—	—	1:160	—	—	—
431	—	—	—	—	—	1:80	—	—	—
435 ^c	—	1:20	—	—	—	—	—	—	—
436	—	—	—	—	—	1:40	—	—	—
437	—	—	1:20	—	—	—	—	—	—
439	—	—	—	—	—	—	1:80	—	—
440	—	1:320	—	—	—	1:80	—	—	—
442	—	—	1:20	—	—	1:320	1:160	—	—
444	—	—	—	—	—	1:80	—	—	—
445	—	—	—	—	—	—	1:20	—	—
449	—	1:80	—	—	—	—	—	—	—
451	—	—	—	—	—	1:20	—	—	—
452	—	—	—	—	—	—	—	—	1:80
457	—	1:320	—	—	—	1:80	—	—	—
458	—	1:320	—	—	—	—	—	—	—
461	—	—	—	—	—	1:160	—	—	—
462	—	—	—	—	—	1:80	—	—	—
463	—	—	—	—	—	1:40	—	—	—
464	—	—	—	—	—	1:40	—	—	—
467	1:40	1:160	1:160	1:320	—	1:40	1:80	—	—
468	—	—	1:20	—	—	—	—	—	—
470	—	—	—	—	1:40	—	—	—	—
474	—	—	—	—	—	1:20	—	—	—
475	—	—	—	—	—	1:40	1:20	—	—
Rogue Reef pups (1985)									
3M	—	—	1:80	—	—	1:320	—	—	—
6M	—	—	1:20	—	—	—	—	—	—
8M	—	—	1:20	—	—	—	—	—	—
12M	—	—	1:160	—	—	—	1:20	—	—
19M	—	—	1:80	—	—	1:80	1:80	—	—

TABLE 3. Continued.

Animal no.	Virus ^a							WCV	TCV
	SMSV-1	SMSV-5	SMSV-6	SMSV-7	SMSV-8	SMSV-10	SMSV-13		
21M	—	—	—	—	—	1:160	1:80	—	—
28M	—	1:320	1:40	—	—	—	—	—	—
33M	—	1:20	—	—	—	—	1:20	—	1:20
40M	—	1:40	—	—	—	—	—	—	—
41M	—	—	1:80	—	—	—	—	—	—
46M	—	—	—	—	—	1:40	—	—	—
9F	—	—	—	1:80	—	—	—	—	—
17F	1:160	—	—	—	—	—	—	—	—
29F	—	—	1:80	—	—	1:80	—	—	—
45F	—	—	1:160	—	—	—	—	—	—

^a SMSV = San Miguel sea lion virus; WCV = Walrus calicivirus; TCV = Tillamook (bovine) calicivirus.

^b Negative result (neutralizing antibody titer $\leq 1:20$).

^c Subadult sea lion.

in 75-cm² flasks. Viruses were harvested by freeze-thawing culture flasks, followed by low-speed centrifugation of culture fluids to remove cell debris. Supernatants were dispensed in 0.2-ml aliquots into screw-cap glass vials and stored at -70 C. Calicivirus serotypes chosen for testing included: viruses isolated exclusively from arctic or subarctic regions (SMSV-5, SMSV-8, SMSV-10, walrus calicivirus); viruses isolated exclusively from the western coast of the lower continental United States (SMSV-6, SMSV-7, SMSV-13, Tillamook calicivirus); one virus isolated from both regions (SMSV-1); viruses isolated exclusively from pinniped species (SMSV-1, SMSV-5, SMSV-8, SMSV-10, SMSV-13, walrus calicivirus); viruses isolated from pinnipeds and fish (SMSV-6, SMSV-7); and a virus isolated from domestic cattle and for which serologic evidence of a marine origin now exists (Tillamook calicivirus) (see Barlough et al., 1986a, for general review).

Serology

A microtiter (96-well) SN procedure using Vero cells was performed, first to screen sera at a dilution of 1:20, and then to titrate positive samples (Monto and Bryan, 1974; Smith et al., 1976). Serum-virus mixtures were incubated for 60 min at room temperature prior to addition of cells. The antibody titer was defined as the highest dilution of serum completely neutralizing 100 TCID₅₀ of virus in all four replicate test wells (100% end-point). Tests for eight of the nine viruses were read after incubation for 72 hr; the walrus calicivirus required 5 days' incubation, owing to its slower, more cell-associated cytopathology (Barlough et al., 1986b).

Type specificities were checked by neutralization and reciprocal cross-neutralization with the appropriate rabbit antisera. The specificities of the individual viruses and antisera have been reported elsewhere (Smith et al., 1977; Smith and Latham, 1978; Smith et al., 1979, 1980b, 1981a, 1983a, b; Berry et al., unpubl. data).

