Electron Microscope Observations on a Virus Transmissible from Pinnipeds to Swine

( Accepted 8 March 1977)

SUMMARY

Evidence from immunological tests and electron microscopy indicates that a virus isolated from an Alaskan fur seal is transmissible to swine. The virus is one of the San Miguel sea lion viruses and a member of the calicivirus groups.

A disease of swine that occurred in northern California in 1932 was originally thought to be foot-and-mouth disease (FMD), but was later shown to be a new virus that was named vesicular exanthema of swine virus (VESV; Madin, 1970). Later outbreaks appeared in 1933 and 1934 even though drastic eradication procedures had been initiated after the original outbreak. The only common feature was that hogs had been fed raw garbage from restaurants which had a wide variety of food scraps. Subsequent outbreaks occurred sporadically in California, and later in the 50’s the disease was transmitted to other states. Finally, after great expense to the government, the disease was considered eradicated in 1956 (Madin, 1973). Further details are outlined by Smith & Akers (1976).

In 1972, during investigations of abortions occurring in sea lions on the San Miguel Islands off the coast of California, a virus was isolated which had many physical and biological properties which were similar to those of VESV. In fact, when inoculated into pigs, the virus produced clinical signs identical with those produced by vesicular exanthema virus and was designated San Miguel sea lion virus (SMSV). The properties would put it in the calicivirus group in which there are only two other members, VESV and feline picornavirus (FPV; Smith et al. 1973). This classification was substantiated by the finding that the capsids of all three of these RNA viruses contained a single major polypeptide (Bachrach & Hess, 1973). A recent summary of the relationship between VESV and SMSV has been published (Sawyer, 1976).

In the present study, the virus was originally isolated from a skin lesion in a fur seal (Callorhinus ursinus) and grown in tissue culture, followed by the inoculation of pigs, demonstration of the virus in pig skin lesions and the final recovery of virus in tissue cultures which had been inoculated with pig skin lesion suspension.

Samples of skin affected with wart-like lesions were removed from the tail of a northern fur seal, taken during the annual culling of seals at St Thomas Island, Alaska, frozen and shipped to our laboratory. The samples were logged as Accession 617-75. The lesions were excised, and homogenized as a 20% suspension in phosphate buffered saline (PBS) containing antibiotics (2000 units penicillin, 2000 µg streptomycin, 100 units polymyxin, 100 units mycostatin, and 50 units kanamycin per ml). The suspension was clarified at 800 g for 20 min in a refrigerated centrifuge and the supernate used for initial inoculum.

Primary swine kidney cell cultures in 4-oz prescription bottles with Hanks’ balanced salt solution containing 5 g/l lactalbumin hydrolysate (HLH) and 5% foetal calf serum were inoculated with two separate suspensions of lesions, in 0.5 ml amounts, as undiluted 20% suspension and in several 10-fold dilutions. The cultures were incubated for 65 min at
37 °C, 10 ml of HLH with 2 % foetal calf serum was added as a maintenance medium, and observed daily for cytopathic changes. Subsequent passages of material recovered from the primary passages were made in swine kidney, MVPK (Dinka, Swaney & McVicar, 1977) and Vero cell cultures, and were examined with the electron microscope.

Pooled tissue culture fluids harvested 48 h after inoculation were used to inoculate each of two 40 lb Hampshire pigs held in isolation with two similar sentinel uninoculated pigs. Each infected pig received 2 ml of a 10⁻¹ dilution in the snout. Sera were prepared from all four pigs before inoculation and at intervals thereafter. Fourteen-day post-inoculation sera from all four pigs were used in virus neutralization tests.

For virus isolation from the infected pigs, vesicular material was harvested from the pigs' feet, macerated, and inoculated as a 10 % suspension into primary swine kidney cell cultures in a manner similar to that of the original isolation. Portions of the vesicle lesion sheath were preserved in 0.5 % glutaraldehyde and processed for electron microscopy. The virus in the 10 % suspension contained 10³ TCD₅₀/ml. Two further passages in primary swine kidney cells increased the titre to 10⁸ TCD₅₀/0.5 ml.

### Table 1. Virus neutralization — pig inoculation

<table>
<thead>
<tr>
<th>Sera</th>
<th>Normal pig</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCD₅₀/ml</td>
<td>10⁻⁴⁻⁵</td>
<td>10⁰⁻⁷</td>
</tr>
<tr>
<td>Virus neutralization index</td>
<td>2.6⁻³.6</td>
<td>5.6⁻₅.6</td>
</tr>
</tbody>
</table>

For comparison of the virus isolated from Accession 617-75 with other strains of SMS V a constant serum, 10-fold virus dilution neutralization assay was used. The viruses (Smith et al. 1974) used – designated 15FT, 21FT, W-6, 1223, IFN, 2MT, 1MR, 205, 7MR and 2MR – were derived from varying passage levels in MVPK cells. Five-tenths of a ml of a 1:1 mixture of serum at 1/10 dilution and virus at successive 10-fold dilutions was inoculated into each of two 2-oz Falcon flasks containing Vero cell monolayers. After incubation for 45 min at 37 °C, maintenance fluid was added, and the infected cultures were held at 37 °C and observed daily for cytopathic changes. A similar test was made with the Accession 617-75 virus and individual serums taken from the four pigs 14 days after the first two had been inoculated.

