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(Accepted for publication 6 November 1986)

Virus and virus-like particles in the faeces of cats with and without diarrhoea

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SUMMARY: Negative staining electron microscopy was used to identify viruses in 166 normal and 62 diarrhoeal faecal samples from 208 cats admitted to an animal shelter during a 16-month period (March 1984 to June 1985). On the basis of size and shape 7 distinct viral types were detected: 24 nm parvovirus-like particles, 30 nm astrovirus, 30 nm picornavirus-like particles, reovirus, rotavirus, coronavirus and a 75 nm "togavirus-like" particle. The incidence of these particles in the 208 cats was 11%, 7%, 6%, 0.4%, 5%, 1% and 1% respectively. Virus isolation studies using 40 of the faecal samples succeeded in isolating reovirus 1 in 2 cases. Immune electron microscope studies demonstrated the presence of antibody in a human serum to cat astrovirus, but failed to clarify the identity of the parvovirus-like particles and picornavirus-like particles, other than showing that some of the parvovirus-like particles were not related to feline panleukopenia virus. It was found that parvovirus-like particles, astrovirus, picornavirus-like particles, reovirus and rotavirus could be excreted by cats with normal faeces as well as cats with diarrhoeal faeces. Parvovirus-like particles, astrovirus, picornavirus-like particles and rotavirus could be excreted in high concentration in normal faeces. There was no simple relationship between age and diarrhoea in the population of cats studied. Age was not a critical factor in the excretion of parvovirus-like particles, astrovirus, picornavirus-like particles and rotavirus. The incidence of diarrhoea was not clearly associated with the seasons.
Aust Vet J **64**: 100-105

Introduction

Viral gastroenteritis has emerged in the last decade as a major cause of morbidity and mortality in many animal species including man. So far, 7 main groups of virus or virus-like particles have been associated with the cat intestinal tract or cat faeces. These include parvovirus (Johnson 1965; Langheinrich and Nielsen 1971; Studdert and Peterson 1973; Siegl 1984), astrovirus (Hoshino *et al* 1981b), calicivirus (Wardley 1976; Studdert 1978), reovirus (Scott 1971), rotavirus (Chrystie *et al* 1979; Hoshino *et al* 1981a), coronavirus (Pedersen *et al* 1981) and coronavirus-like particles (CVLP) (Hoshino and Scott 1980; Stoddart *et al* 1984). In many cases, information on these particles in cats is scant and there appears to have been no major survey of viruses in cat faeces. In this study the nature and frequency of excretion of virus and virus-like particles in a large sample of diarrhoeal and non-diarrhoeal cats were examined, and the significance of the findings to cat and human health briefly reviewed.

Materials and Methods

Collection of Specimens

Faeces were collected, usually on a weekly basis, from March 1984 to June 1985, from the Royal Society for the Prevention of Cruelty to Animals (RSPCA) Centre, Burwood, Victoria. The cats were either strays or boarding and all were thought to be of domestic origin. Faeces were only collected from the cages of cats housed singly so that a given faecal sample could be ascribed to a particular cat.

On admission to the RSPCA Centre cats were routinely vaccinated against feline panleukopenia virus and treated for common parasites. The cats were fed twice a day and their enclosures cleaned at least once a day.

Faeces were classified as normal or diarrhoeal according to their appearance at the time of collection. Faeces that were predominantly firm and well formed were classified as normal and faeces that were predominantly soft, moist and poorly formed were classified as diarrhoeal. A total of 228 faecal samples (166 normal and 62 diarrhoeal) were collected from 208 cats. Details such as age, sex, date of admission and clinical signs were recorded. Apart from minor clinical symptoms all cats sampled appeared healthy. The cats ranged in age from 6 weeks to adults.

Preparation of Faecal Samples

Faeces were processed as described by Oliver *et al* (1985). Briefly, faecal specimens were prepared as a 20% (wt/vol) suspension in Hank's complete balanced salt solution, vigorously shaken, then centrifuged twice at low speed to deposit debris. The clarified supernatant fluid was then concentrated and further purified by ultracentrifugation through a sucrose cushion.

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Negative Staining Electron Microscopy (EM)

The purified concentrated faecal specimens were examined after negative staining with 3% phosphotungstic acid (pH 7) on 400 mesh Formvar-carbon coated grids. At least 4 grid squares were examined for each specimen using a Philips 301 electron microscope. Virus and virus-like particles were photographed and measured from photographic negatives. Catalase crystals, with half the principal lattice spacing taken to be 8.6 nm, were used as calibration standard (Wrigley 1968). Approximate numbers of particles per grid square were recorded.

