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Short Communication

## Shedding of “virus-like” particles in canine faeces

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### Abstract

Diarrhoeic faeces from about 500 dogs were examined by negative stain electron microscopy. As well as parvovirus, and some of the other recognised viral causes of gastroenteritis, unusual “virus-like” particles were observed in about 8% of the samples. The particles were spherical, 100 nm to 300 nm in diameter, and surrounded by a thick wall penetrated by numerous pores. An additional 74 samples of normal faeces yielded no “virus-like” particles. We do not know the nature of these particles.

*Keywords:* “Virus-like” particles; parvovirus; Canine faeces

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### 1. Introduction

Canine parvovirus was identified in 1978. One of the methods used in the diagnosis of canine parvovirus was negative stain electron microscopy of diarrhoeic canine faeces to detect parvovirus particles. Using this technique astroviruses, coronaviruses, coronavirus-like particles, papova-like viruses, parvoviruses, picornaviruses, rotaviruses and small round viruses have been detected (Williams, 1980; Carmichael and Binn, 1981; Hamilton et al., 1982; Hammond and Timoney, 1983; Marshall et al., 1984). We have used this technique to investigate parvovirus disease and to evaluate a diagnostic test (Drane et al., 1994). During this work we observed unusual “virus-like” particles similar to those recorded by Hamilton et al. (1982). Here we report further observations on these particles.

### 2. Materials and methods

About 500 faecal samples or swabs supplied by veterinarians or from our own studies were suspended in either phosphate buffered saline or distilled water and clarified by low

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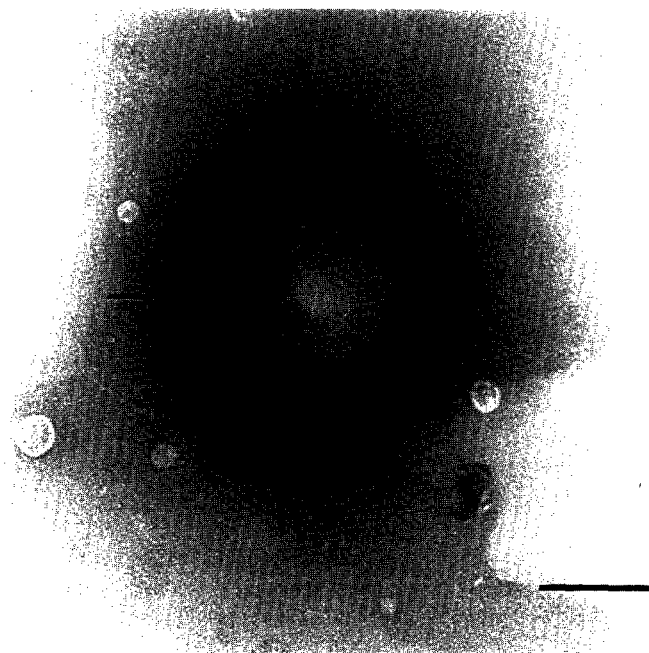


Fig. 1. Single "virus-like" particle (arrow). Bar = 100 nm.

speed centrifugation. A drop of sample was applied to a Formvar-coated copper electron-microscope grid which was then inverted onto 2% (w/v) Noble Agar. After the grid had settled onto the agar, it was picked up then floated on 2% (w/v) ammonium molybdate for thirty seconds at room temperature. The grid was picked up and excess stain removed with filter paper. Three grids were prepared from each sample. The grids were examined with a Philips EM301 electron microscope, at an instrumental magnification of  $50\,000\times$ . All virus or "virus-like" particles that were observed were photographed.

### 3. Results

About 8% of the abnormal faecal samples, and none of the normal faecal samples, contained "virus-like" particles. The "virus-like" particles were usually spherical and had a thick wall which was penetrated by numerous pores (Fig. 1). This wall appears to be rigid as the particles are not readily distorted on the grid. When damaged particles were observed they appeared to be cracked open (Fig. 2). The particles did not disintegrate into a cluster of subunits as has been observed with a number of other viruses.