RESULTS

The prevalence and distribution of SN antibodies to the nine caliciviruses under study are presented in Table 2. Antibodies were found to all seven SMSV serotypes tested and to Tillamook (bovine) calicivirus, but no antibodies were found to the walrus calicivirus. Among the individual populations of sea lions, differences were noted in the prevalence of antibody to the various serotypes. Although admittedly a small sample ($n = 5$), the Bering Sea adults of 1976 had apparently been infected most commonly with SMSV-5 (60% positive), a virus which was first isolated in 1973 in the same geographical area (Pribilof Islands, Alaska) from vesicular lesions on northern fur seal flippers (Smith et al., 1977). Among the adults and subadults from Kodiak Island in 1985, however, antibodies to SMSV-10 were the most prevalent (31.1% positive, $n = 74$). This virus, too, was isolated originally from Pribilof

fur seals, in 1977 (Smith et al., 1981a). Off the coast of southern Oregon, the Rogue Reef pups of 1985 were seropositive most commonly to SMSV-6 (24% positive, $n = 37$), a southern California isolate recovered in 1975 from California sea lions (flipper vesicles) and a northern fur seal (oropharynx), and again in 1977 from the spleen of an opaleye fish (*Girella nigricans* Ayres) (Smith et al., 1979, 1980b).

Overall, antibodies to SMSV-10 were most common (25% positive). Found at a lower frequency were antibodies to SMSV-5 (13.8%), SMSV-6 (12.9%) and SMSV-13 (12%). Least common were antibodies to SMSV-1 (6%), Tillamook calicivirus (3.5%), SMSV-7 (2.6%), SMSV-8 (1.7%) and, of course, the walrus calicivirus (0%).

End-point titration results for seropositive animals (adults, subadults, pups) are shown in Table 3. Antibody titers ranged from 1:20 to 1:320, with many positive reactions at the higher dilutions ($\geq 1:80$). Again with the exception of the walrus calicivirus, only SMSV-8 failed to react at these higher dilutions with any of the test sera. Of the 58 seropositive animals, the majority ($37/58 = 63.8\%$) had antibodies to only a single serotype. Fourteen animals (24.1%) had antibodies to two serotypes, while five (8.6%) had antibodies to three. Two animals (3.5%) from the Kodiak Island vicinity (nos. 412 and 467) were seropositive to four and six serotypes, respectively. No readily recognizable patterns of reactivity to the various serotypes were noted.

End-point titration results for the seropositive mother/fetus pairs from the Kodiak Island vicinity are shown in Table 4. Antibody titers in the seropositive fetuses were virtually always one \log_{10} dilution lower than maternal titers, and most mothers with titers $\leq 1:80$ had seronegative fetuses. These data suggested that the calicivirus antibodies found in the fetuses were probably maternal in origin (Cavagnolo, 1979).

DISCUSSION

Data collected over the past 14 yr indicate that the California sea lion is more intimately involved in marine calicivirus transmission cycles than any other mammalian species thus far examined. Eight of the 12 recognized serotypes of SMSV have been isolated at least once from California sea lion sources, and seroepizootologic surveys conducted over the past decade have consistently demonstrated widespread exposure of California sea lions to many calicivirus serotypes, including the VESV's (see Barlough et al., 1986a, for general review). However, preliminary serologic evidence of exposure of Steller sea lions to SMSV-2, SMSV-5 and VESV types A₄₈, C₅₂, J₅₆ and K₅₆ (Akers et al., 1974; Smith et al., 1976; Smith, unpubl. data) had suggested that the Steller sea lion species might also be involved in calicivirus infection cycles, but wider seroepizootologic data had not been reported. The data presented here now extend these findings, indicating that, indeed, Steller sea lions, like their California sea lion counterparts, have experienced widespread exposure to multiple serotypes of marine caliciviruses.

These marine viruses are believed to be maintained in nature by complex enzootic/epizootic cycles that in some cases may involve phylogenetically diverse marine species (e.g., pinnipeds, cetaceans, fish, nematodes) (Smith and Akers, 1976; Smith et al., 1976, 1980a, b; Smith and Latham, 1978; Smith, 1981; Barlough et al., 1986a). In southern California waters, for example, a near-shore species of fish, the opaleye, appears to serve as a calicivirus reservoir for its natural predator, the California sea lion (Smith et al., 1980a, b, 1981b). Opaleye are omnivorous scavengers that acquire initial calicivirus infections probably by ingestion of contaminated fecal material. Because these fish are recognized intermediate hosts for the sea

TABLE 4. Calicivirus antibody profiles of seropositive Steller sea lion mother/fetus pairs (Kodiak Island vicinity, 1985).

