

Virus and virus-like particles observed in the intestinal contents of the possum, *Trichosurus vulpecula*

Brief Report

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Summary. Intestinal contents derived from the Australian brush-tailed possum, *Trichosurus vulpecula*, were examined by negative stain electron microscopy for the presence of viruses. Out of 100 samples, 23 contained at least one type of vertebrate virus or virus-like particle. Adenovirus was identified in six samples, herpesvirus in two samples, coronavirus in four samples, and coronavirus-like particles in 14 samples. To date no viruses of the brush-tailed possum have been isolated in tissue culture but these results indicate that this species is probably host to several viral species.

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The recorded number of natural viral infections of marsupials as a group is very low. Whether this is because marsupials are less susceptible to viral infections or because they have been insufficiently studied is unclear. Representatives of three virus families have been isolated from or physically detected in marsupials. These are an adenovirus which was isolated from kidney cultures derived from the South American opossum (*Didelphis marsupialis marsupialis*) [15], herpesviruses isolated from rat kangaroos (*Bettongia penicillata* and *Aepyymnus rufescens*) [5], and wallabies (*Macropus parma*, *Dorcopsis muelleri luctuosa* and *Setonix brachyurus*) [7, 24], and a retrovirus detected by the polymerase chain reaction and thin section electron microscopy in peripheral blood leucocytes from the koala (*Phascolarctos cinereus*) [4, 25]. Histological and serological evidence also implicate infection by herpesviruses in marsupial mice (*Phascogale tapoatafa* and *Antechinus stuartii*) [3], and the common wombat (*Vombatus ursinus*) [20], by poxviruses in kangaroo (*Megalia rufescens*, *Macropus fuliginosus* and *Macropus giganteus*) [2, 13], and wallaby (*Setonix brachyurus*) [16], and by an adenovirus [19], and a group A arbovirus [14], in the Australian brush-tailed possum (*Trichosurus vulpecula*).

As discussed elsewhere [19], the Australian brush-tailed possum, which was introduced into New Zealand last century, now has a population of 65 million distributed over 95% of the land area. The pressure exerted by possum numbers on native forests due to overbrowsing and the role of possums in the spread and maintenance of bovine tuberculosis make the search for effective control methods a high research priority in New Zealand.

In a search for viruses that may prove suitable for use in the biological control [23], of the Australian brush-tailed possum, a study was initiated to examine the intestinal tract contents of this species for viral particles by negative staining electron microscopy.

Diagnostically, electron microscopy has been used to detect a range of viruses (eg. adenovirus, coronavirus, rotavirus) in faeces from clinical cases of human and animal diarrhoea [12, 17, 18], and is particularly useful for viruses that are not readily propagated *in vitro*. The same technique has also been used to detect viruses in non-diarrhoeal faeces [10], and is capable of detecting 10^5 – 10^6 virus particles per ml of faeces [1].

This paper reports the detection of four morphologically classifiable viral types present in the intestinal contents of the Australian brush-tailed possum.

Samples of intestinal contents were obtained from 100 possums from three distinct geographical areas in New Zealand: Northland ($n = 50$), Manawatu ($n = 30$), and Westland ($n = 20$). Samples were collected shortly after death from the colon in the area anterior to faecal pellet formation and stored at -70°C until processed. A 20% w/v suspension of each sample was made in 0.1 M phosphate buffered saline, thoroughly mixed, then centrifuged at $2\,800 \times g$ for 30 min to deposit debris. The clarified supernatant was layered over a 1 ml cushion of 45% sucrose and centrifuged at $100\,000 \times g$ for 2 h at 4°C . The resulting pellet was resuspended in 100 μl of distilled water, negatively stained with 2% phosphotungstic acid (pH 7.0) and examined using a Philips 201c, or Philips CM10 electron microscope.

Twenty three samples were found to contain particles that were morphologically consistent with vertebrate viruses. Four distinct types were identified. Adenovirus was found in six samples, herpesvirus in two samples, coronavirus in four samples, and coronavirus-like particles (CVLP) in 14 samples. Examples of each viral type are shown in the electron micrographs in Fig. 1.

