Archives of Virology 76, 257—261 (1983)

Archives of Virology

Ultrastructure of Newly Recognized Caliciviruses of the Dog and Mink

Brief Report

By

J. F. Evermann[1], A. W. Smith[2], D. E. Skilling[2], and A. J. McKeirnan[1]

[1] Department of Veterinary Clinical Medicine and Surgery and the Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, Washington, and

[2] School of Veterinary Medicine, Oregon State University, Corvallis, Oregon, U.S.A.

With 3 Figures

Accepted February 10, 1983

Summary

Two recently recognized viruses obtained from a dog with glossitis and from mink with hemorrhagic pneumonia were characterized by electron microscopy. The results of the negative-stained preparations indicated that the viruses were structurally compatible with the calicivirus group.

*

During studies on the pathogenesis of hemorrhagic pneumonia syndrome in mink, viruses were isolated and initially reported as picornaviruses on the basis of physicochemical criteria (6). This report presents the ultrastructure of this virus, as well as that of another recently recognized calicivirus isolated from a dog with glossitis and gingivitis (4).

The mink virus (MV 20-3) was grown in Vero cells and had a titer of $10^{5.8}$ $TCID_{50}$ per ml. The canine glossitis virus (WSU 79-1831) was grown in Crandell feline kidney (CrFK) cells and had a titer of $10^{5.3}$ $TCID_{50}$ per ml. Specimen preparation for electron microscopy consisted of inoculation of a roller tube of either Vero cells or CrFK cells with 0.1 ml of the respective stock virus. After 1 hour incubation, cell culture medium was added and the tubes incubated at 37° C on a roller drum. When the cells showed 3+ cytopathogenic effect the tubes were frozen and thawed one time and centrifuged at $2000 \times g$ for 10 minutes. An aliquot (480 µl) of supernatant from the tube was further clarified by centrifugation in a Beckman Airfuge 30° fixed angle rotor at $23{,}000 \times g$ for 5 minutes.

0304-8608/83/0076/0257/$ 01.00

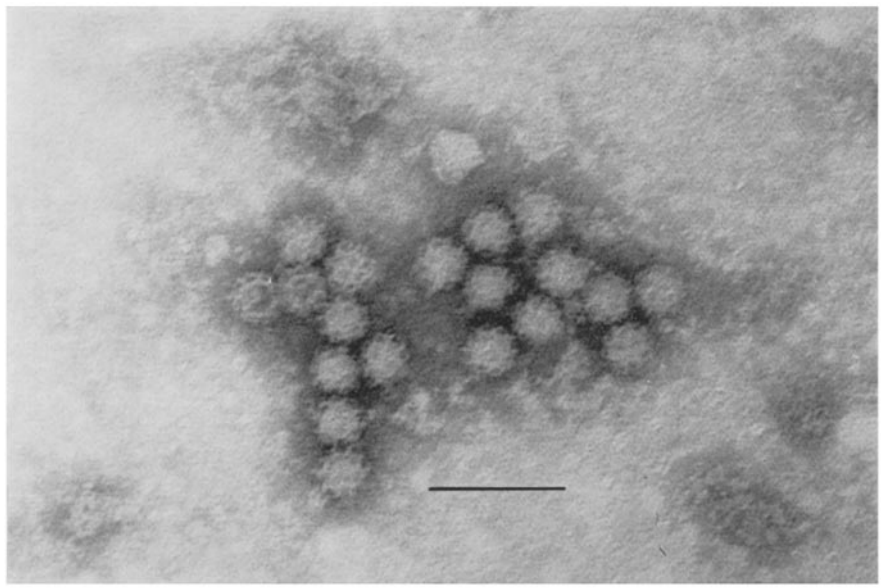

Fig. 1. Negative stained electronphotomicrograph of mink calicivirus, MV 20—3. Bar represent 100 nm

The virus-containing supernatant was then centrifuged in the aforementioned apparatus at 165,000 × *g* for 10 minutes. The pelleted virus was resuspended in 50 μl of distilled water and was pelleted directly onto a carbon coated grid using the Beckman Airfuge EM-90 rotor at 118,000 × *g* for 10 minutes. The grids were stained with 1.5 percent phosphotungstic acid for 1 minute, blotted dry, and examined with a Philips 300 transmission electron microscope at 80 kV. The viruses were sized by using a stock culture of tobacco mosaic virus for internal size standardization.