Immune Electron Microscopy

Negative staining of immune complexes followed by electron microscopy (immune electron microscopy; IEM) was performed in 3 series of experiments as follows:

In order to characterise a typical strain of "picornavirus-like particles" (see Results), the virus was reacted with human hepatitis A antiserum (courtesy of Dr A Coulepis, Fairfield Hospital). In one control experiment hepatitis A virus (courtesy Dr A Coulepis, Fairfield Hospital) was used as test antigen, while in another, distilled water was used in place of the antiserum in reaction with the picornavirus-like particles.

In order to determine if a human serum contained antibodies to feline astrovirus (see Results), 2 strains of astrovirus were reacted with serum from patient AS. In control experiments distilled water was used in place of the serum. Patient AS was a 27-year-old individual who had recently returned from an overseas trip and was admitted to Fairfield Hospital with fever and diarrhoea. At the time the serum was collected, the patient was excreting virus-like particles similar to astrovirus in her faeces.

In order to determine if parvovirus-like particles (see Results) were feline panleukopenia virus, 2 strains of the parvovirus-like particles were reacted with feline panleukopenia parvovirus antiserum (from the study of Studdert and Peterson 1973). In control experiments the antiserum was reacted with the serologically related canine parvovirus which had been purified with propylene glycol (courtesy of Dr P Scott, Veterinary Research Institute, Parkville, Victoria). Control experiments using distilled water in place of antiserum were also carried out with both the feline parvovirus-like particles and the canine parvovirus.

In all experiments the concentrated purified faecal extract was used as a source of antigen and antiserum was added to antigen at a ratio of 1:10, except in experiments with hepatitis A antiserum where antigen and antiserum were mixed at a ratio of 1:1. After mixing, the antigen-antibody combination was incubated at 37°C for 1h, stored overnight at 4°C, and then examined after negative staining.

Virus Isolation

Virus isolation procedures were carried out with 40 clarified but unconcentrated faecal specimens as described previously (Kennett *et al* 1972; Kennett *et al* 1974) using, in all cases, cynomolgus monkey kidney epithelial cells (MK), rhinovirus sensitive HeLa cells (HeLa), and Borrie cells (Bo). In all but one case, heteroploid cynomolgus monkey embryonic cells (MEK) were used as well. The 40 faecal samples were chosen as follows: in 10 cases one or more types of virus were detected by EM (see Results), and in 30 cases, chosen at random, no virus or virus-like particles were detected by EM (see Results). The 2 reoviruses isolated (see Results) were typed by haemagglutination inhibition, using 0.5% human erythrocytes and rabbit antisera prepared against reoviruses 1, 2 and 3.

Results

Virology

Apart from tailed phage, 7 morphologically distinct virus or virus-like particles were recognised in cat faeces. Virus isolation as well as IEM and other serological procedures were used to further classify some of these particles.

Parvovirus-like particles — "Parvovirus-like particles" was the name given to a group of particles morphologically similar to canine parvovirus (Marshall *et al* 1984). These round, typically featureless particles with a diameter of about 24 nm (m ± S.D. 24.4 ± 1.1 nm; n = 205 particles from 23 cats), occurred as discrete particles (Figure 1a), as clumps (Figure 1b) and rarely as immune complexes (Figure 1c). IEM studies using 2 morphologically typical strains showed that neither was serologically related to feline panleukopenia virus. Virus isolation studies, using another 3 strains, showed that the particles failed to induce a detectable cytopathic effect in MK, MEK, HeLa and Bo cells.

