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Faecal viruses of dogs – an electron microscope study

Deborah S. Finlaison

Department of Veterinary Pathology, University of Sydney, Sydney, NSW, 2006, Australia

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Abstract

Faecal samples from 112 dogs both with and without diarrhoea were screened for parvovirus by a haemagglutination titration test and then examined by electron microscopy for the presence of viruses and virus-like particles. On the basis of morphology eight distinct viruses or virus-like particles were identified. Particles identified were coronaviruses, coronavirus-like particles, rotavirus-like particles, papovavirus-like particles, torovirus-like particles, picornavirus-like particles, 27 nm virus-like particles with projections and parvovirus-like particles which did not cause haemagglutination.

Keywords: Faecal virus; Dogs; Coronavirus

1. Introduction

The initial interest in primarily enteric viral pathogens of dogs arose from the recovery of a previously unrecognised canine coronavirus (CCV) from the faeces of dogs stationed in Germany during an outbreak of gastroenteric illness in 1971 (Binn et al., 1975).

More intensive studies of infectious enteritis caused by coronaviruses were not commenced until 1978, following widespread outbreaks in dogs of contagious vomiting and diarrhoea in February of that year. Some deaths resulted from the enteritis but generally the mortality rate was low (Carmichael, 1978; Appel et al., 1979). In August 1978 a series of outbreaks of serious diarrhoeal disease was observed in widely separated areas of the world. In many instances a parvovirus-like particle was associated with the disease. Parvoviral diarrhoea was generally more severe than that associated with the coronavirus outbreaks (Appel et al., 1979; Carmichael and Binn, 1981). Since then rotaviruses, astroviruses and caliciviruses have been demonstrated sporadically from dogs with enteric disease (Eugster and Sidwa, 1979; England and Poston, 1980; Williams, 1980; Fulton et al., 1981; Hammond and Timoney, 1983; Schroeder et al., 1983; Mochizuki and Hsuan, 1984; Evermann et al., 1985; Schaffer et al., 1985; Mochizuki et al., 1993).

While canine parvovirus is common in Australia and its association with enteritis is known, the situation with CCV and other enteric viruses of dogs is unknown. The presence of coronaviruses and coronavirus-like particles (CVLP) in dog faeces in Australia has been reported twice. CVLP were first detected in stools from dogs kept by Aboriginal communities (Schnagl and Holmes, 1978). A clearcut association of these particles with diarrhoea could not be concluded, and work carried out since suggests these particles are not related to coronaviruses and may not be viruses at all (Schnagl et al., 1987). Coronaviruses and CVLP were again detected in dog faeces in a study conducted by Marshall et al. (1984). Four of the 11 dogs in which coronaviruses were detected had diarrhoea. Rotaviruses, astrovirus-like particles, papovavirus-like and 'round' virus particles have been detected also in the faeces of dogs in Australia (Hamilton, 1983; Marshall et al., 1984). Tzipori and Makin (1978) detected antibodies against rotavirus in canine serum samples.

The present study was carried out to identify by electron microscopy viruses and virus-like particles found in the faeces of dogs both with and without diarrhoea. The principal interest lay in the identification of CCV so that studies could be conducted towards isolating the virus in cell culture.

2. Materials and methods

2.1. Specimen collection

114 faecal samples were collected from 112 dogs. The samples were collected from four groups of dogs:

1. Dogs submitted to veterinary practices because of diarrhoea suspected to be viral in origin but usually not consistent in clinical signs with parvoviral enteritis ($n = 41$);
2. Diarrhoeic faecal samples ($n = 5$), soft faecal samples ($n = 6$) and normal faecal samples ($n = 46$) from dogs at the premises of the Royal Society for the Prevention of Cruelty to Animals and a pound;
3. Specimens from clinically normal dogs out of contact with dogs with diarrhoea ($n = 13$), and a dog in contact with a dog with diarrhoea;
4. Normal faecal samples from greyhounds with polyarthritis ($n = 2$).

A brief history of each dog from which a sample was collected was obtained. All faecal samples were stored at 4°C until processed. The majority of samples were collected from dogs within the Sydney metropolitan area, although samples from anywhere in NSW were accepted.