The mink calicivirus (MV 20-3) had exhibited properties consistent with the calicivirus family in earlier studies (6). These features included: physicochemical properties; growth in Vero cells; and electron microscopy of cell-associated virus particles. The results from this study confirmed that the virus was structurally related to the calicivirus family. The virus particles were approximately 35—40 nm in diameter with a patterned dark staining surface (Fig. 1). Higher magnification revealed 10 evenly spaced cup-shaped depressions on the periphery of the virions (Fig. 2).

The canine glossitis virus (WSU 79-1831) had been previously shown to be antigenically related to feline calicivirus on the basis of serum neutralizing tests (4). The electron microscopic studies reported herein (Fig. 3) indicated that the virion structure was also consistent with the Caliciviridae Family (9, 14).

These studies served to substantiate the reports of calicivirus infections of mink and dogs and they can be added to an ever expanding list of caliciviruses now recognized to infect humans, primates, foxes, calves, fish, and chickens (2, 5, 7, 10, 11, 12, 13, 15). Of interest with both of these recently recognized caliciviruses is their origin and pathogenesis in their natural host. Since mink

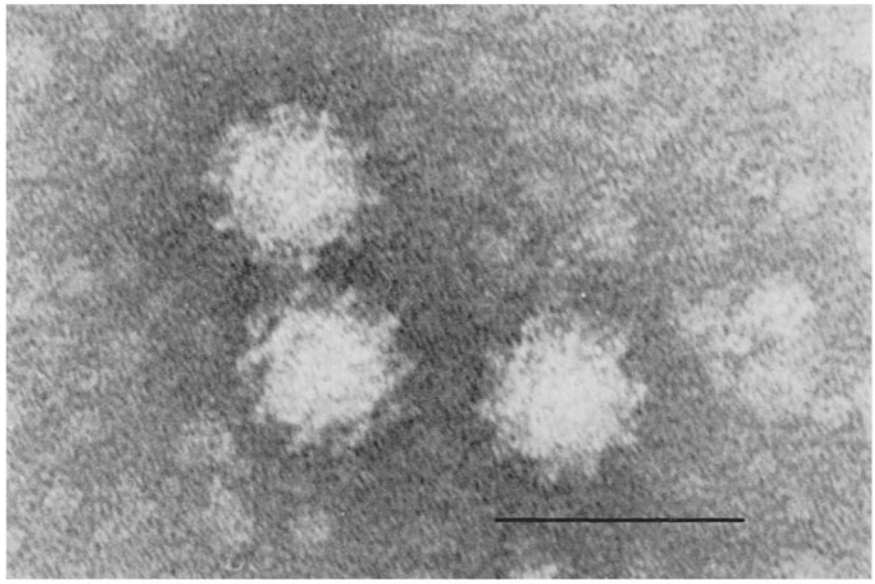

Fig. 2. Negatively stained electronphotomicrograph of mink calicivirus, MV 20—3 showing the ten cup-like surface projections. Bar represents 50 nm

are customarily fed marine by-products (8) there is the possibility that they acquired the calicivirus from marine species. San Miguel sea lion virus (SMSV) has been demonstrated to occur in seal meat fed to mink in the United States

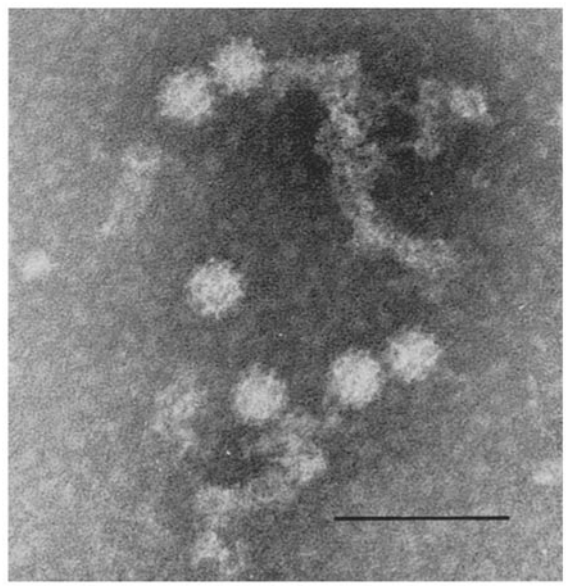

Fig. 3. Negatively stained electronphotomicrograph of canine calicivirus, WSU 79-1831. Bar represents 100 nm

(8). However, the mink calicivirus (MV 20-3) was shown not to be antigenically related to the ten SMSV serotypes available at that time (6).

