in the SPR task by transferring simultaneous (pairs of pictures simultaneously present) same-different performance to a delayed same-different task and then to the SPR discrimination. Approximately 28,000 trials were necessary to train the monkey in the simultaneous procedure, whereas only 10,000 trials were necessary to train the monkey in the delayed and SPR tasks.
9. We identified serial position effects by testing post hoc contrasts across the sample means for the ten serial positions. Variance ratios for each contrast were compared with new critical F values [R. S. Rodger, *Br. J. Math. Statist. Psychol.* **28**, 71 (1975)] in order to control type I errors on a per decision basis. For the monkey, accuracy at serial position 1 (primacy effect) and at serial position 10 (recency effect) was higher than at medial positions 2, 3, and 4 [primacy: $F(9, 300) = 2.05$, $P < .05$; recency: $F(9, 300) = 5.37$, $P < .05$]. For the human subject, similar effects were evident but less pronounced because of a ceiling effect and fewer observations. A recency effect was indicated by higher accuracies at serial positions 9 and 10 than at positions 2 to 6 [$F(9, 90) = 1.44$, $P < .05$]. A primacy effect was also indicated since performance at serial position 1 (and at positions 7 and 8) was intermediate between these extremes [$F(9, 90) = .03$, $P > .05$].
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17. Analysis of the errors committed in the high-interference condition revealed that most incorrect responses were initially "same" responses to probe items not in the current list.
18. We would like to thank Judy Cornish for her technical assistance and Drs. M. R. D'Amato, E. Hearst, W. K. Honig, S. H. Hulse, and P. J. Urcuioli for critical comments on the manuscript. This work was partially supported by NIH grant EY01256-05 and NSF grant BNS 78-07253 to A.A.W.
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Calicivirus Pathogenic for Swine: A New Serotype Isolated from Opaleye *Girella nigricans*, an Ocean Fish

Abstract. *A new calicivirus, designated San Miguel sea lion virus type 7 (SMSV-7), was isolated from fish and produced a disease condition identical to vesicular exanthema in experimentally infected swine. Serotype SMSV-7 was also isolated from four elephant seals and one sea lion trematode, whereas a second calicivirus serotype isolated from fish proved to be SMSV-6.*

We have isolated from the opaleye fish (*Girella nigricans*) a new calicivirus we have designated San Miguel sea lion virus type 7 (SMSV-7) and have experimentally infected swine with this agent. Another calicivirus SMSV-6 (*1*) has also been isolated from the opaleye.

From 1932 through 1955 the calicivirus that causes vesicular exanthema of swine (VESV) was repeatedly introduced into domestic swine in California fed on raw garbage contaminated with the virus; the garbage component carrying the virus remained unknown (*2-4*). In 1972, caliciviruses were isolated from California sea lions (*Zalophus californianus*) and this species was thought to be the reservoir for VESV (*5*). Later, northern fur seals (*Callorhinus ursinus*) and northern elephant seals (*Mirounga agustirostris*) were also shown to shed caliciviruses (*6, 7*).

There are 25 serotypes of calicivirus including the one serotype of feline calicivirus; 22 of these serotypes have been isolated in southern California (*8, 9*). This suggests that California is a possible focus for calicivirus activity, and we have argued that fish are a logical calicivirus reservoir (*4*). One fish, the opaleye, is the intermediate host for the sea lion lung worm (*Parafilaroides decorus*) and thus has a well-established relationship with the California sea lion (*10*).

Fish (*G. nigricans*) were collected from tidal pools on San Nicholas Island (off the southern California coast) and examined for virus. Tidal pools estimated to contain 200 to 1000 liters of water were seeded with up to 500 ml of quinaldine (*11*) diluted 1:10 with isopropyl alcohol. Within minutes the fish could be caught by hand or dip net and transferred to untreated seawater where they quickly regained their coordination.

