Naturally Occurring Picornavirus Infection of Domestic Mink

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ABSTRACT

The isolation and preliminary characterization of a virus from domestic mink are reported. The virus was tentatively classified as a member of the family Picornaviridae on the basis of its physicochemical properties. The mink virus was not neutralized by antiserum to some known members of the calicivirus genus, which included the nine serotypes of vesicular exanthema of swine virus, ten serotypes of San Miguel sea lion virus and feline calicivirus. Seroepidemiological studies indicated that the incidence of mink virus infection was widespread in domestic mink populations. Although the virus was isolated from mink on ranches with a history of hemorrhagic pneumonia (pseudomonas pneumonia), no specific disease process could be attributed to the virus infection.

RÉSUMÉ

Cet article rapporte l'isolation d'un virus chez des visons domestiques, et son identification préliminaire. Ses propriétés physico-chimiques amenèrent les auteurs à le classifier provisoirement comme un membre de la famille des Picornaviridae. Des antisérums spécifiques à certains membres du genre calicivirus, entre autres neuf sérotypes du virus de l'exanthème vésiculaire porcin, dix sérotypes du virus de l'otarie de San Miguel et le calicivirus félin, ne neutralisèrent pas ce virus. Des études séro-épizootiologiques révélèrent que cette infection virale était passablement répandue dans les visonnières. Même si on isola ce virus dans des visonnières où avait sévi la pneumonie hémorragique imputable à Pseudomonas aeruginosa, on ne put attribuer aucune maladie spécifique à cette infection virale.

INTRODUCTION

A number of viral diseases of mink have been characterized. Of these, aleutian disease (AD) is probably the most widespread (8). A picornavirus of mink was originally described as being associated with AD (34). However, the causative agent was subsequently shown to be a temperature sensitive parvovirus (19). Another parvovirus infection of mink, mink virus enteritis, is caused by a virus sharing common antigens with feline panleukopenia virus (11). Other than canine distemper virus (9), no specific respiratory viral infection of mink has been reported.

Viruses of unknown clinical significance in mink include a reovirus isolated from mink with viral enteritis (14). An endogenous C type RNA virus has been detected in mink cells after long-term passage or cocultivation with other cell types (1, 13).

The virus described in this report was isolated during studies on the pathogenesis and epidemiology of hemorrhagic pneumonia (pseudomonas pneumonia) of mink. Hemorrhagic pneumonia is an acute disease of mink in which *Pseudomonas aeruginosa*

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appears to be the primary pathogen (25). In an attempt to identify other agents that could be involved in the pathogenesis of hemorrhagic pneumonia, viral isolation was attempted from mink on ranches experiencing the disease. This communication reports on the isolation, preliminary viral characterization and seroepidemiology of a virus from mink with properties consistent with those of the family Picornaviridae.

MATERIALS AND METHODS

CELL CULTURES

Primary mink kidney (MK) cells, Crandell feline kidney (CrFK) cells and Vero cells were obtained from the Companion Animal Virology Laboratory at Washington State University (WSU), Pullman, Washington. Cells were grown at 37°C in F-15 medium[1] containing 10% fetal bovine serum[2]. Fifty μg gentamicin[3] and 25 μg fungizone[4] were added per mL of medium.

VIRUSES

Virus isolation was attempted from mink on two commercial ranches (A and B) that had experienced recent outbreaks of hemorrhagic pneumonia. Pharynegeal and rectal swabs were obtained from mink housed near those that had died of pneumonia. Swabs were transferred to transport medium consisting of 1% peptone, 0.25% gelatin and 50 μg/mL neomycin sulfate in phosphate buffered saline (PBS). Transport media were inoculated onto MK cells and passaged three times. Cell cultures showing cytopathic effect (CPE) were harvested and the supernatant fluid was inoculated onto Vero cells. Viral isolates were plaque purified in Vero cells by standard agar overlay technique (10). A single isolate, designated MV20-3, was used for all serological and viral characterization studies. A stock virus pool was prepared in Vero cells, harvested at maximum CPE, aliquoted and stored at –70°C. The pool had a titer of $10^{4.7}$ $TCID_{50}$ per 0.025 mL.

