NEW CALICIVIRUS ISOLATES FROM FECES OF WALRUS (ODOBENUS ROSMARUS)

Authors: Smith, A. W., Ritter, D. G., Ray, G. C., Skilling, D. E., and Wartzok, D.

Source: Journal of Wildlife Diseases, 19(2) : 86-89

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-19.2.86

Journal of Wildlife Diseases, 19(2), 1983, pp. 86–89

NEW CALICIVIRUS ISOLATES FROM FECES OF WALRUS (*ODOBENUS ROSMARUS*)

A. W. Smith,[1] D. G. Ritter,[2] G. C. Ray,[3] D. E. Skilling,[1] and D. Wartzok[4]

ABSTRACT: Three calicivirus isolations were made from walrus feces collected in 1977 off sea ice in the south central Chukchi Sea and all were of a single serotype. Individual sera from 40 walruses sampled in 1976 about 100 km east of the Pribilof Islands, Alaska were examined for sera neutralizing antibodies to the walrus calicivirus. Three animals tested positive and these were between the ages of 11 and 18 yr. In 1981, sera from 18 walruses sampled near the Soviet coast were tested and two animals, ages 8 and 12 yr, were positive. This is the first report of a walrus virus isolate and is the first time a calicivirus has been isolated from a host whose natural habitat is confined to northern waters.

INTRODUCTION

Since 1972 11 distinct caliciviruses called San Miguel Sea Lion Virus (SMSV) serotypes have been isolated from a variety of marine animals including California sea lions (*Zalophus californianus*), northern elephant seals (*Mirounga angustirostris*), and opaleye perch (*Girella nigricans*) (Smith et al., 1980b). Although three serotypes (SMSV-5, SMSV-8 and SMSV-10) have been isolated only from northern fur seals (*Callorhinus ursinus*) in the Bering Sea, evidence has indicated that once these become introduced into fur seals, they appear to pass through the herd in epizootic form and then disappear. This suggests that appropriate reservoirs are not present in the Bering Sea for over-wintering or long-term maintenance of the virus (Smith et al., 1976). Fur seals often migrate through southern California waters where caliciviruses are known to occur in both fish and mammal populations, and this has been suggested as the probable source of the caliciviruses causing a fur seal epizootic (Smith et al., 1976; Smith et al., unpubl. data). There has been little evidence to suggest that northern populations of marine mammals other than northern fur seals acquire calicivirus infections (Akers et al., 1974; Smith et al., 1976; Smith and Latham, 1978). For this reason, the finding of caliciviruses among ice-associated marine mammal populations such as the walrus was quite unexpected and is the subject of this report.

Received for publication 17 June 1982.

[1] School of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331, USA.
[2] University of Alaska, Fairbanks, Alaska 99701, USA.
[3] Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903, USA.
[4] Department of Pathobiology, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

MATERIALS AND METHODS

Collection: Walrus feces were collected from resting sites on sea ice in the south central Chukchi Sea in July 1977. The specimens were lifted off the ice using wooden tongue depressors and placed in plastic bags for up to 6 hr until they could be transferred to vials containing 0.5 ml transport medium. The transport medium was 50% glycerol and 50% phosphate buffered saline (pH 7.2), which contained gentamicin (0.75 mg/ml), fungizone (20 mcg/ml), streptomycin (5 mg/ml) and penicillin (15,000 units/ml).

The 52 vials containing walrus feces were stored at −20 C for 3–5 days and then transferred to liquid nitrogen (−196 C) for 8 days. They were then packed in dry ice for air shipment to Baltimore. The samples were kept at −50 C until processing could be arranged. They were again packed in dry ice and shipped to the University of Alaska where they were received at the Virology-Rabies Unit on 31 May 1978, and placed in a freezer at −70 C.

