

Detection of Astroviruses in Feces of a Cat with Diarrhea*

Brief Report

By

Y. HOSHINO^{1, **}, J. F. ZIMMER², N. S. MOISE², and F. W. SCOTT¹

¹ Cornell Feline Health Center and the Departments of Microbiology,
and

² Clinical Sciences, New York State College of Veterinary Medicine, Cornell University,
Ithaca, New York, U.S.A.

With 1 Figure

Accepted September 28, 1981

Summary

Astroviruses were detected by electron microscopy in the feces from a 4 month old kitten with diarrhea. The mean diameter of the viral particles was 28.7 nm, and they showed characteristic five- or six-pointed star-shaped surface configurations. The clinical disease manifested by the cat and the observed morphology of the viral particles are consistent with previous reports on astroviruses of other species.

*

Viral gastroenteritis is one of the leading causes of morbidity and mortality in neonatal and young animals, birds and man throughout the world (2, 3, 5, 17, 19). The development of methods for identification of viruses in fecal samples by direct electron microscopy (EM) has led to the discovery of a number of previously unrecognized enteric viruses, including astroviruses. Astroviruses are isometric, approximately 28 nm in diameter viral particles which, when negatively stained, give the impression of a white, five- or six-pointed star superimposed on the particle. Astroviruses have been detected in the feces of humans (1, 8, 9, 12, 13, 15, 18), calves (23), lambs (20) and turkeys (16) with diarrhea. Since attempts to propagate the mammalian and avian astroviruses in cell cultures have been unsuccessful, their size and morphology as determined by EM are the primary criteria by which they are identified. Viral antigen was detected by indirect immunofluorescent staining within the cytoplasm of cultured cells infected with fecal material of human (10, 11) and bovine (23) astroviruses. However, these infections were abortive in

* This study was supported from unrestricted contributions to the Cornell Feline Health Center.

** Present address: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, U.S.A.

that no specific fluorescence was seen in passaged cell cultures. Recently, HERRING *et al.* (4) reported that ovine astrovirus has a single-stranded RNA genome which resembles that of the picornaviruses and caliciviruses but the polypeptide composition is unlike that of either of these groups. We report here the detection by direct EM of astroviruses in feces from a 4 month old kitten with diarrhea.

An intact female 4-month-old kitten was presented to the Small Animal Hospital at the New York State College of Veterinary Medicine, Cornell University, on October 4, 1979. The chief complaint was that the kitten had had diarrhea, characterized as loose and watery, for approximately one week. A tentative diagnosis of parasite infestation and a diet-induced diarrhea was made, and the cat was vaccinated for feline panleukopenia, rhinotracheitis, and calicivirus. The cat was returned five days later (October 9) with the report that the diarrhea had continued and that the cat had been anorectic for three days. The diarrhea was reportedly more severe than on the initial visit. Physical examination revealed slight dehydration, poor body condition, gas-distended loops of small intestine, and a green, watery diarrhea. A fecal sample was submitted for EM examination for viral particles.

Two days later (October 11), the kitten was hospitalized because of more severe dehydration and diarrhea. The results of a hemogram were normal, but the kitten was mildly acidotic and hypokalemic. During the subsequent nine days of hospitalization, the kitten's feces improved in consistency. The cat was discharged on October 20. Re-examinations one week and 3 months after discharge indicated that the kitten improved steadily and made an uneventful recovery. The feces had become normal, and the appetite was good.

Fecal sample was examined by EM for viral particles using the procedures reported previously (6). A 20 percent (w/v) fecal suspension was prepared in Eagle's minimum essential medium (MEM) with antibiotics and without serum. This suspension was centrifuged at $1,200 \times g$ for 10 minutes (crude fecal suspension), and the supernatant was then ultracentrifuged at $10,000 \times g$ for 10 minutes (clarified fecal suspension). One to 4 drops of crude fecal suspension were mixed with 20 drops of distilled water, one drop of 0.1 percent bovine serum albumin and three to four drops of 4 percent phosphotungstic acid adjusted to pH 7.0. This mixture was applied to a carbon-parlodion-coated copper grid with an all-glass nebulizer and examined in a Philips 201 electron microscope at 80 KV.