2.2. Processing of samples

Each faecal sample was screened for the presence of canine parvovirus using a haemagglutination titration test (HA) (McGavin, 1985). Those samples that were diarrhoeic and had a HA titre greater than 8000 were excluded from further studies because there are few reports of dual infection with canine parvovirus and canine coronavirus in naturally infected diarrhoeic dogs. Therefore 36 diarrhoeic and 68 non-diarrhoeic faecal samples were examined further.

One gram of the faecal sample was homogenised in 15 ml of phosphate buffered saline (PBS, pH 7.3). This suspension was treated with tri-chloro-tri-fluoro-ethane and vortexed until the two layers had become partly miscible. The treated sample was left overnight at 4°C. On the following day the sample was centrifuged at 1400 *g* for 30 min at 4°C. The supernatant fluid was ultracentrifuged at 4°C for 2.25 h at 75 500 *g* using a Beckman 50.2Ti rotor in a Beckman L7-55 ultracentrifuge. The supernatant fluid was discarded and the pellet covered in five to ten drops of 0.1% PBS-azide. This was left overnight at 4°C. The pellet was resuspended in the PBS-azide before being stained.

2.3. *Negative contrast electron microscopy*

Carbon-coated formvar-covered 400 mesh copper grids were floated on one drop of the processed faecal sample for 60 s. Excess fluid was drained. The grid was floated on 2% phosphotungstic acid (pH 7.2) for 30 s and the excess fluid drained. The grids were allowed to air dry for at least ten minutes before being examined by electron microscopy (EM).

The grids were examined using a Phillips 301 or a Phillips CM12 transmission electron microscope. The grids were scanned at an instrumental magnification of 45 000 \times or 31 000 \times respectively for the presence of viral particles. At least three grid squares were examined for each sample.

2.4. *Virus identification*

Viruses detected in any sample were identified morphologically according to established criteria (Doane and Anderson, 1987).

2.5. *Coronavirus isolation*

Coronavirus isolation was attempted from the EM positive samples by inoculating 0.5 ml of a faecal suspension on to a monolayer of either feline kidney (FK) or Madin Darby canine kidney (MDCK) cells. The FK cells were chosen because they are readily available in Australia. The MDCK cells were chosen because they are the only commercial canine cell line available in Australia, even though a previous attempt at growing CCV in them was unsuccessful (Williams, 1980). A 10% faecal suspension was prepared for inoculation into cell culture by centrifugation at 1400 *g* for 25 min at 4°C and filtration of the supernatant fluid through a 0.8 μm and a sterile 0.45 μm filter. Aside from faecal suspension alone being inoculated, faecal suspensions with 50 $\mu\text{g}/\text{ml}$ of DEAE-dextran, 1 $\mu\text{g}/\text{ml}$ of 'Polybrene' (hexadimethrine bromide) or 20 $\mu\text{g}/\text{ml}$ of trypsin were inoculated also. Each sample was passaged 4 times before being considered negative for viral replication.

3. Results

On the basis of morphology eight distinct viruses or virus-like particles, excluding canine parvovirus, were identified in the dog faeces.