Tissue culture cells and lesion fragments were fixed in 0.5 % glutaraldehyde in Sorenson’s buffer, postfixed in 2 % osmium tetroxide in Sorenson’s buffer and dehydrated in alcohols. The samples were then embedded in Epon, sectioned on a Sorvall MT-2 microtome with a diamond knife, and examined in either an RCA-EMU3G or Philips 201 electron microscope. Samples of purified VESV and SMSV (provided through the courtesy of Dr H. L. Bachrach, PIADC) were prepared in negative stain and photographed at high magnification.

The tissue cultures inoculated with suspensions of lesion materials from the fur seals showed cytopathic effects on the second and third days. The second and third passages reached titres of 10⁸ and 10³⁰ TCD₅₀/ml, respectively.

Virus neutralization tests with the several other SMSV isolates showed that the Accession 617-75 virus was identical to the one previously designated as 205.

Both pigs inoculated with the Accession 617-75 virus developed active disease and had lesions 4 days after inoculation. Both developed lesions on the snout. Pig 5184 had lesions on the coronary bands of all four feet, whereas pig 5183 had vesicles only on both hind feet.
Fig. 1. (a) A typical example of virus particles resulting from infection with original isolation material. Arrows point to virus clusters. (b) Virus clusters in foot lesion material from infected pigs. Virus was infrequently seen and was usually in clusters (arrows). (c) Virus particles seen in swine kidney tissue cultures inoculated with lesion material from infected pigs. Virus was generally in large regular arrays in the samples examined (arrows). (d) Purified sea lion virus, PTA stained.
The two contact pigs (5175, 5182) showed no signs of vesicular disease but were bled to test for antibody production.

In the neutralization test with normal pig serum and the sera from the individual pigs (Table 1), sera from the two infected pigs reduced the titre by 5.6 logs. The two contact pigs did not show frank lesions but their sera neutralized 2.6 and 3.6 logs of virus, respectively, indicating the formation of antibodies.

Electron micrographs were made of tissue culture samples from the original isolation and subsequent passages in MVPK and Vero cell cultures. The appearance of virus was similar in all cell systems with small clusters or individual virus particles distributed in the cytoplasm. Fig. 1(a) shows a virus pattern typical of the three cell types.

The pig foot lesion material proved difficult to embed and section since very hard keratinaceous material was found in most samples. However, after trying several embedmers, virus clusters were found with morphology very similar to that in the cell cultures. Fig. 1(b) shows an example of a typical foot lesion virus cluster. The virus was quite difficult to locate and many more sections of lesions had to be examined than with tissue culture samples.

The final visualization of virus was made in the swine kidney cells infected with the pig foot lesion material. In these sections (Fig. 1c), the pattern of virus clusters was more often crystalline. The virus was rarely seen as individual particles or in small loose clusters previously observed. The inset (Fig. 1d) shows purified SMSV prepared from the fur seal isolation with the typical particle structure of the caliciviruses (Wawrzkiewicz, Smale & Brown, 1968).

The isolation, passage in tissue culture, inoculation into pigs, and recovery of the virus identified as SMSV from pigs identifies the virus as one transmissible from pinnipeds to swine. The experimental evidence would lend support to the previous suggestion that the origins of the disease known in California as vesicular exanthema of swine could have come from the feeding of pigs with contaminated scraps from seals or sea lions. While the demonstration in the laboratory tends to fulfill Koch's postulates for the identification of a causative organism, the final step of inoculation of pig virus into seals or sea lions has not taken place. Later studies (Sawyer, 1976) indicate other marine animals may be involved.

The caliciviruses are a group separate from the other members of the family picornaviridae. The pattern of arrays of virus particles in tissue culture and in infected tissue is another characteristic that can be used in their classification and can be added to evidence previously published (Burroughs & Brown, 1974). Virus assembly of SMSV is similar to that shown by Studdert & O'Shea (1975) for feline virus and Zee, Hackett & Madin (1968) for vesicular exanthema of swine. The transmission of the virus from sea lion to tissue culture and then to pigs and tissue culture did not change the morphology of the virus cluster patterns.

We would like to thank J. J. Giovannelli, S. J. Pyne and J. Sorenson for excellent technical assistance.

U.S. Department of Agriculture
Agricultural Research Service
Plum Island Animal Disease Center
Greenport, New York 11944, U.S.A.
Short communications

REFERENCES


(Received 31 January 1977)