Feline calicivirus (FCV) strain FR1-14 (5) was obtained from American Type Culture Collection[5]. Feline herpesvirus (FHV), isolate 79-43, was isolated at WSU and identified by FHV-specific antiserum. Viral stocks of FCV and FHV were produced in CrFK cells and had titers of $10^{5.2}$ and $10^{6.0}$ $TCID_{50}$/0.025 mL, respectively. Microtiter assay results (24) were used to calculate viral titers according to the method of Reed and Muench (21).

PHYSICOCHEMICAL PROPERTIES

Indirect nucleic acid determination was conducted using 5-iodo-2-deoxyuridine (IUDR) according to the method of Crandell and Weddington (6). Lipid solvent sensitivity and virus particle size were estimated by standard techniques (10). Sensitivity to acid was determined using McIlvaine's buffer according to the method of Studdert *et al* (28). The effect of 1 M $MgCl_2$ on the heat stability of the viruses was determined according to the method of Wallis and Melnick (31).

LIGHT AND ELECTRON MICROSCOPY

The cytopathic effect (CPE) of MV20-3 was studied in Vero cells grown on coverslips in 24-well plastic trays. Coverslips were fixed at 12, 24, 36 and 48 hours post-infection with Zenker's fixative and stained with hematoxylin and eosin. At the same time intervals, infected cells in 75 cm^2 plastic flasks were washed in PBS, fixed with glutaraldehyde, embedded in epon-araldite and sectioned for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined in a Phillips 200 electron microscope.

SEROLOGICAL IDENTIFICATION

Antibody to MV20-3 was produced in adult male cross-bred domestic rabbits. Antisera to ten serotypes of San Miguel sea lion virus (SMSV) and nine serotypes of vesicular exanthema of swine virus (VESV) were obtained from A. Smith, Naval Biological Laboratory, San Diego, California. Specific antiserum to FCV was obtained from E. Davis, Norden Laboratories, Lincoln, Nebraska. All the sera were

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[1]Grand Island Biological, Grand Island, New York.

[2]Flow Laboratories, Inglewood, California.

[3]Schering Corporation, Kenilworth, New Jersey.

[4]Squibb and Sons, New York, New York.

[5]Rockville, Maryland.

tested for neutralizing antibody to MV20-3 by standard microtiter assay (24).

SEROEPIDEMIOLOGICAL STUDIES

Sera from mink were tested for neutralizing antibody to MV20-3 by microtiter assay. Sera were obtained at pelting (November-December, 1977) from ranches A and B. Sera were also obtained from two commercial ranches (C and D) without a history of hemorrhagic pneumonia. To determine the incidence of the mink virus infection and to locate a potential source of seronegative mink for pathogenesis studies, sera were obtained from mink located at various institutional ranches throughout the United States and Japan. These included: Washington State University, Oregon State University (courtesy J. Adair), Cornell University (courtesy of H. Travis), Michigan State University (courtesy of T. Bell), University of Wisconsin (courtesy of R. Marsh), Rocky Mountain Laboratory, Hamilton, Montana (courtesy of W. Hadlow) and Hokkaido, Japan (courtesy of J. Y. Homma, Tokyo, Japan). All sera were tested for antibodies to AD virus by immune counterelectrophoresis (7).

RESULTS

VIRAL ISOLATION

Viral isolates were obtained from pharyngeal swabs of one mink on ranch A and two mink on ranch B. These were designated MV20, MV9 and MV13, respectively. Isolates MV9 and MV13 were subsequently found to be completely neutralized by antiserum to MV20-3.

PHYSICOCHEMICAL PROPERTIES

Treatment with IUDR did not affect the replication of MV20-3 and the known RNA virus, FCV. IUDR reduced the yield of the known DNA virus, FHV, by 10^4 $TCID_{50}$/0.025 mL. Isolate MV20-3 was neither inactivated by treatment with chloroform, nor was there any significant loss of viral infectivity observed after filtration through a 50 nm filter.

The sensitivity of MV20-3 to acidic conditions was similar to that of FCV. Neither virus was significantly affected by treatment at pH 4. Treatment at pH 3 decreased the titer of both viruses to less than 1.0 $\log_{10}$ $TCID_{50}$/0.025 mL. Both MV20-3 and FCV were inactivated by incubation at 50°C for one hour. Neither virus was stabilized against the effects of heat by 1 M $MgCl_2$.