A total of 11/50 (22%) of possums from Northland, 10/30 (33%) from Manawatu and 2/20 (10%) from Westland were positive for at least one virus type. Two co-existing virus types were recorded in 3/100 (3%) of the tested samples: one of these contained adenovirus and coronavirus particles; one contained adenovirus and coronavirus-like particles; one contained coronavirus and coronavirus-like particles. Figure 2 shows the distribution of viral types from the three different geographical collection sites. Adenovirus and coronavirus-like particles were detected in all three sites, coronavirus at two sites, and herpesvirus at only one site. Although the number of positive animals for each viral type in individual areas is low, the results indicate that possums in each area are host to a range of viral species.

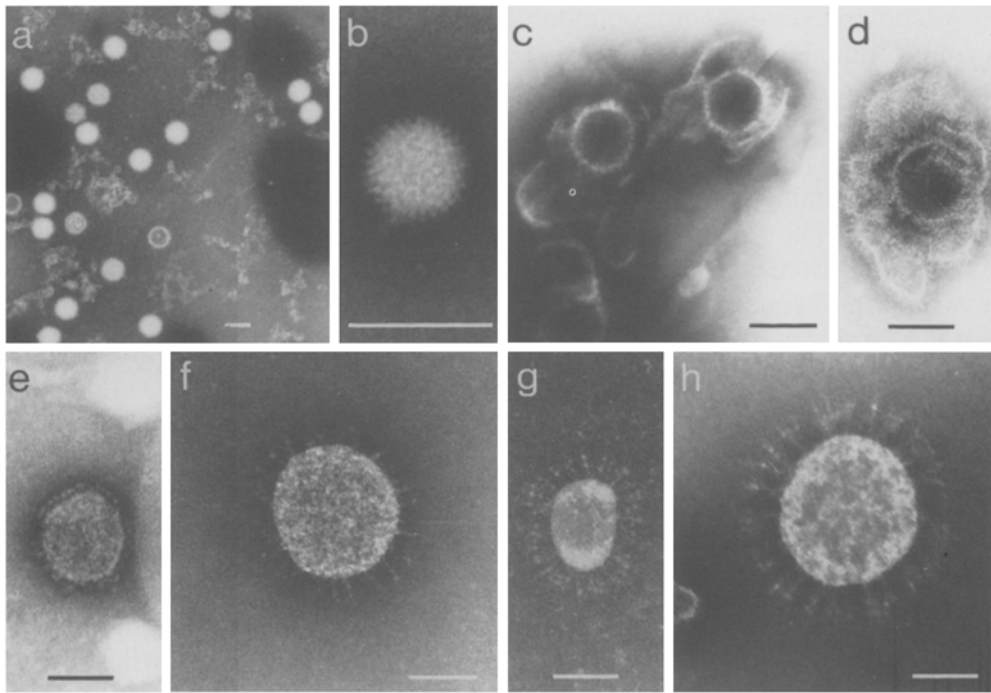


Fig. 1. Electron micrographs of negatively stained virus and virus-like particles seen in the gastrointestinal tract contents of possums: **a, b** adenovirus, **c, d** enveloped herpesvirus, **e** coronavirus, **f-h** coronavirus-like particles. Bars: 100 nm