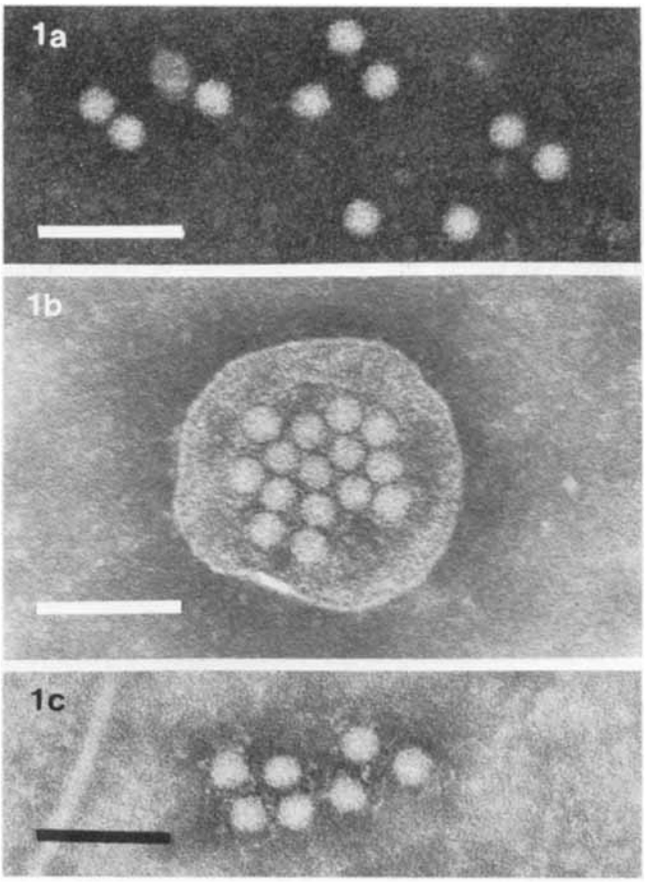


Figure 1. Parvovirus-like particles: (a) free virus; (b) clump of virus (associated with membrane); (c) immune complex of virus. The bar represents 100 nm.

Astrovirus — Astrovirus could be identified with confidence by their size and characteristic staining pattern (Madeley 1979). The particles measured about 30 nm in diameter (m ± S.D. 29.9 ± 1.7 nm; n = 176 particles from 15 cats) and in favourable orientations revealed the characteristic staining pattern of a 5 or 6 pointed, white centred, star (Figure 2). The particles were usually present as free virions or clumps and sometimes appeared to have antibody on their surface.

IEM studies, carried out on 2 strains of the virus using the human "AS" serum, showed clearly that in both cases the human serum reacted with the feline astrovirus (Figure 3a and 3b). Thus human serum can contain antibody to astrovirus from cats.

Attempts to isolate 2 strains of astrovirus in MK, MEK, HeLa and Bo cells were unsuccessful.

Picornavirus-like particles — "Picornavirus-like particles" was the name given to a group of virus-like particles which typically had a circular outline, a featureless surface and a diameter of about 30 nm (m ± S.D. 30.0 ± 2.1 nm; n = 117 particles from 13 cats) (Figure 4). Careful examination of electron micrographs suggested that in 8 of the 13 cases where these particles were detected, there was a hint of surface scalloping reminiscent of astrovirus. The classification of particles in this

group must therefore be considered a loose one and the group may contain particles of more than one type.

IEM and tissue culture studies were carried out on 3 of the 5 strains where there was no evidence of any astrovirus-like surface scalloping. In one case, IEM tests showed the virus-like particles were not hepatitis A. Attempts to isolate 3 strains in MK, MEK, HeLa and Bo cells were unsuccessful.

Reovirus — Reovirus was recognised in one faecal sample by its characteristic morphology (Madeley 1972) (Figure 5) and size (m ± S.D. 74.6 ± 1.0 nm; n = 3 particles from 1 cat). This virus was successfully isolated in MK cells and was typed as reovirus 1. Reovirus was also isolated from another faecal sample (see below), although the virus was not seen by EM.

Rotavirus — Rotavirus was recognised with confidence on the basis of the size and characteristic morphology (Martin *et al* 1975). Rotavirus was of 2 main types: complete particles with an outer shell (Figure 6) measuring about 76 nm in diameter (m ± S.D. 76.3 ± 1.5 nm; n = 33 particles from 8 cats), and particles lacking an outer shell measuring about 67 nm in diameter (m ± S.D. 67.2 ± 1.8 nm; n = 18 particles from 5 cats). Cell culture and serological studies of the rotavirus have been the subject of a separate study (Birch *et al* 1985).

Coronavirus — Coronavirus was recognised with confidence by its size, pleomorphic shape, and characteristic petal shaped spikes (see Madeley 1972) (Figure 7). Coronavirus particles were commonly about 100 to 200 nm along the long axis, while the spikes measured about 19 nm in length (m ± S.D. 19.3 ± 3.6 nm; n = 8 spikes from 8 particles from 2 cats).