Direct EM examination demonstrated the presence of astrovirus-like particles, mostly in aggregates, in the fecal suspension (Fig. 1). These viral particles were roughly spherical in outline, ranged in size from 27.5 nm to 29.4 nm in diameter and had a mean diameter of 28.7 nm. The surface structure had the appearance of characteristic five- or six-pointed stars (Fig. 1, arrows). This star-shaped configuration was not always apparent on all particles. Bridging structures between virus particles were frequently seen (Fig. 1, arrowheads). These structures have been observed on human (14) and ovine (20) astroviruses. It is speculated that these bridging structures may be surface fibers similar to those on adenoviruses.

Attempts at viral isolation with cell cultures were carried out as previously reported (7). Confluent monolayer cultures (16×125 mm culture tubes) of first transfer cells of feline kidney and an established fetal rhesus monkey kidney cell line (MA 104) were inoculated with 0.1 ml of clarified fecal suspension. After one

hour adsorption at 37° C, cell cultures were washed once with phosphate buffered saline, pH 7.2, fed with 1.0 ml of maintenance medium (Eagle's MEM supplemented with 0.01 percent lactalbumin hydrolysate, 0.075 percent sodium bicarbonate, antibiotics and no serum), and incubated at 35° C. Three blind passages were made at one week intervals. Attempts to propagate this feline astrovirus in cell cultures have been unsuccessful. No calicivirus or other cytopathogenic virus was isolated in cell cultures.

Experimental transmission experiments with mammalian astroviruses indicate that they induce mild, constitutional symptoms (10, 20, 21, 23). Studies on the pathogenesis of astrovirus infection in lambs have shown the site of virus multiplication to be the mature villous epithelial cells of the small intestine (21, 22). There is a clinical impression that astrovirus-associated gastroenteritis in man and animals is generally milder than that associated with rotaviruses. Asymptomatic excretion of astrovirus particles has been reported in man (1, 12), calves (23) and lambs (20).

Studies on the pathogenicity of feline astrovirus have been hampered by a shortage of material. As this is the first report of the identification of a feline astrovirus and since no serological test is available, it is unknown how widespread and how significant this virus is. Since cats frequently have close contact with humans, it will be important to study the relationship between feline and human astroviruses.

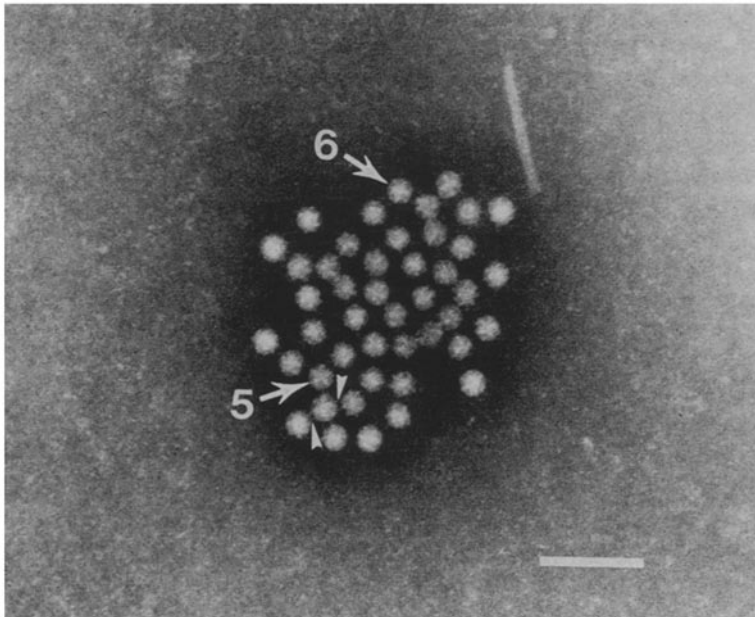


Fig. 1. Electron micrograph of negatively stained astroviruses seen in a fecal sample from a 4 month old kitten with diarrhea. Both five- and six-pointed star-shaped surface configurations are seen (one of each is shown with an arrow and a number). Bridging structures between viral particles are seen (arrowheads). Bar represents 100 nm