Animal no.	Virus ^a								
	SMSV-1	SMSV-5	SMSV-6	SMSV-7	SMSV-8	SMSV-10	SMSV-13	WCV	TCV
408	— ^b	—	—	—	1:20	—	—	—	—
408-A ^c	—	—	—	—	—	—	—	—	—
412	1:320	1:40	1:20	—	—	—	—	—	1:20
412-A	—	—	—	—	—	—	—	—	—
418	—	—	—	—	—	1:20	—	—	—
418-A	—	—	—	—	—	—	—	—	—
422	—	—	—	—	—	1:80	—	—	—
422-A	—	—	—	—	—	—	—	—	—
425	—	—	—	—	—	1:80	—	—	—
425-A	—	—	—	—	—	1:20	—	—	—
427	1:80	—	—	—	—	1:160	—	—	—
427-A	—	—	—	—	—	1:20	—	—	—
431	—	—	—	—	—	1:80	—	—	—
431-A	—	—	—	—	—	—	—	—	—
442	—	—	1:20	—	—	1:320	1:160	—	—
442-A	—	—	—	—	—	1:40	1:20	—	—
449	—	1:80	—	—	—	—	—	—	—
449-A	—	—	—	—	—	—	—	—	—
452	—	—	—	—	—	—	—	—	1:80
452-A	—	—	—	—	—	—	—	—	—
455-A ^d	—	1:40	—	—	—	—	—	—	—
457	—	1:320	—	—	—	1:80	—	—	—
457-A	—	1:40	—	—	—	—	—	—	—
458	—	1:320	—	—	—	—	—	—	—
458-A	—	—	—	—	—	—	—	—	—
461	—	—	—	—	—	1:160	—	—	—
461-A	—	—	—	—	—	—	—	—	—
462	—	—	—	—	—	1:80	—	—	—
462-A	—	—	—	—	—	—	—	—	—
463	—	—	—	—	—	1:40	—	—	—
463-A	—	—	—	—	—	—	—	—	—
464	—	—	—	—	—	1:40	—	—	—
464-A	—	—	—	—	—	—	—	—	—
467	1:40	1:160	1:160	1:320	—	1:40	1:80	—	—
467-A	—	1:20	—	1:20	—	—	—	—	—

^a SMSV = San Miguel sea lion virus; WCV = Walrus calicivirus; TCV = Tillamook (bovine) calicivirus.

^b Negative result (neutralizing antibody titer $\leq 1:20$).

^c A = fetus.

^d Serum from mother (animal no. 455) not available.

lion lungworm (*Paraflaroides decorus*) (Dailey, 1970), the suggestion has been made (Hardy, quoted in Smith et al., 1980a) that lungworm larvae excreted in feces may promote calicivirus transmission by ferrying viruses, either biological-

ly or mechanically, between sea lions and opaleye (Smith et al., 1980a). Whether analogous relationships involving the Steller sea lion and its prey and/or parasite species may have arisen, or whether calicivirus infections in Steller sea lions are

maintained exclusively by intraspecies or interspecies transmission among pinnipeds, is not yet known.

At this time it is not possible to assess the impact, if any, that caliciviruses may have on the health of the Steller sea lion population. It is perhaps notable that vesicular lesions characteristic of caliciviruses were *not* observed among the pups sampled on Rogue Reef, Oregon, in 1985, nor have pathologic changes in tissues collected from the Kodiak Island animals been found that would suggest calicivirus involvement (Goodwin et al., unpubl. data). In contradistinction to this are the relatively frequent observations of calicivirus-induced vesicular lesions among California sea lions, and the recent outbreaks due to the highly virulent SMSV-13 serotype reported at the California Marine Mammal Center in 1984 (Berry et al., unpubl. data) and on San Miguel Island, California, in 1985 (Barlough et al., unpubl. data). Antibodies to this virus were found among all the Steller sea lion populations examined in the present study (Table 2), in the apparent absence of clinical disease.

