Viruses and virus-like particles in the faeces of dogs with and without diarrhoea

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SUMMARY: Negative staining electron microscopy was used to identify viruses in 157 normal and 29 diarrhoeal faecal samples collected from 156 dogs admitted to an animal shelter during an 8 month period (March to October) in 1982. Seven distinct viral types were detected: 21-26 nm parvovirus-like particles, 28-31 nm astrovirus-like particles, a previously undescribed 34-35 nm "round" virus particle, coronavirus, coronavirus-like particles (CVLP), rotavirus and papova-like virus. Parvovirus-like particles alone were detected in 14 diarrhoeal and 50 normal faeces, astrovirus-like particles in 3 normal faeces, "round" viruses in 4 normal faeces, coronavirus in 2 diarrhoeal and 5 normal faeces, CVLP in one diarrhoeal and one normal faeces, rotavirus in 2 normal faeces, papova-like virus in one normal faeces, both parvovirus-like particles and coronavirus in 2 diarrhoeal and 2 normal faeces, parvovirus-like particles and rotavirus in one normal faeces and parvovirus-like and papova-like virus in one normal faeces. The significance of these findings in canine and human disease is discussed.

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Virus Isolation

Virus isolation was attempted by inoculating aliquots of one drop of the purified, concentrated faecal sample into a variety of cell lines including cynomolgus monkey kidney epithelial cells (MK), heteroploid cynomolgus monkey embryonic kidney cells (MEK), rhinovirus sensitive HeLa cells (HeLa), Borrie cells (Bo) (Kennett et al 1974), human embryonic lung fibroblasts (HEL) and human heteroploid epithelial cells (HEp-2 cells). Details of the procedure are given in Kennett et al (1972).

Results

Virology

On the basis of morphology 7 distinct viruses or virus-like particles were identified in the dog faeces. IEM studies

Figure 1. Parvovirus-like particles.
(a) Clump of particles associated with membrane (arrow);
(b) free virus;
(c) immune complex of virus. The bar represents 100 nm.

Figure 2. Immune electron micrographs to show that a typical 24 nm parvovirus-like particle is serologically related to canine and feline parvovirus.
(a) immune complex formed by interaction of the virus and canine parvovirus antiserum;
(b) immune complex formed by interaction of the virus and feline parvovirus antiserum;
(c) uncomplexed virus (arrows) after interaction with the negative control serum. The bar represents 100 nm.

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Parvovirus-like particles — Particles, 21-26 nm in diameter (mean ± S.D. 23.3 ± 1.3 nm; n=70) were the most common detected and will be referred to as parvovirus-like particles. These small, round, typically featureless virions occurred in clumps (Figure 1a), as discrete particles (Figure 1b), and/or as immune complexes (Figure 1c). The particles were sometimes associated with membrane (Figure 1a). IEM studies using a morphologically typical (24 nm) strain showed it to be serologically related to canine and feline parvovirus (Figure 2).

Astrovirus-like particles — These relatively rare virus particles could be distinguished from parvovirus-like particles by their larger size, (28-31 nm) and the observation that they sometimes displayed a star-shaped staining pattern reminiscent of astrovirus (Figure 3). IEM tests for hepatitis A with 2 of these 3 strains, as well as virus isolation studies using MK, MEK, HeLa, Bo and HEL cells indicated they were not hepatitis A or cultivable human enteroviruses despite morphological similarity to these viruses.

"Round" virus particles — Roughly round particles, measuring 34-35 nm in diameter, were detected in 4 faecal samples, and are unlike any virus previously reported in canine faeces. It is possible they are tailless phage. In 2 cases the viruses were frequently clumped, probably due to agglutination by antibody (Figure 4). Although the particles were roughly spherical in outline, they occasionally displayed an irregular surface. Virus isolation studies were attempted with one strain using MK, MEK, HeLa, Bo, HEL and HEp-2 cells.

Coronavirus — Coronavirus could be recognised with confidence as roughly round, ovoid or pear-shaped pleomorphic particles measuring about 70 to 100 nm along the greatest dimension with petal-shaped spikes measuring about 17 nm in length (Figure 5). Virus isolation studies were attempted with two strains using MK, MEK, HeLa, Bo, HEL and HEp-2 cells.

Coronavirus-like particles (CVLP*) — CVLP were recognised as pleomorphic particles with a fringe of club-shaped spikes about 23 nm in length (Figure 6). The particles were highly variable in size and up to about 1000 nm along their greatest dimension. Virus isolation studies were attempted with one strain using MK, MEK, HeLa, Bo, HEL and HEp-2 cells.

Papova-like virus — Papova-like viruses about 50 nm in diameter (Figure 8) were identified by their size and similar morphology to human and simian papovaviruses (see Madeley 1972).

Other viruses — Tailed phage was noted in most specimens; 27 nm particles, intermediate in size between the parvovirus-like particles and astrovirus-like particles, were noted in 2 cases. Their correct classification could not be made on morphological grounds and they are not considered further.