Virus isolation: The fecal samples underwent a single freeze–thaw cycle and were pre-treated prior to inoculation as follows. Aliquots of each sample were transferred into conical 50-ml centrifuge tubes containing sterile glass beads, and adjusted to a final concentration of 1:10. The diluent consisted of a medium containing 80 ml of Hanks' balanced salt solution, 20 ml of fetal bovine serum (heat-inactivated for 30 min at 56 C), 0.5 ml of 7.5% $NaHCO_3$, and 5 ml of penicillin (5,000 units/ml)–streptomycin (2,500 μg/ml). The samples were shaken to emulsify the feces, centrifuged at 200 rpm, 4 C for 15 min, and incubated at 4 C for 1 hr. The supernatants were decanted through gauze into 40-ml tubes, from which 10-ml aliquots were transferred for centrifugation (prechilled Sorvall SS-34 head), at 10,000 g and 4 C for 1 hr. Five ml of supernatant were transferred into vials and treated with an additional 0.3 ml of penicillin and streptomycin (5,000 units and 2,500 μg/ml).

Initial isolation attempts were made by inoculation into the following cell systems: PK-15 (CCL 33,

Porcine Kidney, *Sus scrofa*), Vero (CCL 81, African Green Monkey Kidney, *Cercopithecus aethiops*), and BWL (Bowhead Whale Lung, *Balaena mysticetus*, passage two monolayers, collected by G. H. Jarrell, University of Alaska, and explanted into monolayers by D. G. Ritter, University of Alaska). Three tubes per cell line were utilized for each sample and each tube was inoculated with 0.2 ml of the suspensions. The inoculated cells were incubated for 2 hr at 27 C, then rinsed and maintained at 37 C with medium containing 1% fetal calf serum.

The cell cultures were all read daily for cytopathogenic effect (CPE) for 28 days (two Vero cell passages and four PK-15 and BWL passages) before samples were considered negative. If CPE developed, the cell monolayers were removed, pooled, and passed into new monolayer cell cultures. Re-isolation of virus from the original sample was made. PK-15 and BWL cell cultures were scraped and blind-passed at day 7. Vero cell cultures were scraped and blind-passed at day 14.

Virus identification: For immunizing rabbits and for serum cross-neutralization tests, stock virus was grown in Vero cells (Smith et al., 1973; Smith et al., 1981). The walrus isolates were tested against 11 serotypes of marine calicivirus (SMSV-1, SMSV-2, SMSV-4 through SMSV-12) and feline calicivirus (F-9). They were not tested against any of the 13 known serotypes of vesicular exanthema of swine virus. Virus morphology was examined by electron microscopy using pelleted virus negatively stained as previously described (Ritchie and Fernelius, 1969).

Walrus sera collection and antibody testing: Sera from 40 walruses of mixed age and sex were collected in March and April of 1976, approximately 100 km east of the Pribilof Islands, Alaska. A second group of 18 sera from walruses of mixed age and sex was collected in April 1981 about 15 miles off the coast of the USSR between Cape Navarin and Olyutorsk.

All sera were tested for neutralizing antibody to the prototype walrus virus by serum neutralization testing using 100 $TCID_{50}$ of virus per test and twofold dilutions of heat-inactivated walrus sera. All sera were initially screened at the 1:10 final dilution and those inhibiting cytopathology at this level were titered to terminal dilution.

RESULTS

There were three virus isolates obtained in Vero cell culture (Table 1). These were isolated on days 6 and 10 and were titrated to obtain end points. Third passage 7389 and 7393 and second passage isolate 7420 were cross-passed into Rhesus Monkey Kidney, H.Ep#2, and Wi38 cell monolayers and read daily, but did not produce CPE. The three isolates were passed again and shipped frozen to the senior author on April 1979, at the following passage levels: $7389V_3$, $7393V_3$, and $7420V_2$.

Electron microscopy revealed viral particles 38 nm in diameter with the characteristic symmetry and the surface cup configuration of caliciviruses. All three viruses isolated from walrus were of the same serotype, but none were neutralized by typing serums from the other known caliciviruses tested.

TABLE 1. Walrus[a] virus isolates obtained in Vero cell culture.

Day	VRU accession no.[b]	$TCID_{50}$	Passage no.
10	7389	$10^{-4.5}$	Vero cell 3
10	7393	$10^{-4.5}$	Vero cell 3
6	7420	$10^{-5.5}$	Vero cell 2

[a] There were no specifics available regarding the sex and age of the animals from which feces were collected.