Indented particles were observed (Fig. 3). Often the particles were penetrated by stain but when they were not penetrated the detailed surface texture became apparent (Fig. 1). When the particles were penetrated by stain, no regular internal component was observed, but they did not appear to be completely empty (Fig. 2).

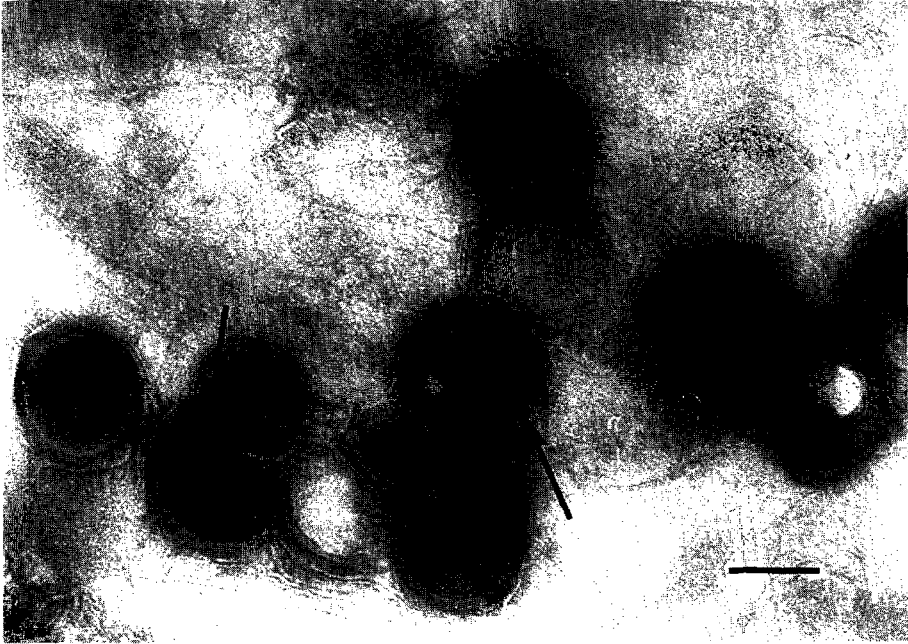


Fig. 2. Cracked "virus-like" particles. (Arrows) bar = 100 nm.

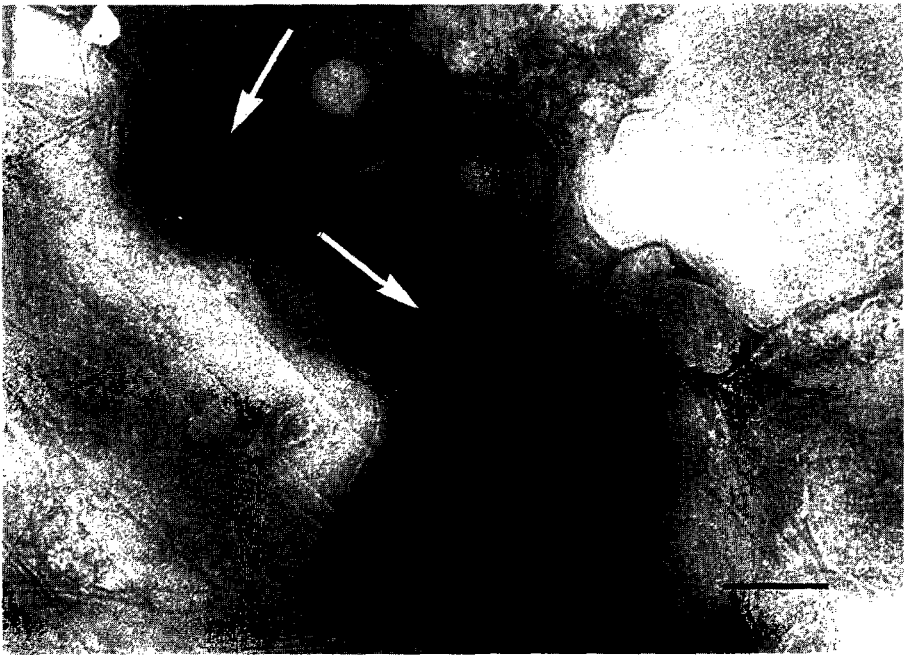


Fig. 3. "Virus-like" particles with indented ends (arrows). bar equals 100 nm.