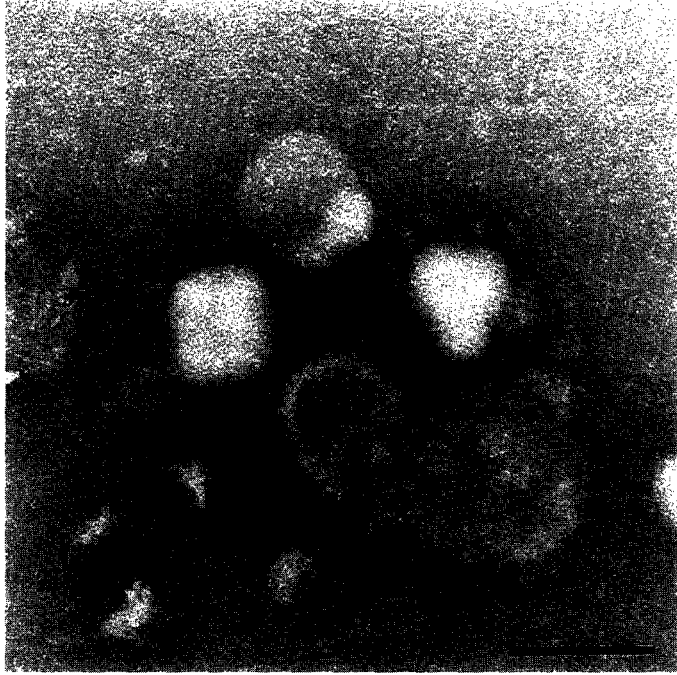


Fig. 1. Group of coronaviruses. Note variation in size and shape. Bar represents 100



Fig. 2. Coronavirus-like particle. Bar represents 100 nm.

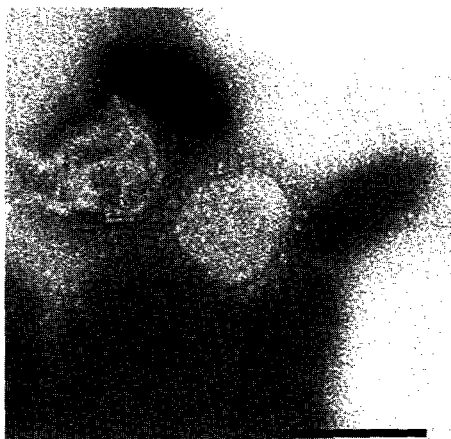


Fig. 3. Coronavirus-like particle. Bar represents 100 nm.

Three faecal samples contained characteristic coronavirus particles (Fig. 1). These particles varied in size from 155 nm to 210 nm, and averaged 150 nm along their greatest dimension. The virus particles were pleomorphic being round, oval or pear shaped. The spikes were club shaped and did not always surround the entire virion. The length of the spikes generally ranged from 19 to 22 nm, although on some particles they were as short as 17 nm. One of these specimens also contained parvovirus. None of the three faecal samples was diarrhoeic, although one of the stools was soft.

CVLP resembling those observed by Schnagl and Holmes (1978) were observed in two samples (Fig. 2). These particles were very pleomorphic and ranged in size from 170 to 555 nm. The spike lengths for the particles in the two samples varied, one measuring 28 nm and the other averaging 23 nm. Neither of these samples was diarrhoeic.

CVLP with shorter fringes measuring approximately 11 nm were identified in two faecal samples (Fig. 3). The spikes were not attached as sparsely as those associated with characteristic coronavirus particles, but they were not as closely attached to one another as the

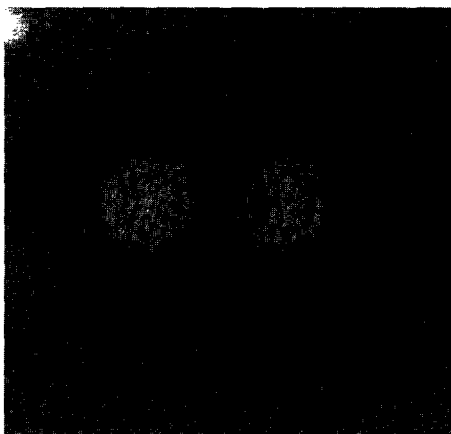


Fig. 4. Rotavirus-like particles. Bar represents 100 nm.



Fig. 5. Papovavirus-like particles. Bar represents 100 nm.