Togavirus-like particles — The term "togavirus-like particle" is used here to designate a virus-like particle which bore some resemblance to the togaviruses (Horzinek 1981) (Figure 8) although the size and appearance of these particles was also consistent with a possible atypical rotavirus.

The particles were round with an overall diameter of about 75 nm (m ± S.D. 75.3 ± 2.0 nm; n = 7 particles from 2 cats). The particles were present as both full and empty structures (Figure 8). The particles appeared to be covered with short protrusions measuring about 8 nm in length (m ± S.D. 8.4 ± 1.3 nm; n = 7 protrusions from 7 particles from 2 cats).

Attempts to isolate these 2 strains of virus-like particle using MK, MEK, HeLa and Bo cells were unsuccessful.

Tissue culture studies of faecal samples where no virus or virus-like particles were detected by EM — Eighteen samples of faeces of normal consistency and 12 faecal samples from cats with diarrhoea, in which no virus or virus-like particles were detected by EM, were also tested for the presence of common cultivable viruses (especially human picornavirus, adenovirus and reovirus) using cell culture techniques. Virus was isolated in one case only: reovirus 1 was grown from one normal faecal sample.

Clinical and Epidemiological Observations

Viruses and diarrhoea — Table 1 summarises the relationship between diarrhoea and the excretion of virus and virus-like particles. It can be seen that, apart from coronavirus and togavirus-like particles, all the other particle types were found in normal faeces, sometimes quite frequently.

Although more than one faecal sample was collected, over time, from 9 of the 208 cats, no virus or virus-like particles were detected more than once in a given cat. Thus the data in Table 1 gives the incidence of the different particles in the cat population when expressed as a percentage of 208.

Table 2 summarises the relationship between the concentration of virus and virus-like particles and the appearance of the faeces for the 4 most common groups of particles (parvovirus-like particles, astrovirus, picornavirus-like particles and rotavirus). It can be seen that there was no simple relationship between virus concentration and the appearance of the faeces; quite high concentrations of virus or virus-like particles could be found in normal faeces in all 4 categories.

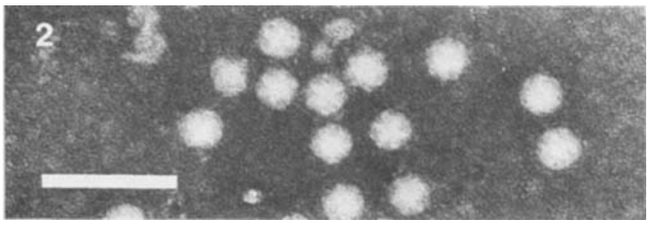
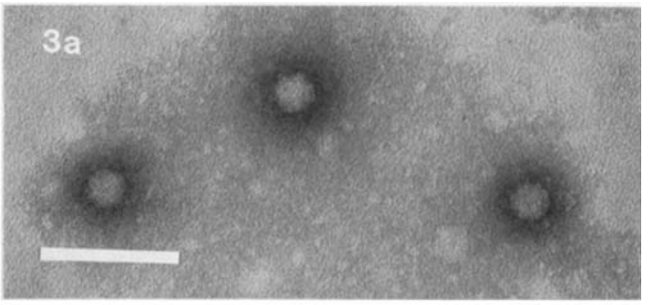


Figure 2. Astrovirus. Note the star-shaped staining pattern in some particles. The bar represents 100 nm.



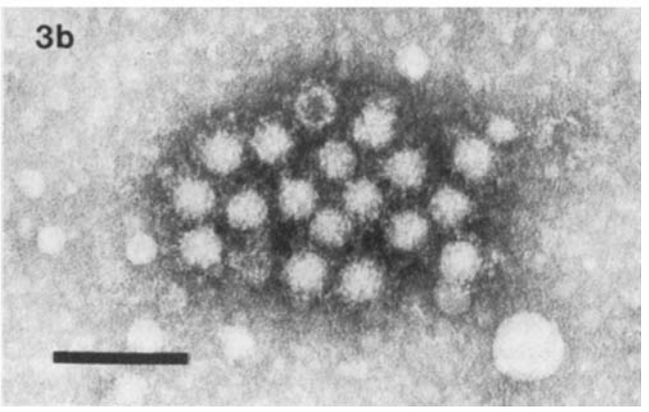


Figure 3. Immune electron micrographs demonstrating that human serum can contain antibody to cat astrovirus. (a) Cat astrovirus without the addition of antibody; (b) immune complex formed after interaction of cat astrovirus with serum from a human with gastroenteritis who was excreting astrovirus-like particles at the time the serum was collected. The bar represents 100 nm.

