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Electron Microscopy Detection and Characterization of Viral Particles in Dog Stools

By

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With 1 Figure

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Summary

A number of apparently normal dog stool samples, randomly collected on the sidewalks of Paris were examined by Electron Microscopy. The study revealed the presence of viral particles in 27 cases.

Morphological criteria lead to the characterization of rotavirus in 2 specimen, coronavirus in 7 and parvovirus in 5. Rotavirus particles appeared always alone while coronavirus and parvovirus particles were present together in 5 cases.

Similar particles have been implicated in animal and human gastroenteritis. The importance of their presence in canine dejections is discussed in view of pollution by dog stools of urban and suburban areas.

Introduction

Electron microscopy has revealed the presence of several types of virus particles in faeces from different avian and mammalian species, including man. Among them, the best characterized are the rotaviruses, whose ultrastructural, biochemical and immunological properties led to the definition of a new taxonomic viral genus (7). More recently other new intestine born viruses have been detected by transmission electron microscopy of both human and non human diarrheic and normal stools (9). These newly recognized viruses include: astroviruses, caliciviruses, coronaviruses, and small round viruses (picorna and parvovirus like) which are easily distinguishable by their particular morphology. All these viruses, and more specially rotaviruses, have been implicated as etiological agents in nosocomial and community acquired diarrhea (8). Therefore, it seems of impor-

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tance to define clearly their natural history and, more particularly, the possible animal sources of contamination. We report in this article our results concerning the electron microscopy study of the viral flore in dog stools and the demonstration of the existence of several different virus particles in randomly collected canine faeces. Some of our results concerning rotaviruses have been published in a preliminary report (17).

Materials and Methods

Dog stools were collected randomly on the sidewalks of Paris streets in June 1979. They were picked up only if they presented a non diarrheic aspect. Treatment of faeces was carried out as described previously (15). Briefly, 5 g of the collected material were suspended in 5 ml of phosphate buffered saline (PBS) and 1 ml of trichlorotrifluoroethane and then homogenized. The homogenates were centrifuged for 30 minutes at $400 \times g$ and 4° C. Supernatants were treated for negative staining with 1 per cent phosphotungstic acid at pH 7.2, and directly inspected under an electron microscope (Phillips 301). Virus particles were sought in at least five grid squares and classified following the criteria defined by FLEWETT (7) and MEDELEY (9).

Results

Three morphological classes of viruses were present in the stools of 27 out of 56 dogs (Table 1). These included rotavirus (3.5 per cent), coronavirus (12.5 per cent), and parvovirus-like (23.2 per cent). In 8.9 per cent of the cases, both coronaviruses and parvoviruses-like particles were observed together. Rotaviruses were always observed alone.

Table 1. *Detection of virus particles in dog stools*[a]

Type	Number of positive samples	%
Rotavirus	2	3.5
Coronavirus	7	12.5
Parvovirus	13	23.2
Coronavirus and Parvovirus	5	8.9
Total	27	48.1

[a] Total number of examined samples: 56

The rotavirus particles were morphologically undistinguishable from the ones described in other species (Fig. 1a) (14). Both double and smooth single shelled types were seen in the preparations. Double shelled particles showed a circular contour which was sharply defined by a membraneous layer; they had a diameter of 70 nm. Single shelled rough particles had a diameter of 60 nm and revealed more clearly the hollow ring shaped capsomeres.

Coronavirus particles showed a typical morphology (6) (Fig. 1b). They were pleomorphic, with a range in diameter from 100 to 500 nm including the projections which were approximately 15 nm in length. Most particles were

between 100 and 200 nm in diameter. The projections formed a single fringe radiating from the core. Only the dilated distal ends of the projections were seen with negative stain. No internal structure was observed. The particles appeared both alone and associated.

The number of particles ressembling parvovirus-like particles (4) was always higher than that of rotaviruses and coronaviruses like particles. By measuring a large number of them, it was possible to determine a mean diameter of 26 nm (Fig. 1 c). The outlines were often round or polygonal and many of the particles