No information is available, however, on the possible effects of caliciviruses on Steller sea lion reproductive efficiency. Although not conclusively proven to cause reproductive disease in pinnipeds, caliciviruses have been isolated from aborting California sea lions and from their fetuses (Smith et al., 1973, 1974, 1977), and have a recognized role in reproductive disorders in other species, such as the domestic pig (Bankowski, 1965). Calicivirus antibodies, presumably of maternal origin (Cavagnolo, 1979), were found in several of the Kodiak Island fetuses (Table 4), but the question of transplacental movement of virus in the Steller sea lion remains unanswered. The Kodiak Island samples were collected at haul-out sites peripheral to the breeding rookeries, in preparation for future studies designed to sample animals

from the rookery islands themselves. It is anticipated that material collected from these areas will provide additional information on the etiologic role (if any) of caliciviruses in disease conditions among these pinnipeds. Certainly the evidence of frequent infection presented in this report, together with the recognized pathogenic potential of caliciviruses for pinnipeds (and other species) (see Barlough et al., 1986a, for review), would suggest that adverse effects on individual animals are a possibility. Whether such effects would be of sufficient magnitude collectively to produce discernible changes in population size and pup production (Braham et al., 1980; Calkins, 1985), however, is uncertain. Perhaps a synergistic interaction with concurrent stressors on the population as a whole would be required to effect such changes.

ACKNOWLEDGMENTS

This work was supported by the Oregon Agricultural Experiment Station of Oregon State University, the National Marine Fisheries Service, Alaska Department of Fish and Game, and Oregon Department of Fish and Wildlife. The authors gratefully acknowledge the contributions of all individuals who assisted in collecting the Steller sea lion samples described in this report. This is Technical Paper No. 7820, Oregon Agricultural Experiment Station.

LITERATURE CITED

- AKERS, T. G., A. W. SMITH, A. B. LATHAM, AND H. M. S. WATKINS. 1974. Calicivirus antibodies in California gray whales (*Eschrichtius robustus*) and Steller sea lions (*Eumetopias jubatus*). Arch. Gesamte Virusforsch. 46: 175-177.
- BACHRACH, H. L., AND W. R. HESS. 1973. Animal picornaviruses with a single major species of capsid protein. Biochem. Biophys. Res. Commun. 55: 141-149.
- BANKOWSKI, R. A. 1965. Vesicular exanthema. Adv. Vet. Sci. 10: 23-64.
- BARLOUGH, J. E., E. S. BERRY, D. E. SKILLING, AND A. W. SMITH. 1986a. Sea lions, caliciviruses and the sea. Avian/Exotic Pract. 3:8-20.
- , ———, ———, ———, AND F. H. FAY. 1986b. Antibodies to marine caliciviruses in the