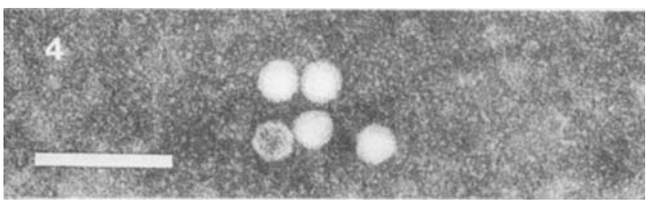


Figure 4. Picornavirus-like particles. The bar represents 100 nm.

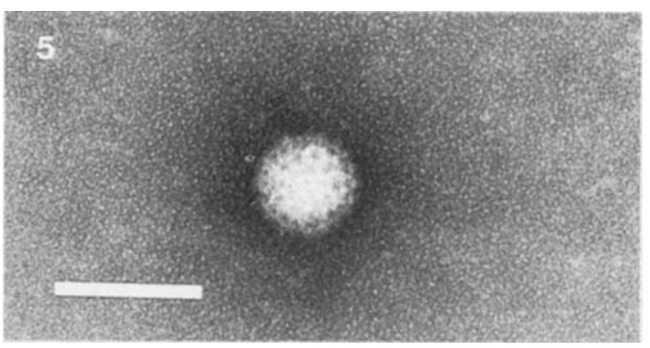


Figure 5. Reovirus. The bar represents 100 nm.

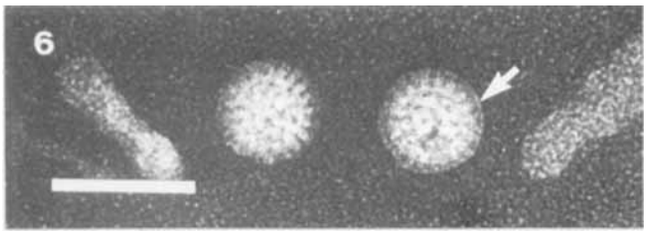


Figure 6. Rotavirus. A distinct outer shell is evident on one particle (arrow). The bar represents 100 nm.

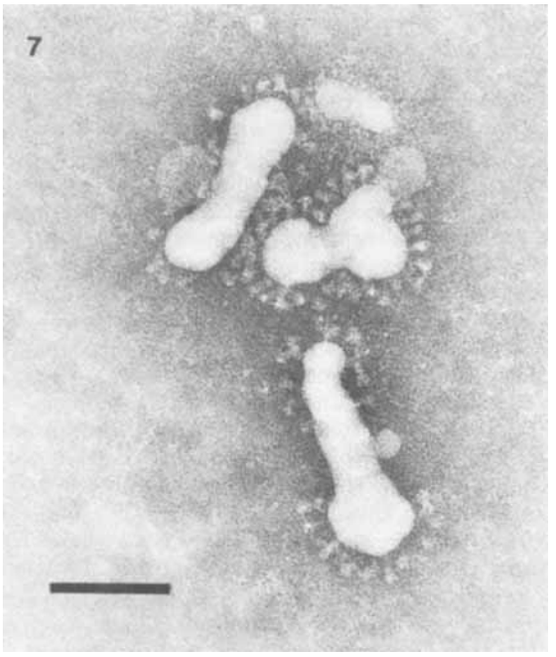


Figure 7. Coronavirus. The bar represents 100 nm.

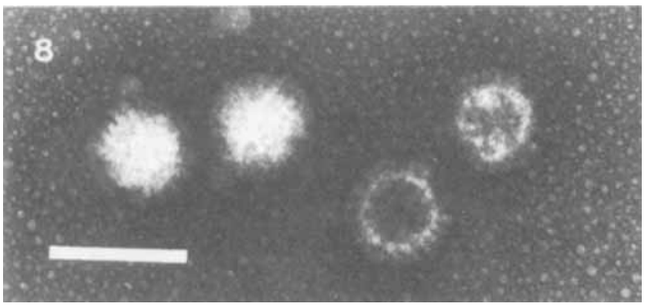


Figure 8. "Togavirus-like" particles. The bar represents 100 nm.