LIGHT AND ELECTRON MICROSCOPY

The CPE caused by MV20-3 in Vero cells was characterized by withdrawal of cell processes and dissociation of rounded cells from the monolayer within 24 hours. Inclusion bodies were not observed. Viral particles were oriented along fibrils within the cytoplasm (Fig. 1) and in proximity to the plasma membrane (Fig. 2). Viral particles had a diameter of 25-30 nm and a center-to-center spacing of 30-45 nm.

ANTIGENIC ANALYSIS

Isolate MV20-3 (100 $TCID_{50}$) was not neutralized by antisera to VESV (nine serotypes), SMSV (ten serotypes), or FCV at serum dilutions of 1:5 or greater. Isolate MV20-3 was neutralized by homologous antiserum at dilutions of up to and including 1:640.

SEROEPIDEMIOLOGY STUDIES

Viral neutralizing antibody to MV20-3 was found in the sera of 80-100% of the mink from each of the four commercial and seven institutional herds (Table I). Titers of sera neutralizing 100 $TCID_{50}$ of virus ranged from 1:5 to 1:320. Precipitating antibody to AD virus was found in approximately 10-90% of the sera from each herd.

DISCUSSION

Preliminary characterization of MV20-3 indicated that it is a member of the family Picornaviridae. Results of seroepidemiological studies showed that infection with MV20-3 is widespread in domestic mink populations. This widespread infection could account for the original isolation of a mink picornavirus by investigators studying the etiology of AD (12). In our study, there was no apparent relationship between the occurrence of AD antibody and titer of MV20-3 antibody.

Isolate MV20-3 had similarities to calciviruses in its cytopathogenicity, pH sensitivity and lack of $MgCl_2$ thermostabilization (18, 28, 32, 35). The ultrastructural

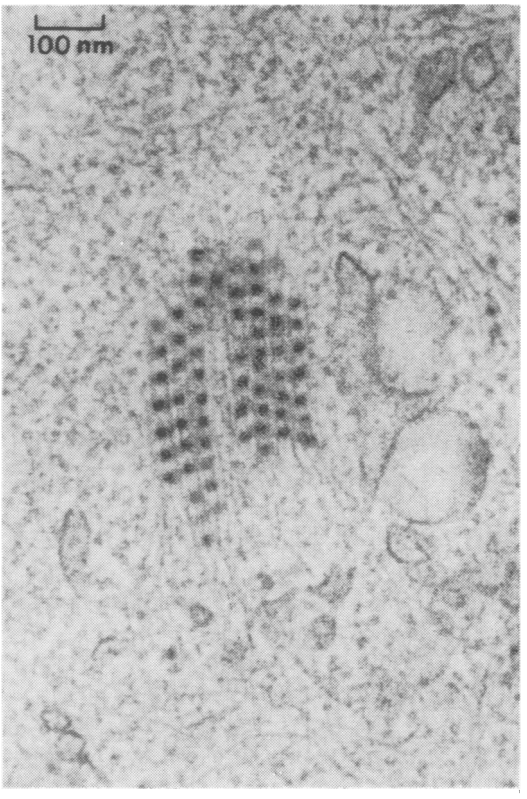

Fig. 1. Mink picornavirus (MV20-3) 24 hours postinoculation of Vero cells. Note the paracrystalline array of viral particles within the cytoplasm.

