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FIRST ISOLATION OF A CALICIVIRUS FROM THE STELLER SEA LION (*EUMETOPIAS JUBATUS*)

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ABSTRACT: A calicivirus was isolated from the rectum of a Steller sea lion (*Eumetopias jubatus*) pup on Rogue Reef, off the southern Oregon coast. Based on the results of neutralization tests with specific typing antisera, the isolate was identified as San Miguel sea lion virus serotype 6 (SMSV-6). Blood obtained from nine of 37 pups (24%) during virus sample collection procedures had specific neutralizing antibodies to SMSV-6. The isolation of SMSV-6 from a Steller sea lion represents, to our knowledge, the first isolation of any virus from this widely distributed marine mammal species, and serves to reconfirm the host-nonspecificity of yet another calicivirus of marine origin.

Key words: Calicivirus, San Miguel sea lion virus, Steller sea lion, *Eumetopias jubatus*, virus isolation, neutralizing antibodies.

INTRODUCTION

Since 1972, 14 distinct calicivirus serotypes have been isolated from a number of marine mammal species, including the California sea lion (*Zalophus californianus californianus*), northern fur seal (*Callorhinus ursinus*), northern elephant seal (*Mirounga angustirostris*), Pacific walrus (*Odobenus rosmarus divergens*), and Atlantic bottlenosed dolphin (*Tursiops truncatus*) (Smith, 1981, 1983; Smith et al., 1985; Barlough et al., 1986). Two of these serotypes have been recovered also from opaleye (*Girella nigricans*), an ocean fish, and one from the sea lion liver fluke *Zalophatrema* spp. (Smith et al., 1980b). These marine viruses are of considerable interest because they are morphologically and physicochemically indistinguishable from the exotic disease agent, vesicular exanthema of swine virus (VESV) (Bachrach and Hess, 1973; Schaffer and Soergel, 1973; Smith et al., 1973, 1977), and are capable of producing VESV-like vesicular disease in exposed pigs (Smith et al., 1973, 1977, 1980b; Breese and Dardiri, 1977; Wilder et al., 1977; Wilder and Dardiri, 1978; Berry et al., 1985). Such findings have served to support the hypothesis that the costly outbreaks of vesicular exanthema of swine 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; Smith and Akers, 1976; Sawyer et al., 1978; Smith, 1981, 1983). The feeding of virus-contaminated fish scraps and marine mammal remains to domestic swine 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).

Historically, most isolations of marine caliciviruses have been made from California sea lions and northern fur seals (Smith, 1983; Smith et al., 1985; Barlough et al., 1986), but serologic studies indicate that Steller sea lions (*Eumetopias jubatus*), the largest and most abundant species of sea lion in the world (Kenyon and Rice, 1961; Schusterman, 1981), may be in-

volved also in calicivirus transmission cycles (Akers et al., 1974; Smith et al., 1976; Barlough et al., 1987). However, prior to 1985 caliciviruses had never been isolated from this marine mammal species, nor had any marine calicivirus serotype ever been reisolated more than 3 yr after its original recovery.

In the summer of 1985, during tagging operations off the southern Oregon coast, samples were collected from Steller sea lion pups for virologic and serologic examination. The purpose of the present report is to describe the isolation and identification of a recognized calicivirus serotype from one of these pups.

MATERIALS AND METHODS

Sample collection

On 30 June 1985 serologic and virologic samples were collected in conjunction with tagging operations on Rogue Reef, 3 km northwest of the mouth of the Rogue River, near Gold Beach, Oregon. The southeastern face of the reef rock was approached at low tide in a small fishing vessel, and landing was accomplished with an inflatable rubber boat (Zodiac of North America, Inc., Stevensville, Maryland 21666, USA). Steller sea lion pups were herded onto a flat outcropping of rock, and yearlings and adult females were driven off. One hundred pups ≤6 wk old were caught in hoop nets, weighed, sexed, flipper-tagged, and checkmarked. Swab samples were collected from the oropharynx and rectum of 58 pups, from the oropharynx of two pups, and from the rectum of two pups. Swabs were broken off into screw-cap glass vials containing 2.5 ml of cell culture medium (Eagle MEM; Whittaker M.A. Bioproducts, Walkersville, Maryland 21793, USA) with 10% fetal calf serum and 100 μg/ml gentamicin. These samples were placed on ice and subsequently frozen at −70 C within 6–8 hr of collection. Blood was obtained from 37 of the tagged pups by drainage from checkmark sites (cartilaginous extensions of the hind-flipper digits). Blood samples were chilled, and cells were removed by low-speed centrifugation after clot retraction. The resulting sera were then heat-inactivated (57 C for 30 min) in preparation for serum neutralization (SN) testing.

