San Miguel Sea Lion Virus Fed to Mink and Pigs

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ABSTRACT

Mink became infected with San Miguel sea lion virus when fed ground meat from seal carcasses showing vesicular-like lesions in the skin. The mink also contracted the infection when they were fed San Miguel sea lion virus infected pig meat or cell culture propagated virus. San Miguel sea lion virus infection in mink was inapparent but the virus was isolated from blood and rectal swabs. Pigs treated similarly with the same virus preparations given to mink developed a severe vesicular disease syndrome similar to that produced by vesicular exanthema of swine. In a separate trial, pigs fed a large sample of commercial ground seal meat did not develop disease signs or antibodies. Further work is needed to assess the hazard of introducing San Miguel sea lion virus into swine on the same premises when potentially San Miguel sea lion virus infective seal meat is fed to mink.

RÉSUMÉ

Le fait d'alimenter des visons avec de la viande hachée provenant de carcasses d'otaries à fourrure qui présentaient des lésions cutanées ressemblant à des vésicules, fit réaliser qu'on les infectait avec le virus de l'otarie de San Miguel. Les visons contractèrent aussi l'infection après avoir absorbé de la viande de porc ou des cultures tissulaires qui contenaient ce virus. L'infection passa inaperçue, mais on isola le virus à partir du sang et d'écouvillons du rectum de ces visons. L'utilisation de porcs au lieu de visons, dans une expérience similaire, se traduisit par l'apparition d'un syndrome vésiculaire grave et semblable à celui que provoque l'exanthème vésiculaire du porc. Au cours d'une autre expérience, les porcs qui avaient mangé un échantillon commercial considérable de viande hachée d'otarie à fourrure ne développèrent pas de signes de maladie, ni d'anticorps. Il faudra effectuer d'autres expériences afin de préciser le danger d'introduire le virus de l'otarie de San Miguel chez des porcs qui vivent sur la même ferme que des visons auxquels on donne de la viande d'otarie à fourrure qui pourrait receler ce virus.

INTRODUCTION

San Miguel sea lion virus type 5 (SMSV-5) has been isolated from a vesicle on a skin sample obtained from the northern fur seal (*Callorhinus ursinus)* found on the Pribilof Islands in the Bering Sea (3). Observers of the annual culling of three and four year old bachelor males from the rookeries on the Pribilof Islands noticed that certain seals had ruptured vesicle-like lesions on the skin of their flippers. San Miguel sea lion virus was isolated from meat ground from seals with skin lesions after the seals had been skinned and evisserated (John C. Sawyer, personal communication). Large amounts of seal meat are a by-product of the annual culling. Ground seal meat is then shipped to the U.S. mainland as mink feed. Some mink ranchers keep pigs to feed on the ground seal meat that drops under the mink cages. Thus, feeding seal meat to both mink and pigs may represent a potential danger to the swine industry because SMSV is indistinguishable physiochemically and morphologically from vesicular exanthema of swine virus (VESV) (2). Furthermore, SMSV and VESV produce similar vesicular disease features in pigs. The purpose of this study was to determine whether mink or pigs would become infected by feeding on seal meat infected with SMSV.

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MATERIALS AND METHODS

SEAL MEAT (ground)

Lot #1 — A suspension prepared from approximately 100 g of ground meat, prepared from seal carcasses with vesicular skin lesions, yielded SMSV-5 with titers of 1 x $10^{5.2}$ tissue culture infective doses ($TCID_{50}$)/g when assayed in green monkey kidney (Vero) cell culture (John C. Sawyer, personal communication). Ten months later virus was not isolated from this lot in two attempts in Vero cultures immediately before feeding.

Lot #2 — Thirty kilograms of ground seal meat processed on the same day as lot #1 originated from seals with no visible skin lesions. In four attempts, no virus was isolated from lot #2 in Vero cell culture.

VIRUSES

San Miguel sea lion virus type 5 from the fourth passage in Vero cell culture was adapted to fetal pig kidney clone 7 (MVPK-7) cell culture (1). At 18 hr postinoculation 100 ml of virus in tissue culture fluid was harvested, centrifuged at 20°C at 800 x g for ten min and the supernatant fluid was used for inoculum. This fluid contained 1 x 10^8 $TCID_{50}$ of virus/ml.

ANIMALS

All mink used were housed singly in wire cages in one animal room. Except as indicated, all mink were males donated by the mink experiment station at Cornell University, Ithaca, New York and were fed the same maintenance ration as that used at Cornell.

Pigs used in parallel studies with the mink were housed in separate rooms for each group.