- Pacific walrus (*Odobenus rosmarus divergens* Illiger). J. Wildl. Dis. 22: 165–168.
- BRAHAM, H. W., R. D. EVERITT, AND D. J. RUGH. 1980. Northern sea lion population decline in the eastern Aleutian Islands. J. Wildl. Manage. 44: 25–33.
- BREESE, S. S., AND A. H. DARDIRI. 1977. Electron microscope observations on a virus transmissible from pinnipeds to swine. J. Gen. Virol. 36: 221–225.
- CALKINS, D. G. 1985. Stellar sea lion pup counts in and adjacent to Shelikof Strait. Final Report Submitted to North Pacific Fish Management Council. Contract 84-1, Anchorage, Alaska, 13 pp.
- CAVAGNOLO, R. Z. 1979. The immunology of marine mammals. Dev. Comp. Immunol. 3: 245–257.
- DAILEY, M. D. 1970. The transmission of *Parafilaroides decorus* (Nematoda: Metastrongyloidea) in the California sea lion (*Zalophus californianus*). Proc. Helminthol. Soc. Wash. 37: 215–222.
- FISCUS, C. H., AND G. A. BAINES. 1966. Food and feeding behavior of Steller and California sea lions. J. Mammal. 47: 195–200.
- JAMESON, R. J., AND K. W. KENYON. 1977. Prey of sea lions in the Rogue River, Oregon. J. Mammal. 58: 672.
- KENYON, K. W., AND D. W. RICE. 1961. Abundance and distribution of the Steller sea lion. J. Mammal. 42: 223–234.
- KING, J. E. 1983. *Seals of the World*, 2nd Ed. Cornell University Press, Ithaca, New York, pp. 20–23.
- MADIN, S. H. 1973. Pigs, sea lions, and vesicular exanthema. Proc. 2nd Int. Conf. Foot Mouth Dis., pp. 78–81.
- MONTO, A. S., AND E. R. BRYAN. 1974. Microneutralization test for detection of rhinovirus antibodies. Proc. Soc. Exp. Biol. Med. 145: 690–694.
- PRATO, C. M., T. G. AKERS, AND A. W. SMITH. 1974. Serological evidence of calcivirus transmission between marine and terrestrial mammals. Nature 249: 255–256.
- SAWYER, J. C. 1976. Vesicular exanthema of swine and San Miguel sea lion virus. J. Am. Vet. Med. Assoc. 169: 707–709.
- , S. H. MADIN, AND D. E. SKILLING. 1978. Isolation of San Miguel sea lion virus from samples of an animal food product produced from northern fur seal (*Callorhinus ursinus*) carcasses. Am. J. Vet. Res. 39: 137–139.
- SCHAEFFER, F. L., AND M. E. SOERGEL. 1973. Biochemical and biophysical characterization of calcivirus isolates from pinnipeds. Intervirology 1: 210–219.
- SCHUSTERMAN, R. J. 1981. Steller sea lion *Eumetopias jubatus* (Schreber, 1776). In Handbook of Marine Mammals, Vol. 1, S. H. Ridgway and R. J. Harrison (eds.). Academic Press, New York, pp. 119–141.
- SMITH, A. W. 1981. Marine reservoirs for calciviruses. In CRC Handbook Series in Zoonoses, Sect. B, Vol. II, J. H. Steele (ed.). CRC Press, Boca Raton, Florida, pp. 182–190.
- , AND T. G. AKERS. 1976. Vesicular exanthema of swine. J. Am. Vet. Med. Assoc. 169: 700–703.
- , ———, A. B. LATHAM, D. E. SKILLING, AND H. L. BRAY. 1979. A new calcivirus isolated from a marine mammal. Arch. Virol. 61: 255–259.
- , ———, S. H. MADIN, AND N. A. VEDROS. 1973. San Miguel sea lion virus isolation, preliminary characterization and relationship to vesicular exanthema of swine virus. Nature 244: 108–110.
- , ———, C. M. PRATO, AND H. BRAY. 1976. Prevalence and distribution of four serotypes of SMSV serum neutralizing antibodies in wild animal populations. J. Wildl. Dis. 12: 326–334.
- , AND A. B. LATHAM. 1978. Prevalence of vesicular exanthema of swine antibodies among feral mammals associated with the southern California coastal zones. Am. J. Vet. Res. 39: 291–296.
- , D. E. MATTSON, D. E. SKILLING, AND J. A. SCHMITZ. 1983a. Isolation and partial characterization of a calcivirus from calves. Am. J. Vet. Res. 44: 851–855.
- , C. M. PRATO, W. G. GILMARTIN, R. J. BROWN, AND M. C. KEYES. 1974. A preliminary report on potentially pathogenic microbiological agents recently isolated from pinnipeds. J. Wildl. Dis. 10: 54–59.
- , ———, AND D. E. SKILLING. 1977. Characterization of two new serotypes of San Miguel sea lion virus. Intervirology 8: 30–36.
- , D. G. RITTER, G. C. RAY, D. E. SKILLING, AND D. WARTZOK. 1983b. New calcivirus isolates from feces of walrus (*Odobenus rosmarus*). J. Wildl. Dis. 19: 86–89.
- , D. E. SKILLING, AND R. J. BROWN. 1980a. Preliminary investigation of a possible lung worm (*Parafilaroides decorus*), fish (*Girella nigricans*), and marine mammal (*Callorhinus ursinus*) cycle for San Miguel sea lion virus type 5. Am. J. Vet. Res. 41: 1846–1850.
- , ———, A. H. DARDIRI, AND A. B. LATHAM. 1980b. Calcivirus pathogenic for swine: A new serotype isolated from opaleye *Girella nigricans*, an ocean fish. Science 209: 940–941.
- , ———, AND A. B. LATHAM. 1981a. Isolation and identification of five new serotypes of calcivirus from marine mammals. Am. J. Vet. Res. 42: 693–694.

- , ———, C. M. PRATO, AND H. L. BRAY. 1981b. Calicivirus (SMSV-5) infection in experimentally inoculated opaleye fish (*Girella nigricans*). Arch. Virol. 67: 165–168.
- WILDER, F. W., AND A. H. DARDIRI. 1978. San Miguel sea lion virus fed to mink and pigs. Can. J. Comp. Med. 42: 200–204.
- , ———, R. J. YEDLOUTSCHNIG, AND P. O. UGSTAD. 1977. Challenge of equines with San Miguel sea lion viruses. Proc. U.S. Anim. Health Assoc. 81: 270–275.

ADDENDUM

Since this work was completed, a calicivirus has been isolated in our laboratory

from a Steller sea lion pup sampled on Rogue Reef, Oregon, during the tagging operations described in this report. The isolate has been identified as SMSV-6, the serotype to which antibodies were most commonly found among Rogue Reef pups (Table 2). Although serum from this particular animal was unavailable for SN testing, the finding lends additional support to the serologic data presented here, and represents also, to our knowledge, the first isolation of any virus from the Steller sea lion.