Virus isolation and identification

Swab samples were thawed, vortexed, clarified by low-speed centrifugation, and filtered through 0.45-μm (APD) polysulfone filters (Acrodisc®, Gelman Sciences, Ann Arbor, Michigan 48106, USA). Each sample (0.2 ml) was adsorbed for 60 min to monolayers of Vero and PK-15 cells (American Type Culture Collection, Rockville, Maryland 20852, USA) in roller tubes. The inocula were then removed, and the cells fed with cell culture medium (Eagle MEM) and incubated at 37 C on a roller drum. Each culture was examined daily for cytopathic effect. All samples were blind-passaged at least three times, at 7-day intervals, before being considered negative.

The virus isolate was purified by three plaque-passages in Vero cells, using 1.5% agar overlays (Dulbecco and Vogt, 1953). Morphologic features of the purified isolate were examined by negative-contrast electron microscopy (Smith et al., 1978; Skilling et al., 1985). Standard physicochemical testing included virus stability in ether, nucleic acid (RNA) determination using 5-fluoro-2-deoxyuridine, pH stability, heat lability, and divalent cation effects (Zee and Hackett, 1967; Howatson and Whitemore, 1973; Revozzo and Burke, 1973; Schaffer et al., 1980). The isolate was identified by standard neutralization tests using 100 $TCID_{50}$ of virus against 20 antibody units of typing serum (Kapikian et al., 1967). Typing sera (maintained in our laboratory) for all extant, established calicivirus serotypes were used (includes: 12 serotypes of SMSV; 12 serotypes of VESV; feline calicivirus; primate calicivirus *Pan paniscus* type 1; mink calicivirus; walrus calicivirus; cetacean calicivirus *Tursiops* type 1; Tillamook (bovine) calicivirus; and canine calicivirus).

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 before addition of cells. The antibody titer (as judged by cytopathic effect after 72 hr incubation at 37 C) was defined as the highest dilution of serum completely neutralizing 100 $TCID_{50}$ of virus in all four replicate test wells (100% end point). Specific rabbit antiserum was used as a positive antibody control, and type specificities were monitored in parallel neutralization tests during end-point titrations (Smith and Latham, 1978; Smith et al., 1979, 1981).

RESULTS AND DISCUSSION

On second passage in PK-15 cells, sample 3FR (rectal swab, pup 3F) showed a

TABLE 1. Neutralizing antibodies to San Miguel sea lion virus type 6 in Steller sea lion pups.

Neutralizing antibody titer (100% end point)	Number of sera
<1:20	28
1:20	2
1:40	1
1:80	4
1:160	2
Number positive/ number examined (% positive)	9/37 (24%)

distinct cytopathic effect that consisted of generalized cell rounding and detachment from the surface. The cell culture fluid was passaged, and calicivirus-like particles were observed on subsequent negative-contrast electron microscopic examination (Skilling et al., 1985). The purified isolate had morphologic and physicochemical charcteristics of the Caliciviridae (Schaffer et al., 1980), and was neutralized only by antiserum to San Miguel sea lion virus serotype 6 (SMSV-6) (Smith et al., 1979). Of 37 pup sera, nine (24%) contained antibodies to this agent, ranging in titer from 1:20 to 1:160 (Table 1). Unfortunately, serum was not available from pup 3F for evaluation.

Traditionally, caliciviruses have been described as host-specific disease agents limited in nature to a restricted number of susceptible host species (Studdert, 1978). Before 1972, only two caliciviruses had been recognized: the exotic swine agent VESV, and feline calicivirus, each of which was reported to occur naturally only in its respective host (Gillespie and Scott, 1973; Madin, 1975). However, since 1972 evidence of a relatively broad host-range spectrum for several new calicivirus serotypes has been presented. One example is SMSV-6, which has been recovered five times over a period of 10 yr from four species of marine animals: from flipper vesicles on two California sea lion pups (San Miguel Island, California, 1975) (Smith et al., 1979); from the oropharynx of a northern fur seal (San Miguel Island, California, 1975) (Smith et al., 1980b); from the spleen of an opaleye fish (San Nicolas Island, California, 1977) (Smith et al., 1980b); and now (1985), from the rectum of a Steller sea lion pup in southern Oregon.