Age — The age of cats was usually recorded by the veterinarians in months up to 1 year and then as "adult", frequently with no further information. For the following analysis cats aged less than 1 year are referred to as "juveniles" while the remainder are referred to as "adults".

When the data for the first faecal collection from each of the 208 cats was analysed, of the 57 diarrhoeal cats, 37 (65%) were adults and 20 (35%) were juveniles with a mean age of 5.4 months. Of 151 cats with normal faeces, 98 (65%) were adults and 53 (35%) were juveniles with a mean age of 5.0 months. Thus, there was no simple relationship between age and diarrhoea, in the population of cats studied.

When the data for the main virus groups was examined (Table 3) to determine if there was any relationship between age and the excretion of a given particle, it was noted that parvovirus-like particles and rotavirus occurred more frequently in older cats than in younger cats, while astrovirus and picornavirus-like particles occurred more frequently in younger cats. However, the differences are small, and it appears that age is not a critical factor for the excretion of parvovirus-like particles, rotavirus, astrovirus and picornavirus-like particles.

TABLE 1
Association between virus type and diarrhoea

Virus type (s) detected by EM	Number of diarrhoeal faecal specimens*	Number of normal faecal specimens†
Parvovirus-like	2 (3)	18 (11)
Astrovirus	6 (10)	5 (3)
Picornavirus-like	3 (5)	8 (5)
Reovirus	0 (0)	1 (1)
Rotavirus	1 (2)	6 (4)
Coronavirus	1 (2)	0 (0)
Togavirus-like	1 (2)	0 (0)
Parvovirus-like and astrovirus	1 (2)	1 (1)
Parvovirus-like and picornavirus-like	0 (0)	1 (1)
Rotavirus and astrovirus	0 (0)	1 (1)
Rotavirus and picornavirus-like	0 (0)	1 (1)
Rotavirus and coronavirus	1 (2)	0 (0)
Togavirus-like and astrovirus	1 (2)	0 (0)

* Figure in brackets gives percentage of 62 diarrhoeal specimens
† Figure in brackets gives percentage of 166 normal specimens

TABLE 2
Relationship between virus concentration and faeces type

Virus type*	Number of diarrhoeal faeces			Number of normal faeces		
	Concentration†			Concentration†		
	1+	2+	3+	1+	2+	3+
Parvovirus-like	2	0	0	13	1	4
Astrovirus	1	1	4	1	1	3
Picornavirus-like	2	0	1	4	1	3
Rotavirus	1	0	0	2	1	3

* Only cases where a single type of virus or virus-like particle was detected in a faecal sample are included here
† 1+ = 1 to 49 particles/grid square
2+ = 50 to 99 particles/grid square
3+ = more than 99 particles/grid square

TABLE 3
The relationship between virus excretion and age of cats for all faeces

Virus type detected by EM*	Total cases	Number of "juveniles"†	Number of "adults"‡
Parvovirus-like	23	7 (10)	16 (12)
Astrovirus	15	7 (10)	8 (6)
Picornavirus-like	13	6 (8)	7 (5)
Reovirus	1	1 (1)	0 (0)
Rotavirus	10	2 (3)	8 (6)
Coronavirus	2	1 (1)	1 (1)
Togavirus-like	2	2 (3)	0 (0)

* Where more than one virus type was excreted by an individual, each virus type is listed
† Percentages of total "juveniles" (73) given in brackets
‡ Percentages of total "adults" (135) given in brackets

Seasonal features — Figure 9 summarises the incidence of diarrhoeal cases throughout the 16 months of the study. The data indicated marked fluctuations in the incidence of diarrhoea which were not clearly associated with the seasons.

