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Detection of Parvoviruses in Wolf Feces by Electron Microscopy

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ABSTRACT: One hundred fifteen wolf (*Canis lupus*) feces were collected between 1980 and 1984 from northeastern Minnesota and were examined for canine parvovirus by negative contrast electron microscopy. Of these, seven (6%) samples revealed the presence of parvovirus. Some of these viruses were able to grow in cell cultures forming intranuclear inclusion bodies and giant cells.

Key words: Wolf feces, electron microscopy, parvoviruses, cell cultures, intranuclear inclusion bodies, giant cell formation.

Fecal samples (n = 115) from wild wolves (*Canis lupus*) were collected in the field between 1980 and 1984 from northeastern Minnesota and were examined by negative contrast electron microscopy (EM). All samples were stored outdoors at ambient temperatures of about −40 to +35 C for up to 5 yr and later in the laboratory at 4 C for 1–6 mo until examined. The samples for EM were prepared by adding 2 g of feces to 10 ml of distilled water, sonicating for 15 sec at 20% maximum power on a W-375 sonicator (Heat Systems-Ultrasonics, Inc., 1938 New Highway, Farmingdale, New York 11735, USA), clarifying by centrifugation at 3,000 g for 10 min, and then centrifuging the supernatant at 50,000 g for 1 hr (Goyal et al., 1987). The pellet was suspended in 200 µl of distilled water. The samples were stained for 2 min by adding 50 µl of 2% potassium phosphotungstate (PTA) to 50 µl of sample suspension according to the drop staining technique of England and Reed (1980). Stained samples were transferred to collodion-carbon-coated copper grids, blotted, dried in air and examined at 100,000× magnification in a Zeiss-10 electron microscope (Carl Zeiss, Inc., One Zeiss Drive, Thornwood, New York 10594, USA). Viruses observed in EM preparations were classified as parvoviruses based on size, morphology and symmetry. Virions ranged from 20 to 25 nm in diameter and were spherical to polygonal in shape. Large aggregates of viruses having complete and empty particles were seen in fecal preparations (Fig. 1). Seven samples (6%) were found to contain parvoviruses (Table 1).

Attempts were made to propagate all parvovirus positive samples in porcine kidney (PK-15), mink (*Mustela vison*) lung (ML), and Crandell feline kidney (CRFK) cells (Nettles et al., 1980). All cells were obtained from the American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland 20852, USA). Inoculated cells did not show significant cytopathic effects except for the presence of intranuclear inclusion bodies and formation of giant cells occasionally in CRFK cells. No evidence of infection was present in ML and PK-15 cells and no attempt was made to further classify the isolated parvovirus particles.

The results in Table 1 indicate an increase in the number of parvovirus carrying wolves from 1980 to 1984. Although these results could conceivably indicate a progressive deterioration of virus in the feces from 1980 to 1984, we do not consider this possibility a cogent explanation. Instead, we believe that these results may be accounted for by the emergence of a new strain of canine parvovirus in dog populations in 1978 (Parrish et al., 1985). It is possible that the wolves with low number of parvovirus may have helped in precipitation and dissemination of parvovirus infections. Parvovirus is excreted in feces and is extremely resistant to heat and desiccation, so it may widely contaminate the environment, thus leading to dissemina-

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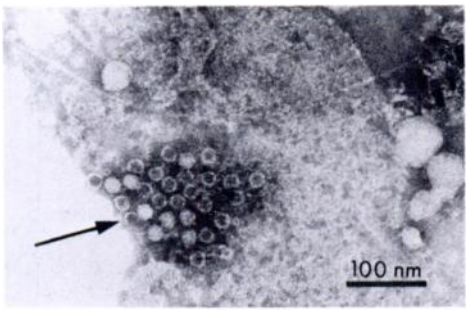


FIGURE 1. Electron photomicrograph of parvovirus particles in wolf feces (100,000×). Both complete and empty virions can be seen.

TABLE 1. Detection of parvovirus in wolf feces by electron microscopy (1980–1984).

Year	Number of samples examined	Number (%) positive
1980	24	0 (0)
1981	24	1 (4)
1982	24	0 (0)
1983	24	1 (4)
1984	19	5 (26)
Total	115	7 (6)

tion of the infection (Thomas et al., 1984). Goyal et al. (1986) and Mech et al. (1986) reported hemagglutination inhibiting antibodies in sera of captive and wild wolves from northeastern Minnesota. By using fluorescent neutralization test, Zarnke and Ballard (1987) detected anticanine parvovirus antibodies in sera of wolves from southcentral Alaska. All of the above mentioned studies indicated an increase in the number of parvovirus seropositive wolves after 1979.

The present study supports the findings of these workers by confirming the presence of parvoviruses in feces of wolves from Minnesota. That the canine parvovirus is capable of infecting many wild carnivores is evidenced by its reported isolation from racoons (*Procyon lotor*) (Nettles et al., 1980), captive wolves (Mann et al., 1980), and foxes (*Vulpes* spp.) (Neuvonen et al., 1982). Thomas et al. (1984) reported the absence of antibodies in free-ranging coyotes from Texas, Utah and Idaho prior to 1979, but observed an increase in the number of antibody carrying coyotes to about 70% by 1982. The onset of canine parvovirus seroprevalence in free-ranging coyotes coincided remarkably with the recognition of clinical disease and seroprevalence in domestic dogs in the United States. The data obtained in the present study suggest that EM of fecal samples, in conjunction with the serological approach, is quite suitable to study inapparent viral infections in wolves. It would be interesting to conduct a large scale epidemiological study on parvoviruses in wild canids. Also, characterization of parvoviruses from wild canids would be useful in understanding their epidemiology in wildlife.

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