The isolation of SMSV-6 from a Steller sea lion is notable for several reasons. First, it represents not only the first calicivirus isolation from a Steller sea lion but also, to our knowledge, the first reported isolation of any virus from this species (Smith et al., 1985). Second, it supports prior seroepizootiologic studies indicating exposure of Steller sea lions to marine caliciviruses (the absence of previous isolations presumably was due to the paucity of suitable material that had been collected from these animals) (Akers et al., 1974; Smith et al., 1976, 1985; Barlough et al., 1987). Third, it supports our hypothesis that calicivirus serotypes can be re-isolated from marine sources long after their original discovery (10 yr for SMSV-6). Thus, eventual isolation from these sources of the "exotic" disease agent VESV, for whose continued existence in the Pacific coastal marine environment there is considerable serologic evidence (Smith and Akers, 1976; Smith and Latham, 1978; Smith, 1981, 1983; Barlough et al., 1986, 1987), now seems even more likely.

Although it is not possible to assess the impact that caliciviruses may have on the health of the Steller sea lion population, these agents are known to cause vesicular lesions, probably abortion, and possibly encephalitis and pneumonitis, in other species of pinnipeds (Smith et al., 1980a, 1981, 1985; Smith, 1981, 1983; Berry et al., 1985; Barlough et al., 1986). Evidence of a general decline in the eastern Aleutian Steller sea lion population (Braham et al., 1980) and of diminished pup production (Calkins, 1985) has been presented, and calicivirus antibodies have been found in many adult animals in the Bering Sea and Gulf of Alaska (Barlough et al., 1987). These

data, together with our isolation of SMSV-6 and the recognized (Bankowski, 1965; Madin, 1975) and suspected (Smith et al., 1973; Smith and Akers, 1976; Smith, 1981) abortigenic properties of VESV and SMSV, respectively, suggest that further investigation of a possible calicivirus role in population fluctuations among Steller sea lions is warranted. Perhaps it is pertinent in this regard to note that all four isolations of SMSV-6 from marine mammals have been made from young pups, whose most probable source of infection was maternal in origin (Smith et al., 1979, 1980b).

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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*). Archiv für die Gesamte Virusforschung 46: 175–177.

BACHRACH, H. L., AND W. R. HESS. 1973. Animal picornaviruses with a single major species of capsid protein. Biochemical and Biophysical Research Communications 55: 141–149.

BANKOWSKI, R. A. 1965. Vesicular exanthema. Advances in Veterinary Science 10: 23–64.

BARLOUGH, J. E., E. S. BERRY, E. A. GOODWIN, R. F. BROWN, R. L. DELONG, AND A. W. SMITH. 1987. Antibodies to marine caliciviruses in the Steller sea lion (*Eumetopias jubatus* Schreber). Journal of Wildlife Diseases 23: 34–44.

———, ———, D. E. SKILLING, AND A. W. SMITH. 1986. The marine calicivirus story, Parts I and II. Compendium on Continuing Education for the Practicing Veterinarian 8: F5–F14 and 8: F75–F83.

BERRY, E. S., J. E. BARLOUGH, D. E. SKILLING, A. W. SMITH, AND N. A. VEDROS. 1985. Calicivirus isolation from an outbreak of vesicular disease in California pinnipeds. Proceedings of the International Association of Aquatic Animal Medicine 16: 102–107.

BRAHAM, H. W., R. D. EVERITT, AND D. J. RUGH. 1980. Northern sea lion population decline in the eastern Aleutian islands. The Journal of Wildlife Management 44: 25–33.

BREESE, S. S., AND A. H. DARDIRI. 1977. Electron microscope observations on a virus transmissible from pinnipeds to swine. Journal of General Virology 36: 221–225.

CALKINS, D. G. 1985. Steller sea lion pup counts in and adjacent to Shelikof Strait. Final Report Submitted to North Pacific Fish Management Council. Contract 84-1. Alaska Department of Fish and Game, Anchorage, Alaska, 13 pp.

DULBECCO, R., AND M. VOGT. 1953. Plaque formation and isolation of pure lines of poliomyelitis viruses. Journal of Experimental Medicine 99: 167–182.

GILLESPIE, J. H., AND F. W. SCOTT. 1973. Feline viral infections. Advances in Veterinary Science 17: 163–200.

HOWATSON, A. F., AND G. F. WHITEMORE. 1973. Development and structure of vesicular stomatitis virus. Virology 16: 466–478.