Each fish (10 to 15 cm long) was eviscerated and the whole viscera were placed into individual Whirl-Pac plastic bags (*12*), frozen on dry ice, transported to the laboratory, and stored at −70°C. Thirty fish were processed in this way and an additional 15 were processed as follows. The major organs (liver, spleen, kidney, gut, muscle, and gills) were sampled individually from each fish and placed in separate 2-dram vials containing 2 ml of cell culture media with 10 percent fetal bovine serum. These were sealed and frozen on dry ice. Later all tissue samples were ground in a mortar with sterile sand and cell culture media,

Table 1. Serotyping of marine calicivirus isolates by means of virus neutralization test. In each test 100 $TCID_{50}$ of virus were reacted against 20 antibody units of typing serum. Symbols: +, neutralization occurred; −, unneutralized virus leading to a cytopathic effect in the cell monolayers at 72 hours. All typing serums were of rabbit origin.

Virus	Antiserums										
	SMSV-1	SMSV-2	SMSV-4	SMSV-5	SMSV-6	57-T	Gn-14	Gn-26	2837	2839	Fluke
SMSV-1	+	−	−	−	−	−	−	−	−	−	−
SMSV-2	−	+	−	−	−	−	−	−	−	−	−
SMSV-4	−	−	+	−	−	−	−	−	−	−	−
SMSV-5	−	−	−	+	−	−	−	−	−	−	−
SMSV-6	−	−	−	−	+	+	+	−	−	−	−
57-T*	−	−	−	−	+	+	+	−	−	−	−
Gn-14†	−	−	−	−	+	+	+	−	−	−	−
Gn-21‡	−	−	−	−	−	−	−	+	+	+	+
Gn-26‡	−	−	−	−	−	−	−	+	+	+	+
2816§	−	−	−	−	−	−	−	+	+	+	+
2837§	−	−	−	−	−	−	−	+	+	+	+
2839§	−	−	−	−	−	−	−	+	+	+	+
2844§	−	−	−	−	−	−	−	+	+	+	+
Fluke¶	−	−	−	−	−	−	−	+	+	+	+

*Throat swab of a 4-month-old northern fur seal pup bearing tag No. 57 sampled on San Miguel Island in October 1977. †Isolated from the spleen of *G. nigricans* No. 14 taken on San Nicholas Island, California, in February 1977. ‡Isolated from whole viscera homogenate of opaleye Nos. 21 and 26 taken on San Nicholas Island, California, in February 1976. §Tag numbers of northern elephant seal pups sampled on San Miguel Island in February 1976. ¶Trematode of the genus *Zalophatrema* from a sea lion dying of verminous pneumonia (*23*).

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then clarified by centrifuging at 3500 rev/min for 10 minutes. The supernatant was withdrawn and used for isolating virus. Most tissue homogenates were cytotoxic and had to be diluted 1:10 and sometimes 1:100 before 0.2 ml could be satisfactorily adsorbed for 60 minutes on monolayers of Vero [African green monkey (*Cercopithicus aethiops*) kidney] cells in roller tubes. We placed 2 ml of cell culture media on rinsed monolayers and incubated the cultures at 37°C on roller drums as previously described (*1*).

Two viruses, Gn-21 and Gn-26 (*13*), were isolated from homogenates of whole viscera and another, Gn-14, was recovered from a spleen. Cytopathic changes were observed after three passages. After passage 7 the virus titers were 10^7 median tissue culture infective doses ($TCID_{50}$) for isolates Gn-26 and Gn-14; for Gn-21 after passage 10 the titer was 10^5.

The viruses were plaque purified and their nucleic acid content determined by means of 5-fluoro-2-deoxyuridine (*14*). Ether sensitivity, *p*H stability, heat lability, and divalent cation (0.1*M* Mg^{2+}) effects were all tested by previously described methods (*5, 15–18*). Typing serums were prepared in rabbits for each virus isolate as described (*19*), and serotyping was accomplished with cross-neutralization tests in a microtiter system where 100 $TCID_{50}$ were tested against 20 antibody units of serum (*19*).