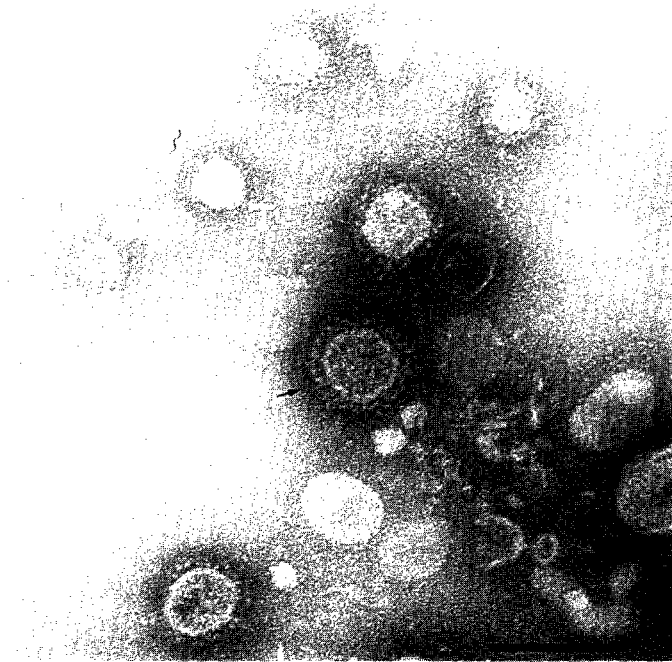


Fig. 6. Fringed torovirus-like particles. One is demonstrated by arrow. Bar represents 100

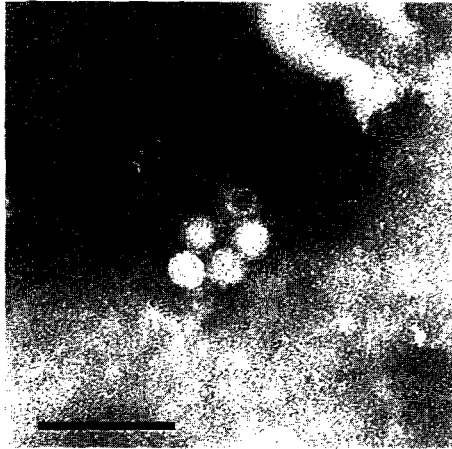


Fig. 7. Picornavirus-like particles. Bar represents 100 nm.

spikes surrounding the torovirus-like particles. These particles averaged 115 nm in diameter. Both of these samples were diarrhoeic.

Rotavirus-like particles were observed in one faecal sample (Fig. 4). The particles observed were characteristic of single-shelled rotavirus particles and measured approximately 74 nm in diameter. This sample also contained parvovirus and was not diarrhoeic.



Fig. 8. Group of parvovirus-like and picornavirus-like particles. Picornavirus-like particles indicated by arrows. Bar represents 100 nm.

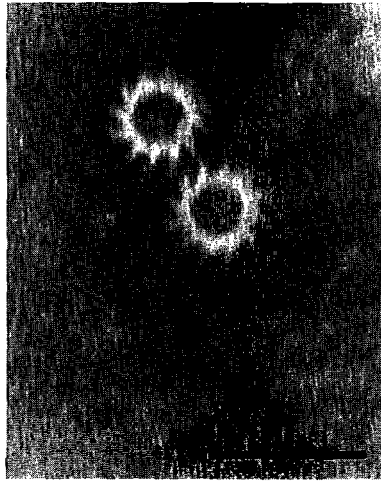


Fig. 9. 27 nm virus-like particles. Bar represents 40 nm.

Table 1
Details about dogs from which viruses or virus-like particles were identified

Virus	Sample	Clinical Signs	Age	HA titre ^a
Coronavirus	DF74	None	2.5 years	320
Coronavirus	DF89	Soft faeces	10 months	negative
Coronavirus	DF93	None	2 years	negative
Coronavirus-like	DF25	None	> 6 months	negative
Coronavirus-like	DF83	None	7 months	negative
Coronavirus-like	DF103	Diarrhoeic	8 weeks	negative
Coronavirus-like	DF107	Anorexia, depression, dehydration, vomiting, abdominal pain, tenesmus, vomiting and diarrhoea. 40.0°C. Faeces loose and contained mucus.	13 years	negative
Fringed torovirus-like particles	DF44	Anorexia, depressed, dehydrated, vomiting, abdominal pain and diarrhoea. Faeces were watery, red/orange, with flecks of blood. Later diagnosed with an intussusception.	7 months	negative
Papovavirus-like	DF39	None	7 years	negative
Picornavirus-like	DF22	Anorexia, vomiting, depression, dehydration and diarrhoea. 36.6°C. Watery, green, mucoid faeces, malodorous, couple of flecks of blood.	8 weeks	negative
Picornavirus-like	DF87	Yellow/brown diarrhoeic faeces	18 months	negative
Picornavirus-like and 27 nm virus-like particles	DF99	None	> 5 years	160 positive
Picornavirus-like and parvovirus-like particles	DF110	Sudden onset of diarrhoea. Lethargy and death within 24 h.	9 weeks	negative
Rotavirus-like	DF79	None	5.5 months	320