SMSV INFECTED PIG MEAT

Six pigs were inoculated intradermally (ID) in the snout and coronary bands with SMSV-5. Each pig received 2 ml of a 10% vesicular tissue suspension containing 1 x 10^8 $TCID_{50}$ of SMSV-5/ml. At the peak of vesicle formation, 25 g of vesicular epithelial tissue were collected from the snout and feet. This vesicular tissue was mixed with 249 gm of minced muscle obtained from a pig with a 40.5°C febrile reaction and vesicular lesions after inoculation with SMSV-5. The vesicular tissue and muscle mixture was ground and reduced to a smooth paste in a Waring blender. Each gram of this homogenate contained 5 x $10^{4.5}$ $TCID_{50}$ of SMSV-5 when assayed in MVPK-7 cell culture.

ANIMAL GROUPS AND INOCULUM

Five animal groups were tested (Table I). Each of four mink in group 1 was fed 25 g of lot #1 ground seal meat. Each of four mink in group 2 was fed 22 g and each of two pigs fed 84 g of SMSV infected pig meat described above. Each of three mink in group 3 was fed 60 g and each of the two pigs was fed 85 g of a combination of Cornell mink feed mixed with tissue culture produced virus in the form of a paste with a titer of 2.5 x $10^{7.5}$ $TCID_{50}$ of SMSV-5.

Each of the three mink in group 4 was given ID injections (coronary band and tongue) and intranasal inoculates of SMSV-5 infective tissue culture fluid amounting to a total of 2.0 ml of inoculum and each of the two pigs was given a total of 5.0 of inoculum in similar sites. This inoculum contained 1 x 10^8 $TCID_{50}$ of SMSV-5/ml.

Each of the 12 pigs in group 5 was fed 30 kg of ground seal meat (lot #2) that had been frozen and thawed during its storage and shipment.

CLINICAL OBSERVATIONS AND SAMPLING

Mink were restrained by the subcutaneous injection of a mixture of 25 mg of ketamine hydrochloride (Ketajet)[1] and 5 mg of xylazine (Rompun)[2]. Rectal swabs and heparinized blood samples were taken every two or three days for 14 days from the mink and pigs. Two ml of heparinized blood was taken by jugular vein puncture. Rectal swabs were stirred vigorously into

[1]Bristol Laboratories, Syracuse, New York 13201.

[2]Chemagro, Kansas City, Missouri 64120.

TABLE I. Experimental Design for the Challenge of Pigs and Mink with Ground Seal Meat and San Miguel Sea Lion Virus (SMSV)

Animal Group No.	Animals	Total No.	Inoculum	Amount per Animal	Inoculation Route[a]	Virus $TCID_{50}$[b]
1	Mink	4	Lot #1 ground seal meat	25 g	Oral	$1 \times 10^{5.2}$/g
2	Mink Pigs	4 2	SMSV-5 pig ground meat and vesicular tissue homogenate	22 g 84 g	Oral	$5 \times 10^{4.5}$/g
3	Mink Pigs	3 2	SMSV-5 in tissue culture fluid absorbed in mink food	60 g 85 g	Oral	$2.5 \times 10^{7.5}$/g
4	Mink Pigs	3 2	SMSV-5 in tissue culture fluid	2 ml 5 ml	ID, IN	1×10^{8}/ml
5	Pigs	12	Lot #2 ground seal meat	2.5 kg	Oral	

[a]ID — Intradermal; IN — intranasally
[b]$TCID_{50}$ = tissue culture infective $doses_{50}$

TABLE II. Virus Isolations and Serum Antibody Titers to San Miguel Sea Lion Virus (SMSV-5) from Mink in Group 1 (Fed Lot #1 Ground Seal Meat)

Animal No.	Vesicle Formation on Feet	SMSV-5 Virus Isolation[a] 0 DPI R	0 DPI B	2 DPI R	2 DPI B	4 DPI R	4 DPI B	7 DPI R	7 DPI B	10 DPI R	10 DPI B	14 DPI R	14 DPI B	SMSV-5 Virus Neutralization[b] 0 DPI	14 DPI	21 DPI
Mink 30	–	–	–	–	–	–	–	–	–	–	–	–	–	NE	0	0
Mink 33	–	–	–	+	–	–	–	–	–	–	–	–	–	0	0	0
Mink 34	–	–	–	–	–	–	–	–	–	–	–	–	–	0	4	0
Mink 35	–	–	–	–	–	–	–	–	–	–	–	–	–	0	4	4

[a]DPI = Days postinoculation, R = rectal swab and B = heparinized blood samples; + = positive reaction and NE = not examined
[b]Reciprocal of highest serum dilution that neutralized 50 to 100 tissue culture infective $doses_{50}$

2 ml of minimum essential medium (MEM) containing 500 U of penicillin and 250 μg of streptomycin/ml and discarded. This fluid and the heparinized blood samples were stored separately at -70°C until testing. Serum was stored at -20°C until examined.