KAPIKIAN, A. Z., R. M. CONANT, V. V. HAMPARIAN, R. M. CHANOCK, P. J. CHAPPLE, E. C. DICK, J. D. FENTERS, J. M. GWALTNEY, D. HAMRE, J. C. HOLPER, W. S. JORDAN, E. H. LENNETTE, J. L. MELNICK, W. J. MOGABGAB, M. A. MUFSON, C. A. PHILLIPS, J. H. SCHIEBLE, AND D. A. J. TYRRELL. 1967. Rhinoviruses: A numbering system. Nature (London) 213: 761–763.

KENYON, K. W., AND D. W. RICE. 1961. Abundance and distribution of the Steller sea lion. Journal of Mammalogy 42: 223–234.

MADIN, S. H. 1973. Pigs, sea lions, and vesicular exanthema. Proceedings of the Second International Conference on Foot and Mouth Disease, Gustav Stern Foundation, New York, New York, pp. 78–81.

———. 1975. Vesicular exanthema. *In* Diseases of swine, 4th ed., H. W. Dunne and A. D. Leman (eds.). Iowa State University Press, Ames, Iowa, pp. 286–307.

MONTO, A. S., AND E. R. BRYAN. 1974. Microneutralization test for detection of rhinovirus antibodies. Proceedings of the Society for Experimental Biology and Medicine 145: 690–694.

REVOZZO, G. C., AND C. N. BURKE. 1973. A manual of basic virological techniques. Prentice-Hall, Englewood Cliffs, New Jersey, pp. 150–151.

SAWYER, J. C., 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. American Journal of Veterinary Research 39: 137–139.

SCHAFFER, F. L., H. L. BACHRACH, F. BROWN, J. H. GILLESPIE, J. N. BURROUGHS, S. H. MADIN, C. R. MADELEY, R. C. POVEY, F. SCOTT, A. W. SMITH, AND M. J. STUDDERT. 1980. Caliciviridae. Intervirology 14: 1–6.

———, AND M. E. SOERGEL. 1973. Biochemical and biophysical characterization of calicivirus 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, New York, pp. 119–141.

SKILLING, D. E., J. E. BARLOUGH, E. S. BERRY, AND A. W. SMITH. 1985. A simple, rapid method for preparation of virus isolates from cell culture for electron microscopy. Journal of Tissue Culture Methods 9: 217–220.

SMITH, A. W. 1981. Marine reservoirs for caliciviruses. *In* CRC handbook series in zoonoses, Sect. B, Vol. II, J. H. Steele (ed.). CRC Press, Boca Raton, Florida, pp. 182–190.

———. 1983. Focus on . . . caliciviral disease. United States Department of Agriculture Foreign Animal Disease Report 11(3): 8–16.

———, AND T. G. AKERS. 1976. Vesicular exanthema of swine. Journal of the American Veterinary Medical Association 169: 700–703.

———, ———, A. B. LATHAM, D. E. SKILLING, AND H. L. BRAY. 1979. A new calicivirus isolated from a marine mammal. Archives of Virology 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. Journal of Wildlife Diseases 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. American Journal of Veterinary Research 39: 291–296.

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

———, D. E. SKILLING, J. E. BARLOUGH, AND E. S. BERRY. 1985. The calicivirus: An ocean organism comes ashore. Proceedings of the International Association of Aquatic Animal Medicine 16: 94–101.

———, ———, 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. American Journal of Veterinary Research 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 calicivirus from marine mammals. American Journal of Veterinary Research 42: 693–694.

———, ———, AND A. E. RITCHIE. 1978. Immunoelectron microscopic comparisons of caliciviruses. American Journal of Veterinary Research 39: 1531–1533.

STUDDERT, M. J. 1978. Caliciviruses: Brief review. Archives of Virology 58: 157–191.

WILDER, F. W., AND A. H. DARDIRI. 1978. San Miguel sea lion virus fed to mink and pigs. Canadian Journal of Comparative Medicine 42: 200–204.

———, ———, R. J. YEDLOUTSCHNIG, AND P. O. UGSTAD. 1977. Challenge of equines with San Miguel sea lion viruses. Proceedings of the United States Animal Health Association 81: 270–275.

ZEE, Y. C., AND A. J. HACKETT. 1967. The influence of cations on the thermal inactivation of vesicular exanthema of swine virus. Archiv für die Gesamte Virusforschung 20: 473–476.

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