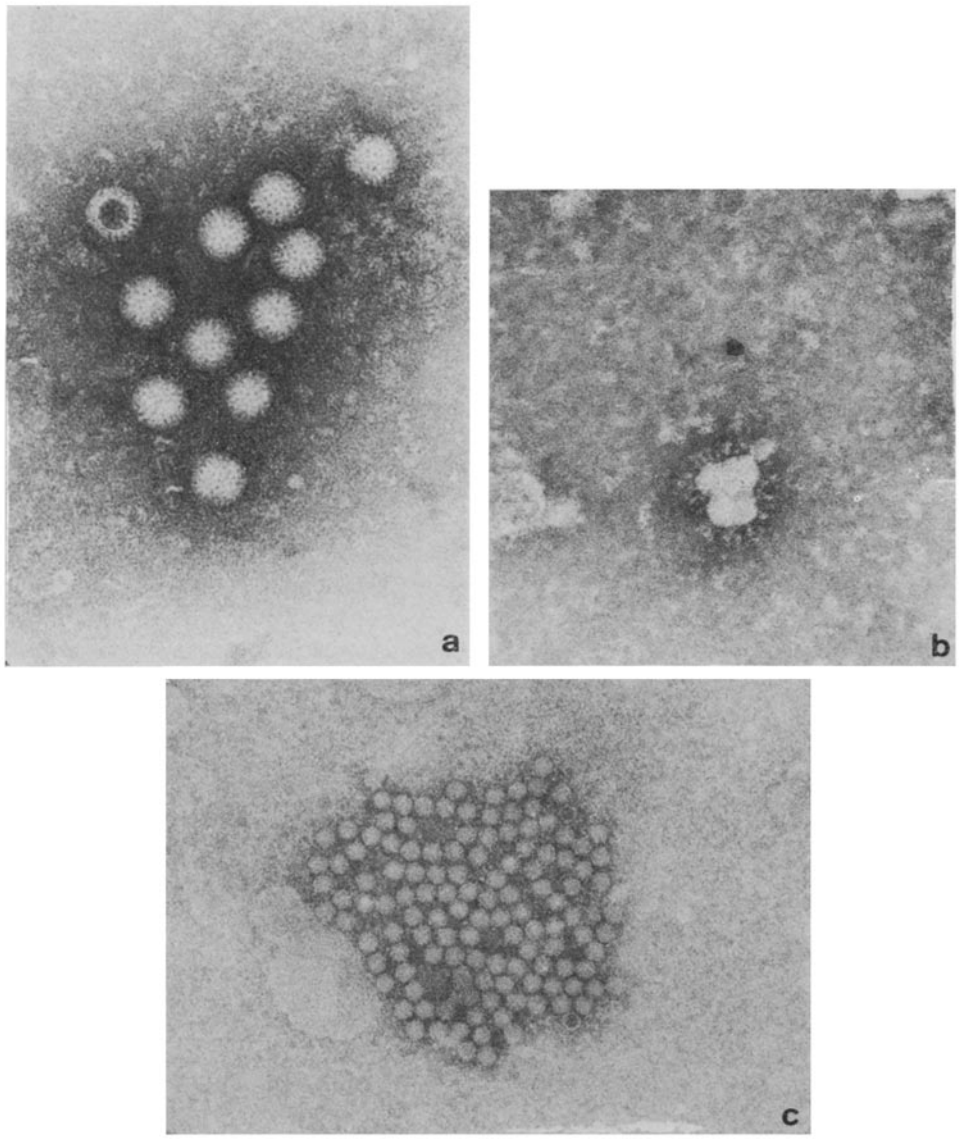

Fig. 1. *a* Rotavirus (100,000× magnification), *b* Coronavirus (100,000× magnification), *c* Parvovirus-like particles (100,000× magnification)

were penetrated by the phosphotungstate, so that they appeared as empty virions with no perceptible surface structure.

Discussion

Since the assessment of the viral etiology of severe gastroenteritic diseases both in man and in other species, the studies concerning the suspected viral agents have rapidly multiplied (7, 8, 9). However, many of the questions posed by these viruses are not yet fully understood, specially their natural history and their epidemiology. We decided to focus our attention on dogs for three reasons: 1) even if dogs have been shown to be occasionally infected by coronaviruses (18) and parvoviruses (4), no systematic studies of the canine intestinal flora has yet been published, 2) antibodies against rotaviruses have been found in dogs (11), 3) pollution by dogs stools constitutes a serious sanitary problem in most cities and suburban areas.

Our results indicate that particles with the ultrastructural morphology of rotaviruses, coronaviruses, and parvoviruses exist commonly in apparently normal dog faeces randomly collected in the sidewalks of a big city such as Paris.

The finding of rotaviruses in dog stools could be relevant concerning the epidemiology of rotaviruses associated human diseases. The particles we found could either be merely canine or originated in other species. In any of both cases, they may be pathogenic for man. In fact, a significant number of experimental cross species transmission of rotaviruses have now been reported (12, 3, 13, 23, 21) and some evidence of bovine rotaviruses in pigs has already been published (20, 22). In our case, we cannot answer this important question. We can only say that the two isolates originated in dogs cross-reacted with bovine rotaviruses in an ELISA test (16), which would suggest a cross reactivity with human strains. Experiments concerning this subject are going on in our laboratory.

The presence of coronaviruses in the stools of dogs with and without gastroenteritis has been observed in United States and Australia (19, 18). We fully confirm these results: an important number of examined specimens showed particles having the ultrastructure morphology of coronaviruses. At present, coronavirus are classified almost entirely by means of their characteristic morphology (6, 10, 19). Our strains presented all of them identical morphological aspect and they were strictely comparable to previous coronavirus isolates. However biochemical and serological studies should be necessary to further ascertain the coronavirus identity of these strains. In this context, it is interesting to point out that the coronaviruses are known to cause gastroenteritis (5) and respiratory diseases (10) in man.