Clinical and Epidemiological Observations
Since more than one faecal sample was collected from some dogs, the clinical and epidemiological analysis below is based on both total faecal samples and total dogs. Table 1 summarises the chief clinical findings for total faecal samples.

Parvovirus-like particles — Although the proportion of diarrhoeal faeces with only parvovirus-like particles was greater than the proportion of normal faeces with only these

*Coronavirus-like particles (CVLP) is an alternate term for contagious viral lesions (CVLP) by Williams (1980).
Virus type(s)

- Astrovirus-like
- Parvovirus-like
- CVLP

Figure in brackets gives percentage of Parvovirus-like and Rotavirus. The chi-square test showed the difference between these percentages was significant (P < 0.01). Thus, when dogs are excreting only parvovirus-like particles, it is very high levels of virus excretion rather than infection per se which is associated with diarrhoea.

Of the 156 separate dogs studied, parvovirus-like particles were detected at least once in 63 cases (40%). It is probable, however, that most, if not all dogs remaining in the shelter longer than 5 or 6 days became infected with the virus (Figure 9), since excretion of virus rose progressively from the first to the sixth day post admission then levelled off to about 30 to 40% of all dogs thereafter.

Serial collection of faeces from 2 dogs (Figure 10), furthermore, suggested that while the period of excretion might be shortlived (dog 2), dogs could show a cyclic pattern of virus excretion over very long periods (dog 1).

No relationship between age and excretion of the virus was found. Dogs of all ages excreted the virus.

The parvovirus-like particles were detected in each of the 8 months of the survey although the percentage of faecal samples positive for the virus in any given month varied from 19% to 64%.

Six of the 70 faecal samples positive for the parvovirus-like particles were also positive for coronavirus, rotavirus or papova-like virus (see Table 1 and below).

Astrovirus-like particles — The astrovirus-like particles were detected in 3 dogs (1.9%), aged about 3 to 6 months; none had diarrhoea.

"Round" virus particles — These particles were detected in 4 dogs (2.6%), 2 of which were described as "mature" and the others aged about 8 and 20 months respectively. None had diarrhoea.

Coronavirus — Coronavirus was detected in 11 dogs (7.1%) of which 4 had diarrhoea; (2 diarrhoal and 2 non-diarrhoal dogs had both coronavirus and parvovirus-like particles). Ages varied from about 3 months to "old".

CVLP — CVLP was detected in 2 dogs (1.3%), one aged about 5 months and the other about 5 years. The younger dog, whose faecal sample had counts of hundreds of particles per grid square, had diarrhoea; the older dog, whose faecal sample had counts of about 10 particles per grid square, did not.

Rotavirus — Rotavirus was detected in 3 dogs (1.9%). The dogs were recorded as "young", about 18 months old and "at least 2 years old". None had diarrhoea. One of these dogs had both rotavirus and parvovirus-like particles.

Papova-like virus — Papova-like virus was detected in faeces from 2 dogs (1.3%) collected about 2 months apart. Data was inadequate to determine if the same dog had been re-admitted; both dogs were listed as female, about 5 years old. Both faecal samples were normal. One of these dogs was also excreting parvovirus-like particles.

TABLE 1

| Virus type(s) present | No. of diarrhoeal faecal specimens | No. of normal faecal specimens† |
|-----------------------|-----------------------------------|---------------------------------|
| Parvovirus-like       | 14 (48.3)                         | 50 (31.9)                       |
| Astrovirus-like       | 0 (0)                             | 3 (1.9)                         |
| "Round"               | 0 (0)                             | 4 (2.6)                         |
| Coronavirus           | 2 (6.9)                           | 5 (3.2)                         |
| CVLP                  | 1 (3.5)                           | 1 (0.6)                         |
| Rotavirus             | 0 (0)                             | 2 (1.3)                         |
| Papova-like virus     | 0 (0)                             | 1 (0.6)                         |
| Parvovirus-like and   | 2 (6.9)                           | 2 (1.3)                         |
| coronavirus           |                                    |                                 |
| Parvovirus-like and   | 0 (0)                             | 1 (0.6)                         |
| rotavirus             |                                    |                                 |
| Parvovirus-like and   | 0 (0)                             | 1 (0.6)                         |
| papova-like virus     |                                    |                                 |

* Figure in brackets gives percentage of 29 diarrhoeal specimens.
† Figure in brackets gives percentage of 157 normal specimens.
‡ Coronavirus-like particles.

Discussion

The chief finding of this study is that viruses of 7 distinct types, some of known pathogenicity, can be excreted by non-diarrhoeal, mature, apparently healthy dogs; of 157 normal faeces studied 34% contained parvovirus-like particles, 5% contained coronavirus, 3% contained a previously undescribed 34-35 nm "round" virus particle, 2% contained rotavirus, 2% contained astrovirus-like particles, 1% contained papova-like virus and 1% contained CVLP.