Virus-infected cells were stained negatively for examination with the electron microscope as previously described (*20*).

The physicochemical characteristics of the virus were those of a naked RNA virus whose size and morphology were typical of the calicivirus group. The results of cross-neutralization tests with other known caliciviruses and their typing serums are given in Table 1. The isolates Gn-21, Gn-26, 2816, 2837, 2839, 2844, and fluke all belong to the same serotype, whereas Gn-14 and 57-T belong to serotype SMSV-6. The three opaleye isolates were not neutralized by typing serums for the VESV types (A_{48}, B_{52}, C_{52}, D_{53}, E_{54}, F_{54}, G_{55}, H_{54}, I_{55}, J_{56}, and K_{56}) nor did the Gn-26 or Gn-14 virus antibodies neutralize the VESV types tested (*21*). Domestic swine, experimentally exposed to the prototype virus (isolate Gn-26) developed typical clinical vesicular exanthema with secondary vesicular lesions and spread the disease horizontally to other swine with which they had contact.

The range for *G. nigricans* extends from Point Conception south along the California coast into Mexico. This places the opaleye within the geographic location where marine calicivirus activity appears to be most intensive. The replication ($10^{7.5}$ infective units per gram) of calicivirus in the spleen of experimentally infected *G. nigricans* has been shown and the possibility of a nematode carrier for SMSV has been investigated (*22*). The isolation of a calicivirus from fish and its demonstrated virulence for swine does not prove that fish reservoirs were the primary sources for the original outbreaks of VESV in the 1930's and 1940's. However, these findings show that marine caliciviruses, pathogenic for swine, do survive in ocean fish, thereby supporting our theory that raw fish scraps contained in garbage were the most probable source of the virus causing vesicular exanthema of swine (*4*). This exotic disease that mimics foot-and-mouth disease was first introduced into swine in California in 1932. In 1952 VESV spread throughout the swine areas of the United States and was contained only by implementing eradication measures costing $30 million and enforcing laws prohibiting the feeding of raw garbage to swine.

It is noteworthy that SMSV-7 has also been isolated from the throat or rectum of four elephant seals sampled on San Nicholas Island, California, and from a California sea lion liver fluke belonging to the genus *Zalophatrema* (*7*). Repeated attempts to isolate calicivirus from rectal swabs and other tissues of the same California sea lion failed. While the intermediate hosts and life cycle of the *Zalophatrema* are unknown, the possible association of a calicivirus with this metazoan parasite raises some intriguing speculations on parasite vectors in the marine environment.

The third opaleye isolate, Gn-14, was serotye SMSV-6. This antigenic type was first isolated from vesicles on the flippers of sea lion pups on San Miguel Island in October 1975 (*1*), whereas Gn-14 was isolated some 16 months later from an opaleye taken on San Nicholas Island 46 miles south of San Miguel. One additional isolate of the SMSV-6 type, 57-T, was recovered on San Miguel Island from the throat of a 4-month-old northern fur seal.

The serotypes of the virus isolates reported here were determined by using 20 antibody units of serum against 100 $TCID_{50}$ of virus. Some possible subtype differences between isolate numbers Gn-21, Gn-26, 2816, 2837, 2839, 2844, and the fluke isolate were noted but have not been investigated further.

These data illustrate that the marine caliciviruses infect a variety of species including poikilotherms and that fish may be an important reservoir for the exotic virus that caused the historic outbreaks of vesicular exanthema in swine. We believe this to be the first report of a virus pathogenic for terrestrial mammals that has a saltwater fish as one of its natural reservoirs.

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24. This work was supported by the Office of Naval Research and the National Marine Fisheries Service. We thank R. Delong, National Marine Fisheries Service, Seattle, Wash., and R. Gunnels, Naval Ocean Systems Center, Hawaii, for their assistance in obtaining samples from the field. Send reprint requests to A.W.S.

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