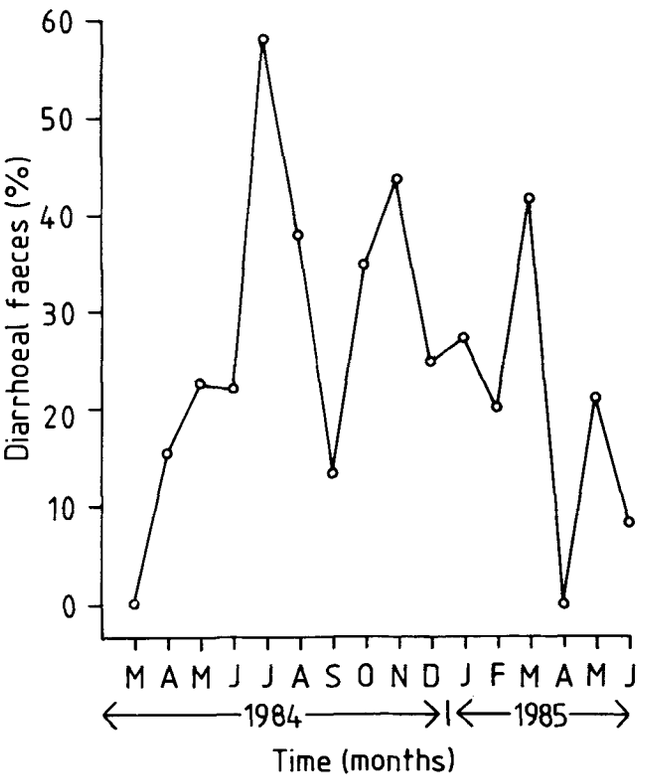


Figure 9. Incidence of diarrhoeal faeces during the course of the study.

Discussion

The chief finding of this study was that virus and virus-like particles of 7 types could be excreted in the faeces of domestic cats. Two categories of particles do not appear to have been previously reported in cats and at least one of these particle types may not have been previously described at all. At least 5 of the 7 virus types could occur in apparently healthy, non-diarrhoeal cats. There is a possibility that some of the particles detected were related to human viruses, but no human picornaviruses or adenoviruses were isolated.

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 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 provide valuable information in the first instance.

Parvovirus-like particles, indistinguishable from canine panleukopenia virus (Marshall *et al* 1984) were detected in about 11% of cats studied. They were quite commonly found in cats without diarrhoea and could be excreted in high concentration by such cats. Attempts to identify some of these particles by culture techniques and IEM were unsuccessful although IEM showed that at least some of these particles were not serologically related to feline panleukopenia virus. The particles therefore may represent a parvovirus serologically different from feline panleukopenia virus or bacteriophage lacking a distinct tail (Tikhonenko 1970; Hung 1976).

Astrovirus was detected in about 7% of cats studied. It was frequently associated with diarrhoea, but could also be excreted in high concentration by cats with normal faeces.

Although astrovirus and astrovirus-like particles have now been reported in a number of animal species including mice (Kjeldsberg and Hem 1985), cattle (Bridger *et al* 1984), dogs (Williams 1980), sheep (Herring *et al* 1981), man (Konno *et al* 1982), cats (Hoshino *et al* 1981b) and pigs (Bridger 1980), the serology of astrovirus is poorly understood. Within a given species such as man (Lee and Kurtz 1982; Kurtz and Lee 1984) and cattle (Woode *et al* 1985) more than one serotype has been described, but it is not clear if astroviruses from different species can be serologically related. Bridger (1980) failed to demonstrate any serological relationship between porcine astrovirus antiserum and bovine astrovirus, while Lee and Kurtz (1982) failed to demonstrate any relationship between 2 strains of human astrovirus and antiserums to bovine and ovine astrovirus. The results of this study show that human serum can contain antibody to feline astrovirus, but the precise interpretation of this finding must await further study.

Picornavirus-like particles were detected in the faeces of about 6% of cats, including some with faeces of normal consistency. While there was a strong suspicion that some of these particles were astrovirus with poorly defined surface markings, some of the particles certainly did not resemble astrovirus. IEM and culture methods failed to clarify the identity of some of these latter particles. Similar particles do not appear to have been reported previously in cat faeces.

Reovirus was found by EM and culture procedures in about 1% of cats. Both strains were typed as reovirus type 1 and were from normal faeces. The relationship between disease and infection by reovirus is problematic, at least in humans (England 1985). Nevertheless, the study does show that cats can act as carriers of reovirus 1.

Rotavirus was detected in about 5% of all cats examined. The particles could be excreted in high concentration in the absence of diarrhoea. Birch *et al* (1985) recently reported on the electropherotypes and serotypes of 6 strains of feline rotavirus identified in this study. These workers demonstrated that all isolates were serotype 3 rotaviruses and noted that cats might act as a source of rotavirus infection in man.

Coronavirus is a known agent of enteritis in cats (Pedersen *et al* 1981) and in this study was found in 2 cats with diarrhoea. No attempt was made to further identify these particles.