[b] VRU—Virology Rabies Unit, University of Alaska, Fairbanks, Alaska.

Three of the animals sampled east of the Pribilof Islands had neutralizing antibody titers of between 1:10 and 1:20. One was an 11-yr-old male, another a 14-yr-old female and the animal with the antibody titer of 1:20 was an 18-yr-old male. Two animals from off the Soviet coast, an 8-yr-old male and a 12-yr-old male, each had antibody titers of 1:10.

DISCUSSION

There is evidence suggesting that SMSV-2 prior to 1972 and SMSV-5 in 1973 were introduced into the northern fur seal herd in Alaska and caused epizootics (Smith et al., unpubl. data). Both have subsequently disappeared from the herd and neither has reappeared, even though young susceptible seals continue to enter the population annually. This, combined with the finding that northern fur seals in general show almost no evidence of exposure to other marine calicivirus types, has led to our postulating that even though these viruses are occasionally introduced into northern marine mammal populations such as fur seals, they do not over-winter in the Bering Sea (Smith et al., 1976; Smith et al., unpubl. data).

Recently, however, Smith et al. (unpubl. data) have tested four bowhead whales and have shown that they carry antibodies to three serotypes of marine caliciviruses and two serotypes of vesicular exanthema of swine calicivirus (VESV). However, all tested negative for antibodies to walrus calicivirus. This combined with the isolation of calicivirus from walrus is cause for us to reconsider the question of Bering Sea reservoirs capable of maintaining calicivi-

ruses from year to year. It is possible that gray whales (*Eschrichtius robustus*) migrating from Baja along the California coast into the Bering Sea could carry calicivirus from the southern California reservoirs northward and, in support of this, there is strong evidence suggesting repeated contact between gray whales and marine caliciviruses (Smith et al., 1976). Another source of exposure for both the walrus and the bowhead whale could be the northern fur seal. As evidence, all three marine calicivirus serotypes that bowhead whales were shown to have developed antibodies against were isolated from northern fur seals (Smith et al., 1981). Conversely, no common relationships have yet been shown between the walrus virus isolates and other marine mammals. It now appears to us that the walrus calicivirus may overwinter and persist in reservoirs within the walrus population or habitat. This supposition is based on three observations. First, walrus and northern fur seals are not generally described as two species having close contactual relationships, either in the water or on land and thus, the fur seal is not an attractive candidate as a calicivirus source for walrus. Neither do these two pinnipeds share similar major food chain species and, in the case of the fur seal, the source of calicivirus is presumed to be a known calicivirus reservoir (opaleye perch) found from Point Conception California south (Smith et al., unpubl. data). The available evidence suggests that bowhead whales have frequent contact with a variety of caliciviruses, yet these species do not migrate south of the Bering Sea and, therefore, contact with these caliciviruses must depend either on contact with carrier animal species or on virus reservoirs within the bowhead habitat. Finally, direct contact on the rookeries is a reasonably well documented mode of calicivirus transmission for fur seals (Smith et al., unpubl. data), yet on San Miguel Island, where fur seals and California sea lions share some of the same hauling grounds and do have intermittent physical contact, the calicivirus disease profiles of these two species, side by side on the same island, are quite dissimilar. This would suggest that food chain or other sources of virus contact may be much more important for between-species transmission than occasional or casual physical contact (Smith et al., 1976; Smith and Latham, 1978). It must be remembered that if an animal becomes persistently infected, a walrus for instance, and continues to shed calicivirus for many months as can occur with other species (Madin, 1975), then that animal constitutes a disease reservoir. Although it is not possible at this time to assess the impact of caliciviruses on the health of the walrus population, caliciviruses are known to cause skin lesions, probably abortion, and possibly encephalitis and pneumonia in other pinniped species (Smith et al., 1973; Smith et al., unpubl. data; Smith et al., 1980a). In cats, caliciviruses cause ulceration in the mouth and pneumonia, and in swine, vesicular and ulcerative lesions around the mouth and feet, diarrhea, abortion, agalactia, and, at times, myocarditis (Madin, 1975; Saif et al., 1975; Woode and Bridger, 1975). In calves and humans, caliciviruses cause infant diarrhea and gastroenteritis (Madeley and Cosgrove, 1975; Woode and Bridger, 1975). It is apparent that, as a group, the caliciviruses are pathogens and cause several diverse disease conditions. On this basis, we believe that the calicivirus of walruses will ultimately be shown to be a pathogen for that species. The serologic surveys of two walrus groups have shown evidence of exposure to this specific virus type in two groups of animals widely separated by geography and time. Similar testing of other ice-associated marine mammals, and marine mammals far removed from the walrus habitat would be helpful in elucidating the natural history of this first virus isolate of walrus origin.