^aParvovirus haemagglutination titre (A titre > 160 is considered positive)

Papovavirus-like particles were observed in one sample and measured approximately 47 nm in diameter (Fig. 5). These particles were not associated with diarrhoea.

Pleomorphic particles, with fringes 8 to 11 nm, that resembled torovirus particles were observed in one sample (Fig. 6). The particles varied in size from about 45 nm to 140 nm and averaged 85 nm in diameter. The spikes were positioned closely to one another.

Particles resembling members of the family Picornaviridae were observed in four samples. In three of these samples the particles measured about 27 nm in diameter, while in the fourth sample the particles were larger at about 30 nm in diameter. The particles in the first three samples had featureless surfaces and both intact and 'empty' virions were observed (Fig. 7). The particles in the fourth sample were generally 'empty' and ten capsomeres could be counted around the edge of the particle (Fig. 8). Some smaller virus-like particles were detected in this sample also. These particles were about 22 nm in diameter and resembled parvoviruses, although a positive HA titre was not observed. Three of these samples were from diarrhoeic dogs. In two cases the diarrhoeic dogs were less than nine weeks old.

Two previously undescribed virus-like particles were observed in one sample. These particles were circular and had 12 projections extending from their surface (Fig. 9). They measured approximately 27 nm in diameter, including the projections. This sample was normal and parvovirus and picornavirus-like particles were present also.

Table 1 records the details of the dogs from which viruses or virus-like particles were identified.

Coronaviruses were not isolated in either FK or MDCK cells.

4. Discussion

The major aims of this study were to identify the presence and if possible the significance of CCV infection in dog faeces in Australia. CCV was detected in three samples only out of 104 tested. Electron microscopy is the only method available in Australia currently for detecting CCV. Development of more sensitive techniques here may give a higher yield of coronavirus-excreting dogs but "what will this mean?"

CVLP in two of the four samples in which they were identified were associated with diarrhoea. Further characterisation of these particles needs to be carried out to determine if they are viral and/or related to the characteristic coronaviruses.

Picornavirus-like particles were detected in four faecal samples, and in three cases their presence was associated with diarrhoea. Although picornavirus-like particles have been previously identified in dog faeces (Carmichael and Binn, 1981) little work has been conducted to determine if these are human picornaviruses in dogs or dog specific picornaviruses. Two of the diarrhoeic samples were from neonates, suggesting a possible relationship of picornavirus infection with neonatal diarrhoea in dogs.

Rotavirus-like particles were detected in one sample only. This sample was taken from a dog less than one year old which was not diarrhoeic. A study of neonatal and young dogs may be more appropriate in determining the role of rotavirus in canine enteritis.

One sample contained fringed virus-like particles which resembled toroviruses. Toroviruses have not been previously described in dogs but are recognised as enteric pathogens in humans, horses and cattle.

The 27 nm virus-like particles observed in one sample are unlike any virus particles previously reported. Purification and further characterisation of these particles is necessary to determine if they are viral.

The viruses detected in this study are largely similar to those detected in another Australian study conducted by Marshall et al. (1984). The current study identifies the presence of previously unrecognised virus-like particles in dog faeces which need further investigation to uncover their role in canine enteritis.