Since the viruses were classified chiefly on morphological grounds, each category could include more than one virus type. However, since the characteristics of the viruses described in this study are either imperfectly understood, or in some cases totally unknown, and since methods of detection other than EM are in most cases difficult or unavailable, the clinical and epidemiological analysis below provides valuable information in the first instance.

The data indicates that excretion of parvovirus-like particles is widespread in the dog community and occurred throughout the 8-month period of the study. No relationship between age and excretion of these particles was found. The data suggests that infection is readily spread when dogs are in close contact. A periodicity in the shedding of parvovirus-
The emergence of canine parvovirus in 1978, presumably as a host range mutant of the closely related feline or mink parvoviruses, and the dramatic pandemic which rapidly followed, also focuses on the possibility of interspecies transfer of enteric viruses. Early reports of association of human diarrhoea with canine parvovirus were refuted (Lenzhaus and Studdert 1980), but the emergence of further host range mutants of parvovirus must be considered a distinct possibility.

Although human picornaviruses have been isolated from canine faeces (Carmichael and Binn 1981), no such viruses were isolated in the 2 experiments carried out in this study. The 2% detection rate of astrovirus-like particles probably corresponded to the previously described canine astrovirus (Williams 1980).

A round virus particle 34 to 35 nm in diameter was found in about 3% of dogs sampled, and does not appear to have been described previously. While the identity of this virus is uncertain — it could be tailless phage — it is worth noting its similarity to the recently described Otofuke and Sapporo agents, which have been associated with outbreaks of human gastroenteritis in Japan (Taniguchi et al. 1979, 1981; Kogasaka et al. 1981).

Coronavirus was detected in about 7% of all dogs; a third of these had diarrhoea. Some limited success has been achieved in the cultivation of the human coronavirus strain 229E in this laboratory (M. L. Kennett personal communication), but attempts to cultivate the virus in this study were unsuccessful.

CVLP was clearly distinguishable from coronavirus and has previously been described in both dogs (Schnagl and Holmes 1978) and people (Caul et al. 1975; Schnagl et al. 1979; Marshall et al. 1982). The nature and association of CVLP with disease in dogs and man, however, is problematic. CVLP has been detected in apparently healthy dogs and people (Schnagl and Holmes 1978; Schnagl et al. 1979;
Marshall et al. (1982) and dogs and people with diarrhoea (Caul et al. 1975; Clarke et al. 1979; Williams 1980; Marshall et al. 1982). In this study, a dog excreting very high concentrations of the particles had diarrhoea, while another excreting low concentrations did not. If virus, then pathogenicity may be dose related.

It is now known that rotavirus from one species may infect another and sometimes cause diarrhoea (Bridge et al. 1975; Middleton et al. 1975). Furthermore Engleberg et al. (1982) recently reported a correlation between dog ownership and rotavirus gastroenteritis in a population of Indians in the San Carlos Apache Reservation. The finding of rotavirus in mature non-diarrhoeic dogs in this study may therefore have epidemiological significance for both canine and human gastroenteritis.

The detection of papova-like virus particles in canine faeces does not appear to have been reported previously. The origin of this virus was probably oral or gastrointestinal papilloma.

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Death in sheep following dosing with copper diethylyamine oxyquinoline sulphonate as a commercial injectable copper preparation

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SUMMARY: Death occurred in sheep following diethylyamine oxyquinoline sulphonate (DOS) copper injections given at recommended dose rates. The copper content in unused portions of DOS copper packs was normal and free of bacterial contamination. Liver and blood copper levels in dead and sick sheep were not high. Sick sheep showed signs of hepatic encephalopathy and dead sheep were generally piled against fences and scrub. Deaths were associated with acute, severe, generalised, centrilobular, hepatocellular necrosis and live sheep had elevated circulating levels of liver enzymes consistent with liver damage. In recovered sheep there were no residual complications. It would appear that even at 0.5 mg/kg of DOS copper the safety threshold may sometimes be exceeded in some sheep.

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Introduction

In the treatment of copper deficiency, injectable copper preparations have an advantage over orally administered copper, because they bypass the inhibitory effects on copper availability of sulphur and molybdenum in the gut (underwood 1981). However, in sheep some injectable copper compounds such as copper-methionine, and to a lesser extent copper calcium EDTA, produce tissue reactions at the injection site and result in variable and short-lived protection when compared with copper as diethylyamine oxyquinoline sulphonate (copper DOS) which produces uniform long-lived protection and negligible tissue reaction (Suttle 1981). Because of the rapid availability of copper from copper DOS it is potentially more toxic than other injectable copper preparations (Cunningham 1959; Mahmoud and Ford 1981). Nevertheless, few cases of copper DOS poisoning have been reported. In Australia deaths have occurred in sheep and goats in Western Australia after normal doses of DOS copper

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