"Togavirus-like particle" was the name given to a possible new type of virus that has not previously been described in cats. This type of particle was found in 2 cats both of which had diarrhoeal faeces. Recently Williams (1985) reported the presence of "virus-like particles", similar in size and shape to those seen here, in the faeces of a person with diarrhoea. Therefore this particle type may be of significance to both cat and human health. Further studies are needed to exclude the possibility that these togavirus-like particles are an atypical rotavirus.

Two virus particle types previously identified in cat faeces were not found in this study. Coronavirus-like particles (CVLP), which have a morphology distinct from coronavirus, have been reported in cat faeces (Hoshino and Scott 1980; Stoddart *et al* 1984) but were not found in this survey. Calicivirus has been isolated from cat faeces (Wardley 1976) but was not found in this study, possibly because EM, the chief method of investigation here, is insensitive for their detection in faeces.

A number of authors have used culture methods to examine the faeces of domestic animals and non-human primates for viruses of human interest (Gelfand 1961; Grew *et al* 1970; Kalter 1982) although there does not appear to have been any detailed survey of cat faeces. Although 40 faecal samples were tested by methods appropriate for the isolation of human picornaviruses, adenoviruses and reoviruses in this study, only reovirus was isolated.

Acknowledgments

The authors would like to thank the staff of the RSPCA Burwood, Dr S Liu, S Land and K Dickson of the Virology Department, Fairfield Hospital and the staff of the entero-respiratory laboratory at Fairfield Hospital for their advice and assistance.

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(Accepted for publication 30 October 1986)

Neurological disease and lipofuscinosis in horses and sheep grazing *Trachyandra divaricata* (branched onion weed) in south Western Australia

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SUMMARY: A severe paretic syndrome accompanied by intense neuronal lipofuscinosis is described in sheep and horses exposed to *Trachyandra divaricata*. This is a newly recognised toxic hazard for grazing livestock in the coastal region of the south west of Western Australia. Animals appear to become affected over a period of weeks when summer conditions induce a scarcity of alternative feed. The disease is discussed in relation to its recent documentation in South Africa where the plant is indigenous.
Aust Vet J **64:** 105-108

Introduction

Trachyandra divaricata (Jacq) Kunth. is a species of the family Liliaceae, indigenous to south western regions of South Africa. In Western Australia it is commonly known as branched onion weed (Figures 1, 2) and it has become well established and widely distributed on the Calcareous tuart and beach sands of the south western coastal belt (MacFarlane 1986). Disease in grazing livestock in South Africa has recently been attributed to this plant (Newsholme *et al* 1985) and to a related species, *T. laxa* (Grant *et al* 1985). The disease is characterised clinically by a severe paretic syndrome, and pathologically by intense lipofuscin storage in neurons in the brain, spinal cord and peripheral ganglia, and to a lesser extent, in the Kupffer cells of the liver, renal tubular cells and macrophages in the spleen, lung and intestinal mucosa. In this paper we report the occurrence of a similar clinical and pathological entity in sheep and horses exposed to *T. divaricata* in south western Australia. It is suggested that the evidence is sufficient for *T. divaricata* to be recognised as a hazard to grazing livestock in this region. According to the Western Australian Herbarium, *T. divaricata* is the only species of *Trachyandra* known to occur in Australia and appears to be confined to the area indicated above, although it is poorly recorded (MacFarlane 1986).

The species it is most likely to be confused with is *Asphodelus fistulosus,* another naturalised plant common in the region (MacFarlane 1986).

The following description of the plant is from Obermeyer (1962):

"Plants robust up to 90 cm high (Figure 1). *Roots* many, not much thickened, occasionally growing to a great depth. *Rhizome* woody, thick, irregular in shape. *Squamae* narrow, tubular, surrounding each leaf-and scape-base separately, *Leaves* liner, up to 100 cm long, 4-12 mm wide, tapering gradually to the apex, flat, glabrous, somewhat fleshy, flexible, erect or usually prostrate, straight or with a lax spiral twist, bright green, occasionally orange at the base.
Inflorescence stout, usually with accessory branches, divaricately branched; scape 10-50 cm high, stout glabrous; bracts small, 4 mm long, membranous, widely ovate at the base; pedicels 4-12 mm long. *Flowers* erect, perianth segments 7-12 mm long, white, green-keeled with a yellow dot near the base, spreading, recurved from the middle; stamens yellow in lower half, dimorphous, 3 outer spreading, 3 inner commivent around

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