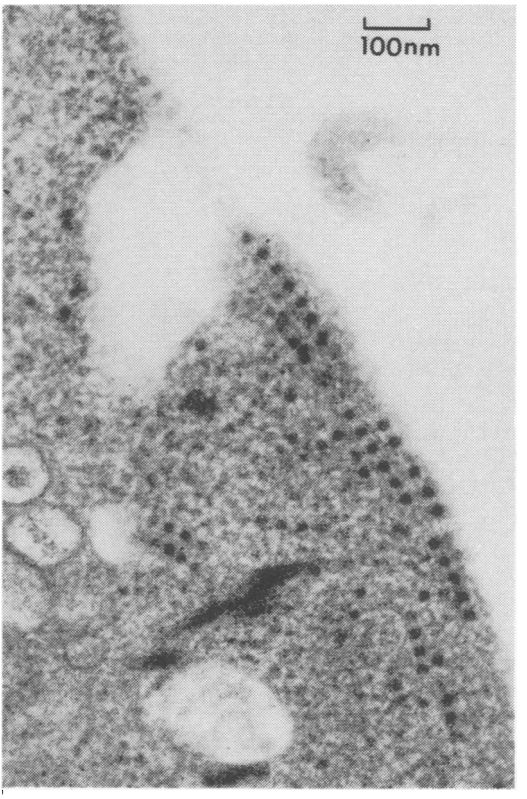

Fig. 2. Mink picornavirus (MV20-3) 36 hours postinoculation of Vero cells. Note linear arrays of viral particles in proximity to the cell membrane.

appearance of viral particles in infected Vero cells was similar to that observed with certain caliciviruses (16, 30, 36). This was especially evident in the size and spacing of particles and the formation of paracrystalline arrays along microfibrils. Further characterization of MV20-3 is necessary to clarify its taxonomic position. This would include ultrastructural examination and determination of biochemical characteristics such as the composition of polypeptides and RNA (2, 4).

Virus neutralization studies showed no detectable serological relationship between MV20-3 and the defined mammalian and marine caliciviruses. Distinct serological types of VESV and SMSV have been reported (26, 29) and FCV, VESV and SMSV are readily differentiated by neutralization tests (3). Other immunological tests have shown VESV and SMSV to be closely related to each other, but not to FCV (3, 27).

The geographical relationship of occurrence of VESV and SMSV led some to propose that both viruses may have arisen from a marine source (17). The common use of marine by-products in mink diets prompted us to look for possible antigenic relationships between MV20-3 and VESV and SMSV. Northern fur seal meat is fed to mink in certain areas of the United States and SMSV has been isolated from this mink food (23). None of the mink sampled in the present study were known to be fed meat from fur seals. Marine fish products were included in the diet on some ranches.

Experimental infection of mink with SMSV has been reported (33). Except for one mink with vesiculation at the site of intradermal inoculation, the infection was inapparent. Mink had no detectable virus neutralizing antibody to SMSV before infection and developed titers of 1:4 to 1:16 after infection. Mink are also apparently resistant to infection with VESV (22).

TABLE I. Prevalence of Naturally Occurring Virus-Neutralizing Antibody to MV20–3 in Mink

Herds	No. Positive[a] / No. Sera Tested	% Positive	Titer Ranges[b]
Commercial			
A	24/24	100	1:20 – ≥ 1:320
B	24/24	100	1:10 – ≥ 1:320
C	23/24	96	1:5 – ≥ 1:320
D	24/24	100	1:10 – ≥ 1:320
Institutional			
Michigan	14/14	100	1:10 – 1:160
Montana	20/20	100	1:5 – ≥ 1:320
New York	20/20	100	1:20 – ≥ 1:320
Oregon	24/24	100	1:10 – ≥ 1:320
Washington	12/12	100	1:5 – ≥ 1:320
Wisconsin	18/19	95	1:5 – ≥ 1:320
Japan[c]	32/40	80	1:10 – ≥ 1:320

[a]Number of mink sera with neutralizing titer to 100 $TCID_{50}$ of MV20–3 of 1:5 or greater
[b]Sera were tested at dilutions of 1:5 to 1:320
[c]Sera from Japan were tested at an initial dilution of 1:10

Because the isolations of MV20-3 were from pharyngeal swabs of apparently normal mink, the possibility of a carrier state exists. The observation that FCV can be carried in the nasopharyngeal region of cats for prolonged periods without demonstrable clinical symptoms (20) supports this contention.

Further work on the pathogenesis of the mink picornavirus would be facilitated by locating a source of seronegative mink. Experimental infection of ten mink kits (ten to 12 weeks old) from ranch D resulted in no clinical disease or development of lesions (15). Sixty mink from ranch D examined serologically for two months (eight to 18 weeks of age) demonstrated an overall increase in viral neutralizing titers, but no significant clinical disease was recognized in these animals. The relationship of MV20-3 to pseudomonas pneumonia could not be determined on the basis of the data presented. However, it is possible that infection by this virus may predispose mink to a more severe pseudomonas infection that may result in the clinical disease of hemorrhagic pneumonia.

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