References

1. ASHLEY, C. R., CAUL, E. O., PABER, W. K.: Astrovirus-associated gastroenteritis in children. *J. clin. Pathol.* **31**, 939—943 (1978).
2. BANATVALA, J. E.: The role of viruses in acute diarrhoeal disease. *Clinics in Gastroenterol.* **8**, 569—598 (1979).
3. EUGSTER, A. K., SNEED, L.: Viral intestinal infections of animals and man. *Comp. Immun. Microbiol. Infect. Dis.* **2**, 417—435 (1980).
4. HERRING, A. J., GRAY, E. W., SNODGRASS, D. R.: Purification and characterization of ovine astrovirus. *J. gen. Virol.* **53**, 47—55 (1981).
5. HOLMES, I. H.: Viral gastroenteritis. *Prog. med. Virol.* **25**, 1—36 (1979).
6. HOSHINO, Y., SCOTT, F. W.: Coronavirus-like particles in the feces of normal cats. *Arch. Virol.* **63**, 147—152 (1980).
7. HOSHINO, Y., BALDWIN, C. A., SCOTT, F. W.: Isolation and characterization of feline rotavirus. *J. gen. Virol.* **54**, 313—323 (1981).
8. KJELDSBERG, E.: Small spherical viruses in faeces from gastroenteritis patients. *Acta Path. Microbiol. Scand.* **B 85**, 351—354 (1977).
9. KURTZ, J. B., LEE, T. W., PICKERING, D.: Astrovirus-associated gastroenteritis in a children's ward. *J. clin. Pathol.* **30**, 948—952 (1977).
10. KURTZ, J. B., LEE, T. W., CRAIG, J. W., REED, S. E.: Astrovirus infection in volunteers. *J. med. Virol.* **3**, 221—235 (1979).
11. LEE, T. W., KURTZ, J. B.: Astroviruses detected by immunofluorescence (letter). *Lancet* **2**, 406 (1977).
12. MADELEY, C. R., COSGROVE, B. P.: Viruses in infantile gastroenteritis (letter). *Lancet* **2**, 124 (1975).
13. MADELEY, C. R., COSGROVE, B. P.: 28-nm particles in feces in infantile gastroenteritis (letter). *Lancet* **2**, 451—452 (1975).
14. MADELEY, C. R.: Comparison of the features of astroviruses and caliciviruses seen in samples of feces by electron microscopy. *J. inf. Dis.* **139**, 519—523 (1979).
15. MADELEY, C. R.: Viruses in the stools. *J. clin. Pathol.* **32**, 1—10 (1979).
16. McNULTY, M. S., CURRAN, W. L., MCFARRAN, J. B.: Detection of astroviruses in turkey faeces by direct electron microscopy. *Vet. Rec.* **106**, 561 (1980).
17. MONTO, A. S., LOOPMAN, J. S.: The Tecumseh study. XI. Occurrence of acute enteric illness in the community. *Amer. J. Epidemiol.* **112**, 323—333 (1980).
18. PEIGUE, H., BEYTOU-MONGHAL, M., LAVERAN, H., BOURGES, M.: Coronavirus et "astrovirus" observés dans les selles d'enfants atteints de gastroentérites. *Ann. Microbiol. (Inst. Pasteur)* **129 B**, 101—106 (1978).
19. SCHREIBER, D. S., TRIER, J. S., BLACKLOW, N. R.: Recent advances in viral gastroenteritis. *Gastroenterol.* **73**, 174—183 (1977).
20. SNODGRASS, D. R., GRAY, E. W.: Detection and transmission of 30-nm virus particles (astroviruses) in faeces of lambs with diarrhoea. *Arch. Virol.* **55**, 287—291 (1977).
21. SNODGRASS, D. R., ANGUS, K. W., GRAY, E. W., MENZIES, J. D., PAUL, G.: Pathogenesis of diarrhoea caused by astrovirus infections. *Arch. Virol.* **60**, 217—226 (1979).
22. GRAY, E. W., ANGUS, K. W., SNODGRASS, D. R.: Ultrastructure of the small intestine in astrovirus-infected lambs. *J. gen. Virol.* **49**, 71—82 (1980).
23. WOODE, G. N., BRIDGER, J. C.: Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *J. med. Microbiol.* **11**, 441—452 (1978).

Authors' address: Dr. Y. HOSHINO, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, U.S.A.

Received November 19, 1980