The parvovirus-like particle which we identified, are undistinghishable from the parvovirus described in canine hemorrhagic enteritis (4) and from the viral agent detected in apparently healthy animals (2). Recently outbreaks of canine parvovirus-like viral enteritis have been reported in various locations throughout the United States (1).

We are not yet in a position to draw a conclusion on the possible hazards to human health deriving from the presence of all these viruses in dogs. Nevertheless we believe that this new field should be throughly explored.

References

1. Appul, M. J. G., Cooper, B. J., Greisen, H., Carmichael, L. E.: Status report canine viral enteritis. J. Amer. vet. med. Ass. **173**, 1516—1518 (1978).
2. Binn, L. N., Lazar, E. C., Eddy, G. A., Kajima, M.: Recovery and characterization of a minute virus of canines. Infect. Immun. **1**, 503—508 (1970).
3. Bridger, J. C., Woode, G. M., Jones, J. M., Flewett, T. H., Bryden, Z. S., Davies, H.: Transmission of human rotavirus to gnotobiotic piglets. J. med. Microbiol. **8**, 565—569 (1975).
4. Burtonboy, G., Coignoul, F., Delferriere, N., Pastoret, P. P.: Canine Hemorrhagic Enteritis: Detection of viral particles by electron microscopy. Arch. Virol. **61**, 1—11 (1979).
5. Caul, E. O., Clarke, S. K. R.: Coronavirus propagated from patient with non bacterial gastroenteritis. Lancet **ii**, 953—954 (1975).
6. Caul, E. O., Egglestone, S. I.: Further studies on human enteric coronaviruses. Arch. Virol. **54**, 107—117 (1977).
7. Flewett, T. H., Woode, G. N.: The rotaviruses. Arch. Virol. **57**, 1—23 (1978).
8. Holmes, I. H.: Viral gastroenteritis. Prog. med. Virol. **25**, 1—36 (1979).
9. Madeley, C. R.: Viruses in stools. J. clin. Pathol. **32**, 1—10 (1979).
10. McIntosh, K.: Coronaviruses: A comparative review. Curr. Top. Microbiol. Immunol. **63**, 85—129 (1974).
11. McNulty, M. S., Allan, G. M., Thompson, D. N., O'Boyle, J. D.: Antibody to rotaviruses in dogs and cats. Vet. Rec. **102**, 534—535 (1978).
12. Mebus, C. A., Wyatt, R. G., Sharpee, R. L., Sereno, M. M., Kalica, A. R., Kapikian, A. Z., Twiehaus, M. J.: Diarrhea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. Infect. Immun. **14**, 471—474 (1976).
13. Mitchell, J. D., Lambeth, L. A., Sosula, L., Murphy, A., Albrey, M.: Transmission of rotavirus gastroenteritis from children to monkey. Gut **18**, 156—160 (1977).
14. Roseto, A., Escaig, J., Delain, E., Cohen, J., Sherrer, R.: Structure of rotaviruses as studies by the freeze-drying technique. Virology **98**, 471—475 (1979).
15. Roseto, A., Gonzalez, J. P., Edlinger, E.: Rotavirus et gastro-entérite du nouveau-né. Premières observations. C. R. Acad. Sc. Paris **284**, 2307—2308 (1977).
16. Roseto, A., Peries, J.: Manuscript in preparation.
17. Roseto, A., Lema, F., Sitbon, M., Cavalieri, F., Dianoux, L., Peries, J.: Detection of Rotaviruses in dogs. I.R.C.S. Medical Science **7**, 478 (1979).
18. Schnagl, R. D., Holmes, I. H.: Coronavirus-like particles in stools from dogs from some country areas of Australia. Vet. Rec. **102**, 528—529 (1978).
19. Takeuchi, A., Binn, L. N., Jervis, H. R., Kennan, K. P., Hildebrandt, P. K., Valas, R. B., Bland, F. F., III: Electron microscope study of experimental enteric infection in neonatal dogs with a canine coronavirus. Lab. Invest. **34**, 539—549 (1976).
20. Todd, D., McNulty, M. S.: Characterization of pig rotavirus RNA: J. gen. Virol. **33**, 147—150 (1976).
21. Tzipori, S.: Human rotaviruses in young dogs. Med. J. Aust. **ii**, 922—923 (1976).
22. Woode, G. N., Bridger, J., Hall, G. A., Jones, J. M., Jackson, G.: The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. med. Microbiol. **9**, 203—209 (1976).
23. Wyatt, R. G., Sly, D. L., London, W. T., Palmer, A. E., Kalica, A. R., van Kirk, D. H., Chanock, R. M., Kapikian, A. Z.: Induction of diarrhea in colostrum-deprived newborn Rhesus monkeys with the human reovirus-like agent of infantile gastroenteritis. Arch. Virol. **50**, 17—27 (1976).

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