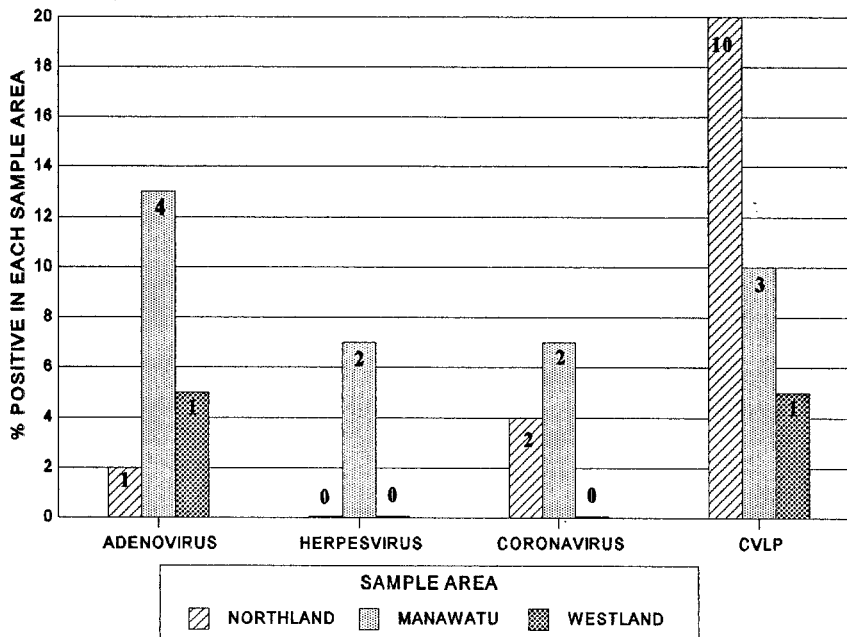


Fig. 2. Distribution of viral and virus-like particles in each of the three sites for which possums were sampled. Numbers within bars represent actual number of positive possums

Adenovirus particles are commonly found in gut contents and faeces especially of young animals and infants and can be associated with diarrhoea [5, 11]. Of the six samples positive for adenovirus, one was fluid in consistency and contained a large number (> 25 particles per photographic field at $40\,250\times$ magnification) of adenovirus particles (Fig. 1). The non-enveloped nucleocapsids measured 72–79 nm in diameter, exhibited icosahedral symmetry and typical adenovirus morphology. The remaining five adenovirus positive samples were non-diarrhoeal in consistency and contained only the occasional adenovirus particle (one or two per grid square on 200 mesh grids).

One sample contained a small number of unenveloped herpesvirus nucleocapsids. Enveloped herpesvirus particles were observed in the preparation from a second sample (Fig. 1c, d). The envelope was up to 366 nm across and bore surface projections of 10–14 nm in length. The nucleocapsids measured 95–120 nm in diameter and displayed icosahedral symmetry. Individual capsomeres were seen as hollow hexamers. The intact envelope of the herpesvirus in the second sample suggests that the virus source was distal to the stomach as it seems unlikely that the envelope would survive intact as the virus passed through the strongly acidic environment of the stomach. Peyer's patches or the gut epithelial cells are possible candidate sites for replication of herpesvirus. Earlier reports, including one marsupial infection, have described herpesviruses in these tissues [8, 24].

Coronavirus particles were seen in four samples. They showed variation in size from 54 nm to 187 nm (mean = 112 nm) and were primarily round to elliptical in shape, with the occasional pleomorphic form. The distinguishing feature of this genus, which is often found in the gastrointestinal tract of animals is the presence of characteristic surface projections, peplomers, which consist of a thin stalk (total length 10–20 nm) with a terminal sphere or flattened sphere (Fig. 1e).

The coronavirus-like particles found in 14 samples showed a range in size and variations in the morphology of their surface projections. The particles were round to ovoid in shape, 75–245 nm diameter (mean 147 nm) with very long surface projections (31–67 nm) consisting of a thin stalk with one or two knobs discernable along their length (Fig. 1f–h). Some projections appear to have one or two laterally squashed knobs along the stalk giving the appearance of T-shaped structures (Fig. 1h). In some cases the stalk is seen to project beyond the attached knob (Fig. 1g, h). A similar range of coronavirus-like morphologies have been reported in other mammals including cats [9], mice [11], dogs [21], and man [10, 22].

This study has provided electron microscopic evidence of four recognisable virus types present in the possum population in New Zealand. It further supports the interpretation of our earlier serological study that possums are host to at least one species of adenovirus [19]. Not unexpectedly, it appears that they are also host to herpesvirus, coronavirus and coronavirus-like particles. While the finding of viral particles in the intestinal tract is not absolute evidence that they are the result of active replication in the possum, the possibility that they have merely been ingested and are in transit seems unlikely. Our findings suggest that

the small number of reports of viruses in marsupials is due in part to the paucity of comprehensive studies of diseases that affect this group of mammals. Further work is underway to propagate the different viral types obtained from the Australian brush-tailed possum to allow their further characterisation.

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