On the basis of seroepizootiologic data infection by the mink calicivirus is widespread in the mink population (6). While associated with hemorrhagic pneumonia of mink, the virus has not been shown experimentally to cause disease in mink. Further studies are necessary to determine if the mink calicivirus predisposes mink to *Pseudomonas aeruginosa* infection as has been proposed (6).

The canine glossitis virus was isolated in 1979 coincident with a world-wide epizootic of canine parvovirus (CPV), which was the cause of myocarditis and enteritis in susceptible wild and domestic canids (1, 3). Once it was determined that CPV was antigenically related to feline panleukopenia the use virus vaccines of feline origin containing FPL virus in dogs was encouraged in an effort to control the CPV infection (1).

However, since some of the virus vaccines of feline origin also contained modified live feline calicivirus and feline herpesvirus together with FPL virus, it remains a possibility that the canine glossitis virus was transmitted from a dog receiving modified live feline calicivirus in combination with FPL virus. Additional studies are necessary to determine the pathogenesis of the canine glossitis virus in susceptible dogs and cats.

References

1. Carmichael, L. E., Binn, L. N.: New enteric viruses in the dog. Adv. Vet. Sci. Comp. Med. **25**, 1—37 (1981).
2. Cilli, V., Castrucci, G.: Viral diarrhea of young animals: A review. Comp. Immunol. Microbiol. Infec. Dis. **4**, 229—242 (1981).
3. Evermann, J. F., Foreyt, W., Maag-Miller, L., Leathers, C. W., McKeirnan, A. J., LeaMaster, B.: Acute hemorrhagic enteritis associated with canine coronavirus and parvovirus infections in a captive coyote population. JAVMA **177**, 784—786 (1980).
4. Evermann, J. F., Bryan, G. M., McKeirnan, A. J.: Isolation of a calicivirus from a case of canine glossitis. Canine Prac. **8**, 36—39 (1981).
5. Flewett, T. H.: Electron microscopy in the diagnosis of infectious diarrhea. JAVMA **173**, 538—543 (1978).
6. Long, G. G., Evermann, J. F., Gorham, J. R.: Naturally occurring picornavirus infection of domestic mink. Cand. J. Comp. Med. **44**, 412—417 (1980).
7. Prato, C. M., Akers, T. G., Smith, A. W.: Calicivirus antibodies in wild fox populations. J. Wildl. Dis. **13**, 448—450 (1977).
8. Sawyer, J. C., Madin, S. H., Skilling, D. E.: Isolation of San Miguel sea lion virus from samples of an animal food product produced from northern fur seal *(Callorhinus ursinus)* carcasses. Am. J. Vet. Res. **39**, 137—139 (1978).
9. Schaffer, F. L.: Caliciviruses. In: Newly Characterized Vertebrate Viruses. Comp. Virol. **14**, 249—284 (1979).
10. Smith, A. W., Akers, T. G., Latham, A. B., Skilling, D. E., Bray, H. L.: A new calicivurs isolated from a marine mammal. Arch. Virol. **61**, 255—259 (1979).
11. Smith, A. W., Prato, C., Skilling, D. E.: Caliciviruses infecting monkeys and possibly man. Am. J. Vet. Res. **39**, 287—289 (1978).
12. Smith, A. W., Skilling, D. E., Dardiri, A. H., Latham, A. B.: Calicivirus pathogenic for swine: A new serotype isolated from Opaleye *(Girella nigricans)*, an ocean fish. Science **209**, 940—941 (1980).
13. Smith, A. W., Skilling, D. E., Latham, A. B.: Isolation and identification of five new serotypes of calicivirus from marine mammals. Am. J. Vet. Res. **42**, 693—694 (1978).

14. STUDDERT, M. J.: Caliciviruses. Arch. Virol. **58**, 157—191 (1978).
15. WYETH, P. J., CHETTLE, N. J., LABRAM, J.: Avian calicivirus (Letter). Vet. Rec. **109**, 477 (1981).

Authors' address: Dr. J. F. EVERMANN, Department of Veterinary Clinical Medicine and Surgery and the Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, U.S.A.

Received August 23, 1982