Virus isolation

Rectal swab washings and heparinized blood samples were thawed and centrifuged 800 x g at -20°C for ten min. Then 0.2 ml of the supernatant was inoculated onto each of four Vero cell cultures in Leighton tubes. The cultures were incubated at 37°C for 30 min before MEM was added. Cultures were observed for seven days and were passaged two more times. The third passage cell fluids from all cultures in which cytopathic effect (CPE) occurred were frozen at -70°C.

Virus isolation from lesions

Epithelial tissues from the vesicular lesions were harvested, ground, diluted 1:10 in MEM with antibiotic, clarified (800 x g) at 20°C for ten min, and inoculated onto Vero cell cultures as described above.

Virus neutralization tests

Preinoculation and 14 and 21 days postinoculation (DPI) serums were assayed for antibodies by virus neutralization (VN) tests. All tests were conducted in MVPK-7 cell cultures. Serial twofold dilutions of serum from animals in groups 1 through 4 were mixed with equal volumes containing 100 $TCID_{50}$ of homologous virus. Serums from animals in group 5 were diluted 1:5 and mixed with equal volumes of tenfold virus dilutions.

RESULTS

GROUP 1 ANIMALS

Group 1 mink (Table II) had no signs of clinical disease but virus was isolated from the rectal swab at two DPI from one mink. Antibodies against SMSV-5 were present at a 1:4 dilution of 14 DPI serums from two mink and the 21 DPI serum from one of these mink.

GROUP 2 ANIMALS

Group 2 results are summarized in Table III. By two DPI, pigs fed the virus-infected pig meat and vesicular tissue homogenate had severe depression and one pig had a vesicle on the posterior portion of the coronary band of each hind foot. Over the next ten days several signs of vesicular disease were seen which included lameness, vesicles on the soles of all four feet, secondary vesicle formation on the snout, diarrhea and weight loss. Temperatures were elevated as high as 41°C before they began to recede.

Virus was recovered from rectal swabs and blood of the two pigs at four DPI and from the rectal swab of one pig at seven DPI. At 21 DPI both pig serums had a 1:16 antibody titer against SMSV.

Mink had no signs of clinical disease nor were VN antibodies to SMSV shown in any of the mink serums but SMSV was recovered from the blood of one mink at four DPI.

GROUP 3 ANIMALS

Group 3 animals were fed SMSV-5 in cell culture fluid mixed with mink feed. Pigs did not have signs of vesicular disease until seven DPI. Lameness was not severe and only one of four feet was effected on each pig. At four DPI, SMSV was recovered from the rectal swab of one pig and from the blood of the other. Serum from one pig had a 1:64 antibody titer at 14 DPI. Both pigs had a 1:16 antibody titer at 21 DPI.

None of the three mink had signs of disease but one had SMSV-5 in rectal swabs taken at ten DPI (Table III). At 14 DPI the three mink had serum antibodies against SMSV at a 1:4 dilution. At 21 DPI only one mink still had the 1:4 serum antibody titer.

GROUP 4 ANIMALS

Pigs inoculated intradermally and intranasally had severe signs of vesicular di-

TABLE III. Virus Isolations and Serum Antibody Titers to San Miguel Sea Lion Virus Type 5 (SMSV-5) from Animals in Group 2 (Fed Infective Pig Meat), Group 3 (Fed Cell Culture Virus) and Group 4 (Inoculated Intradermally and Intranasally)

Animal Group No.	Animal No.	Vesicle Formation on Feet	SMSV-5 Virus Isolation[a]										SMSV-5 Virus Neutralization[b]		
			0 DPI		2 DPI		4 DPI		7 DPI		10 DPI		0 DPI	14 DPI	21 DPI
			R	B	R	B	R	B	R	B	R	B			
2	Mink 37	−	−	−	−	−	−	−	−	−	−	−	0	0	0
2	Mink 38	−	−	−	−	−	−	−	−	−	−	−	0	0	0
2	Mink 40	−	−	−	−	−	−	−	−	−	−	−	0	0	0
2	Mink 41	−	−	−	−	−	−	+	−	−	−	−	0	0	0
2	Pig 5179	+	−	−	−	−	+	+	−	−	−	−	0	NE	16
2	Pig 5181	+	−	−	−	−	+	+	+	−	−	−	0	NE	16
3	Mink 42	−	−	−	−	−	−	−	−	−	−	−	0	4	4
3	Mink 43	−	−	−	−	−	−	−	−	−	+	−	0	4	0
3	Mink 44	−	−	−	−	−	−	−	−	−	−	−	0	4	0
3	Pig 5176	+	−	−	−	−	+	−	−	−	−	−	0	64	16
3	Pig 5180	+	−	−	−	−	−	+	−	−	−	−	0	NE	16
4	Mink 45	−	−	−	−	−	−	−	−	−	−	−	0	4	8
4	Mink 46	+	−	−	−	−	−	−	−	−	−	−	0	4	16
4	Mink 49	−	−	−	−	−	−	−	−	−	−	−	0	16	16
4	Pig 5177	+	−	−	−	−	−	−	−	−	−	−	0	NE	8
4	Pig 5178	+	−	−	+	+	+	+	−	−	−	−	0	NE	16