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References

- Appel, M.J.G., Cooper, B.J., Greisen, H., Scott, F. and Carmichael, L.E., 1979. Canine viral enteritis. I. Status report on corona-and parvo-like viral enteritides. *Cornell Vet.*, 69: 123–133.
- Binn, L.N., Lazar, E.C., Keenan, K.P., Huxsoll, D.L., Marchwicki, R.H. and Strano, A.J., 1975. Recovery and characterization of a coronavirus from military dogs with diarrhoea. Proc. 78th Ann. Meeting U.S. Animal Hlth. Assoc., pp 359–366.
- Carmichael, L.E., 1978. Infectious canine enteritis caused by a Corona like virus: current status and request for information. *Baker Fast. Lab. Rep. Series*, 2 No. 9.
- Carmichael, L.E. and Binn, L.N., 1981. New enteric viruses in the dog. *Adv. Vet. Sci. Comp. Med.*, 25: 1–37.
- Doane, F.W. and Anderson, N., 1987. *Electron Microscopy in Diagnostic Virology. A practical guide and atlas.* Cambridge University Press.
- England, J.J. and Poston, R.P., 1980. Electron microscopic identification and subsequent isolation of a rotavirus from a dog with fatal neonatal diarrhea. *Am. J. Vet. Res.*, 41: 782–783.
- Eugster, A.K. and Sidwa, T., 1979. Rotaviruses in diarrhetic feces of a dog. *Vet. Med. small Anim. Clin.*, 74: 817–819.
- Evermann, J.F., McKeirnan, A.J., Smith, A.W., Skilling, D.E., and Ott, R.L., 1985. Isolation and identification of caliciviruses from dogs with enteric infections. *Am. J. Vet. Res.*, 46: 218–220.
- Fulton, R.W., Johnson, C.A., Pearson, N.J. and Woode, G.N., 1981. Isolation of a rotavirus from a newborn dog with diarrhoea. *Am. J. Vet. Res.*, 42: 841–843.
- Hamilton, R., 1983. Particles in canine faeces. *Aust. Vet. Practit.*, 13: 36.
- Hammond, M.M. and Timoney, P.J., 1983. An electron microscopic study of viruses associated with canine gastroenteritis. *Cornell Vet.*, 73: 82–97.
- Marshall, J.A., Healy, D.S., Studdert, M.J., Scott, P.C., Kennett, M.L., Ward, B.K. and Gust, I.D., 1984. Viruses and virus like particles in the faeces of dogs with and without diarrhoea. *Aust. vet. J.*, 61: 33–38.
- McGavin, D., 1985. PhD thesis, pp 86–87.
- Mochizuki, M. and Hsuan, S., 1984. Isolation of a rotavirus from canine diarrhoeal faeces. *Jpn. J. Vet. Sci.*, 46: 905–908.
- Mochizuki, M., Kawanishi, A., Sakamoto, H., Tashiro, S., Fujimoto, R. and Ohwaki, M., 1993. A calicivirus isolated from a dog with fatal diarrhoea. *Vet. Rec.*, 132: 221–222.
- Schaffer, F.L., Soergel, M.E., Black, J.W., Skilling, D.E., Smith, A.W. and Cubitt, W.D., 1985. Characterization of a new calicivirus isolated from feces of a dog. *Arch. Virol.*, 84: 181–195.
- Schnagl, R.D., Brookes, S., Medvedec, S. and Morey, F., 1987. Characteristics of Australian human enteric coronavirus-like particles: comparison with human respiratory coronavirus 229E and duodenal brush border vesicles. *Arch. Virol.*, 97: 309–323.

- Schnagl, R.D. and Holmes, I.H., 1978. Coronavirus-like particles in stools from dogs, from some country areas of Australia. *Vet. Rec.*, 102: 528–529.
- Schroeder, B.A., Kalmakoff, J., Holdaway, D. and Todd, B.A., 1983. The isolation of rotavirus from calves, foals, dogs and cats in New Zealand. *N.Z. vet. J.*, 31: 114–116.
- Tzipori, S. and Makin, T., 1978. Propagation of human rotavirus in young dogs. *Vet. Microbiol.*, 3: 55–63.
- Williams, F.P., 1980. Astrovirus-like, coronavirus-like, and parvovirus-like particles detected in the diarrhoeal stools of beagle pups. *Arch. Virol.*, 66: 215–226.