ACKNOWLEDGMENTS

A. W. Smith and D. E. Skilling wish to acknowledge the support from the Office of Naval Research, the Naval Ocean Systems Center, and the San Diego Zoologic Society for the virus identification and retrospective serology. D. G. Ritter made the original virus isolations and wishes to acknowledge support from the National Institutes of Health. G. Carleton Ray and D. Wartzok made the original collections with the assistance of I. L. Groves and J. C. Beier and were supported by grants and contracts to The Johns Hopkins University from the Office of Naval Research and the National Geographic Society. We wish to thank Dr. Sheldon Diamond at the Naval Regional Medical Center, Balboa Hospital for electron microscopic support, Dr. Tom Albert, University of Maryland, for making bowhead whale sera available, Dr. F. Fay, University of Alaska, for providing wal-

rus sera from the Eastern Bering Sea and Mr. G. A. Antonellis, Jr., National Marine Mammal Laboratory, National Marine Fisheries Service, for providing serums taken during a joint Soviet–American marine mammal research expedition off the Soviet coastline.

LITERATURE CITED

AKERS, T. G., A. W. SMITH, A. G. LATHAM, AND H. M. S. WATKINS. 1974. Calicivirus antibodies in California gray whales (*Eschrichtius robustus*) and the Steller sea lion (*Eumatopias jubatus*). Arch. Gesamte Virusforsch. 46: 175–177.

MADELEY, C. R., AND B. P. COSGROVE. 1975. Calicivirus in man. Lancet i: 199.

MADIN, S. H. 1975. Vesicular exanthema. *In* Diseases of Swine, 2nd Ed., H. W. Dunne and A. D. Leman (eds.). Iowa State University Press, Ames, Iowa, pp. 307–486.

RITCHIE, A. E., AND A. L. FERNELIUS. 1969. Characterization of bovine viral diarrhea viruses. V. Morphology of characteristic particles studied by electron microscope. Arch. Gesamte Virusforsch. 28: 369–389.

SAIF, L. J., E. H. BOHL, K. W. THEIL, R. F. CROSS, AND J. A. HOUSE. 1975. Rotovirus-like, calicivirus-like, and 23 nm virus-like particles associated with diarrhea in young pigs. J. Clin. Microbiol. 12: 105–111.

SMITH, A. W., T. G. AKERS, A. B. LATHAM, C. M. PRATO, AND H. L. BRAY. 1976. Distribution and incidence of serum neutralizing antibodies for four San Miguel Sea Lion Virus serotypes in wild animal populations. J. Wildl. Dis. 12: 326–334.

———, ———, S. H. MADIN, AND N. A. VEDROS. 1973. San Miguel Sea Lion Virus isolation, preliminary characterization and relationship to vesicular exanthema of swine. Nature 244: 108–110.

———, AND A. B. LATHAM. 1978. Prevalence of vesicular exanthema of swine antibodies among feral mammals association with the southern California coastal zone. Am. J. Vet. Res. 39: 291–296.

———, D. E. SKILLING, AND R. J. BROWN. 1980a. Preliminary investigation of a possible lungworm (*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. Calicivirus pathogenic for swine: A new serotype isolated from opaleye (*Girella nigricans*), an ocean fish. Science 209: 940–941.

———, ———, AND A. B. LATHAM. 1981. Isolation and identification of five new serotypes of caliciviruses from marine mammals. Am. J. Vet. Res. 42: 693–694.

WOODE, G. N., AND J. C. BRIDGER. 1975. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. J. Med. Microbiol. 11: 441–452.