[a]DPI = Days postinoculation, R = rectal swab, B = heparinized blood samples, + = positive reaction, − = negative reaction and NE = not examined

[b]Titer expressed in reciprocal of high serum dilution that neutralized 50 to 100 tissue culture infective dose$_{50}$ of virus

sease. Temperatures were elevated as high as 40.5°C before receding to normal values in ten to 11 days. SMSV-5 was isolated from rectal swabs and blood of one pig at two and four DPI. Serum antibody titers to SMSV at 21 DPI were 1:8 and 1:16.

At four DPI, one mink had a febrile response and a ruptured vesicle on one toe at the inoculation site. Attempts to isolate a cytopathogenic agent from this lesion were negative. Other than this one mink, signs of clinical disease were not seen in any of the mink. The three mink had serum antibody titers ranging from 1:4 to 1:16 at 14 and 21 DPI.

GROUP 5 ANIMALS

Clinical disease signs were not noticed during the 27 day observation period in the 12 pigs fed lot #2 seal meat. Antibodies to SMSV-5 were not detected in the 12, 20 and 27 DPI serums.

DISCUSSION

Results of virus isolation and antibody detection studies in groups 1, 2, 3 and 4 indicate that SMSV did infect mink and pigs. Although the numbers of pigs and mink were limited, information developed suggests that the disease produced by SMSV in mink is inapparent. Clinical signs of disease seen in mink are difficult to assess because of the active nature and aggressiveness of mink. These mink did not show any depression. In contrast, a severe vesicular disease occurred in pigs exposed to SMSV. Furthermore, animals in groups 1, 2 and 3 were infected via the oral route.

Ground seal meat which originally had a virus titer of $1 \times 10^{5.4}$ $TCID_{50}/g$ and obtained from four seals with ruptured vesicle-like lesions on their flippers was fed to mink in group 1. Immediately before the feeding trial two virus isolation attempts from this meat revealed no virus. However, the isolation of SMSV-5 at two DPI from the rectum of one mink and a twofold increase in serum titer of two mink at 14 DPI indicates that if SMSV was present it was not of sufficient concentration to cause viral replication in the pigs. Storage and shipment conditions may have affected the virus content in both lots of sealburger since the time span from processing to feeding was one year.

In a prior unpublished trial, six pigs were force fed 5.0 ml of 1×10^{8} $TCID_{50}/ml$ of SMSV-1 (4) by emptying a syringe into their mouth. The pigs developed no temperatures or vesicles. But 21 DPI serums (1:10 dilution) from five of six of these pigs neutralized at least one and one-half tenfold dilutions of SMSV-1. SMSV-1 was isolated from rectal swabs of three of these pigs at six DPI and from two pigs at three DPI. This information coupled with the results of group 3 (fed tissue culture virus in mink feed) suggests that the oral route is a less susceptible route of inoculation for SMSV of cell culture origin than of SMSV of swine meat origin.

Previous trials in this laboratory have shown that pigs inoculated ID with SMSV tissue suspensions of pig passage 1 or 2 have more severe clinical signs of vesicular disease than pigs inoculated ID with virus from tissue culture.

Incidence of SMSV isolation from rectal swabs and blood was greater in pigs than in mink. Results with mink in groups 1 and 3 indicate that virus can be recovered in rectal swabs from mink but generally not from the blood, while virus was isolated from blood of pigs at two and four DPI and from the rectal swabs as late as seven DPI.

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REFERENCES

1. **DINKA, S. K., L. M. SWANEY and J. W. McVICAR.** Selection of a stable clone of the MVPK-1 fetal porcine kidney cell for assays of foot-and-mouth disease virus. Can. J. Microbiol. **23**: 295-299. 1977.
2. **SMITH, A. W., C. M. PRATO, W. G. GILMARTIN, R. J. BROWN and M. C. KEYES.** A preliminary report on potentially pathogenic microbiological agents recently isolated from pinnipeds. J. Wildl. Dis. **10**: 54-59. 1974.
3. **SMITH, A. W., C. M. PRATO and D. E. SKILLING.** Characterization of two new serotypes of San Miguel sea lion virus (SMSV). Intervirology **8**: 30-36. 1977.
4. **SMITH, A. W., S. H. MADIN and T. G. AKERS.** Pinnipeds as a possible natural reservoir for a virus disease of domestic swine. pp. 1-10. Proc. 10th Annual Conf. on Biol. Sonar and Diving Mammals. Stanford Research Inst., Menlo Park, California. 1973.