Fig. 4. "Virus-like" particles of two different sizes, larger particle (thick arrows) smaller particle (thin arrow), bar equals 100 nm.

The particles ranged from 100 nm to about 300 nm. Usually in any one sample there were particles of one size but occasionally particles of two different diameters were present in one sample (Fig. 4 and Fig. 8).

The particles were usually scattered singly or in small groups throughout the sample (Fig. 1) but, at times, larger groups or groups of over a hundred particles were observed bound within membranes (Fig. 5, Fig. 6 and Fig. 8). The particles often absorbed to gut bacteria (Fig. 7). Parvovirus particles were observed in some samples containing the virus-like particles (Fig. 8).

#### 4. Discussion

We have observed these "virus-like" particles in diarrhoeic canine faeces from 1981 to 1994. Similar particles have been observed in canine faeces in Portugal (Alves de Matos, 1988, personal communications) which indicates the particles have a wide temporal and geographic distribution.

Other comprehensive negative-stain electronmicroscopy studies of canine faeces, however, have not reported on these particles (Williams, 1980; Carmichael and Binn, 1981; Hammond and Timoney, 1983; Marshall et al., 1984).

Initially we thought that the particles may be a new family of viruses (Hamilton et al., 1982). We now think that this is unlikely. Although the particles have a regular patterned



Fig. 5. Group of "virus-like" particles enclosed within a membrane, (thin arrow) bar equals 100 nm.

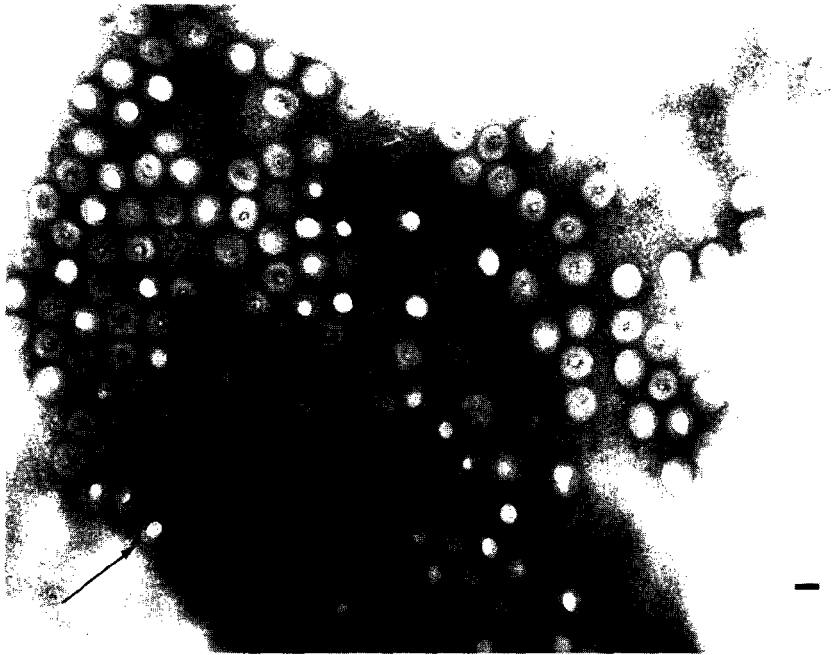


Fig. 6. Over a hundred "virus-like" particles enclosed within a membrane, (thin arrow) bar equals 100 nm.

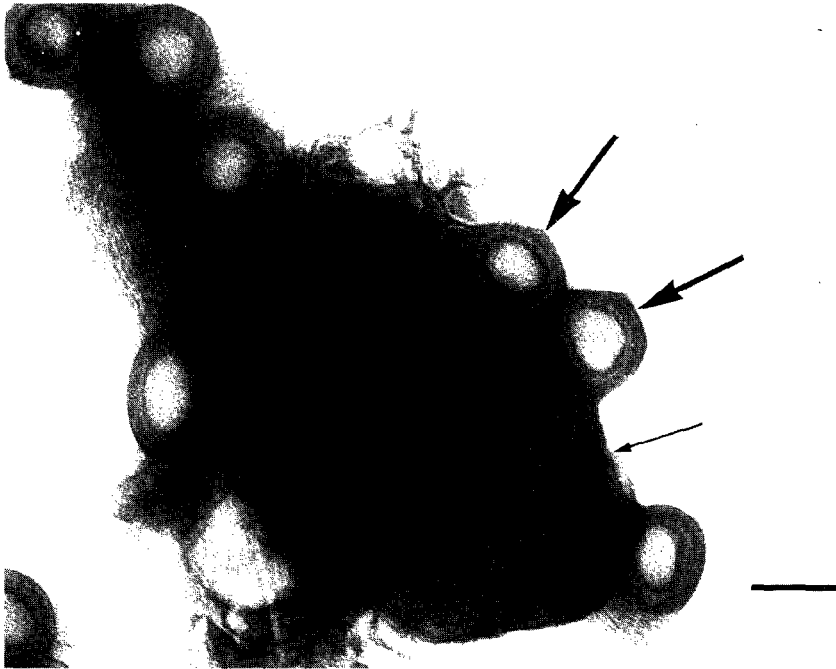


Fig. 7. "Virus-like" particles (thick arrows) adsorbed to a bacterium. (thin arrow) bar equals 100 nm.

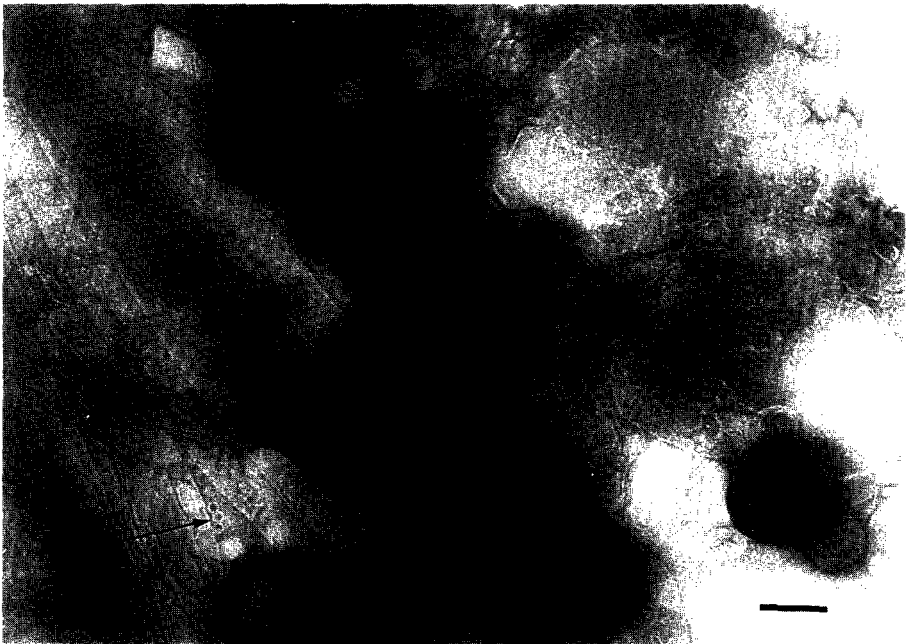


Fig. 8. "Virus-like" particles and parvovirus particles (thin arrow). Bar equals 100 nm.

surface they do not appear to be composed of typical viral sub-units nor do they appear to be composed of ‘‘spikes’’ inserted into a membrane. Perhaps the particles are botanical or mineral components of dog’s food. Perhaps the ‘‘virus-like’’ particles are some structures associated with bacteria or parasites in the gut of dogs. The fact that the particles are found enclosed within membranes would suggest this. They may be spore or egg structures that are formed within a membrane, usually released from the membrane within the gut then shed in the faeces.

We do not know if these particles are involved in any disease process but they are found in diarrhoeic faeces. However, the faeces often also contained parvovirus, a well known cause of gastroenteritis in dogs. They have also been observed associated with picornaviruses (Alves de Matos, 1988, personal communication).

We do not know the true nature of these ‘‘virus-like’’ particles.

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### **References**

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