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A Serologic Survey for Antibodies to Three Canine Viruses in Wolverines *(Gulo gulo)* from the Brooks Range, Alaska

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ABSTRACT: Canine distemper virus (CDV), canine parvovirus type 2 (CPV-2), and canine adenovirus type 1 (CAV-1) are pathogens that are typically associated with canids but may cause serious disease in a wide range of other carnivores. From 1998 to 2002, serum samples from 64 wolverines (*Gulo gulo*) from the Brooks Range, Alaska, were tested for antibodies to CDV, CPV-2, and canine adenovirus (CAV). Four animals tested positive for antibodies to CDV (7%), one for antibodies to CPV-2 (2%), and none for antibodies to CAV. These are similar to antibody prevalence estimates for other large and medium carnivores in North America.

Key words: Canine adenovirus, canine distemper virus, canine parvovirus, *Gulo gulo*, serology, wolverine.

Canine distemper virus (CDV), canine parvovirus type 2 (CPV-2), and canine adenovirus type 1 (CAV-1) are common pathogens of canids (Appel, 1987). Both CDV and CPV-2, however, have been detected in a wide range of noncanid carnivores, often causing serious disease with high mortality rates (Deem et al., 2000; Steinel et al., 2001). A CDV epidemic caused a serious decline in lion (*Panthera leo*) abundance in the Serengeti National Park, Tanzania (Roelke-Parker et al., 1996), and mortality associated with CDV infection in black-footed ferrets (*Mustela nigripes*) approached 100% (Williams et al., 1988). Antibodies to CPV-2 has been reported from a variety of non-canid species, including the spotted hyena (*Crocuta crocuta*) (Harrison et al., 2004), the giant panda (*Ailuropoda melanolueca*) (Mainka et al., 1994), and the brown bear (*Ursus arctos*) (Madic et al., 1993; Marsilio et al., 1997). The potential importance of CAV-1, the causative agent of infectious canine hepatitis, in noncanid carnivores is unknown, but antibodies to this virus have been detected in American black bear (*Ursus americanus*), striped skunk (*Mephitis mephitis*), and raccoon (*Procyon lotor*) (Grate et al., 1987).

The wolverine (*Gulo gulo*) is a medium mustelid that has recently received attention because of declining populations in both Europe and western North America (Banci, 1994; Landa et al., 1999; Kyle and Stobeck, 2001). The potential importance of disease in regulating wolverine populations is poorly understood, and the occurrence of CDV, CPV-2, and CAV-1 in wolverine populations has not been documented (Murray et al., 1999). Wolverines typically scavenge carcasses of large ungulates (Magoun, 1987) and potentially share carcasses with other carnivore species, such as red foxes (*Vulpes vulpes*) and gray wolves (*Canis lupus*). Carcasses could therefore act as transmission sites between wild canids and wolverines. In Alaska, CDV, CPV-2, and CAV-1 are known to occur in gray wolf populations (Zarnke et al., 2004). To investigate if wolverines are also infected or exposed to these viruses, we conducted a serologic survey for antibodies to CDV, CPV-2, and canine adenovirus (CAV) among wild wolverines from the Noatak and Kobuk river drainages in the western Brooks Range, Alaska. Apart from a recently published survey of Canadian carnivores (Philippa et al., 2004), this is, to our knowledge, the first serologic survey for these viral agents in this species.

Samples were collected from two sources. As part of a radiotelemetry research program, wolverines were captured either by darting them from a helicopter or by trapping them in steel-barrel traps. We anesthetized 23 animals from 1998 to 2002

(Golden et al., 2002), and blood was collected from the cephalic vein. Serum samples were stored at −20° C until analysis. We also purchased carcasses of harvested wolverines from hunters. During 2001 and 2002, we collected blood from 41 carcasses on Nobuto blood filter strips (Toyo Roshi Kaisha Ltd., Tokyo, Japan). Filters were air-dried and were eluted in 500 μl of phosphate-buffered saline (pH=7.4) at room temperature for 1 hr. Eluted samples were stored at −80° C until analysis. All surveyed animals were from the Noatak or Kobuk river drainages, in the western Brooks Range (68°35′N–65°15′N; 162°55′W–159°15′W). We estimated the age of 34 of the harvested animals by using cementum annuli counts from canine teeth (Matson's Laboratory, LLC, PO Box 308, Milltown, Montana, USA), as previously described (Matson, 1981).

All serologic tests were conducted at the Washington Animal Disease Diagnostic Laboratory (WADDL, Washington State University, Pullman, Washington, USA). Serum samples were tested for antibodies to CDV using virus neutralization; eluted samples from Nobutu strips were tested by an immunofluorescent assay (Guo et al., 1986). Serum and eluted samples were tested for antibodies to CPV-2 using an immunofluorescent assay (Evermann et al., 1980; Helfer-Baker et al., 1980). Sera were tested for antibodies to CAV by virus neutralization (Foreyt et al., 1986). This test does not distinguish between CAV type 1 and type 2. Positive threshold antibody titers were 50, 25, and 4 for serum tested for CDV, CPV-2, and CAV, respectively. For eluted samples, a 1:1 dilution for CDV and CPV-2 and a 1:2 dilution for CAV were considered positive.

Our sample was biased toward young animals, with 73% of surveyed wolverines estimated at <2 yr of age; 58% of the samples were from males and 42% from females. Four animals tested positive for antibodies to CDV (7%); positive antibody titers ranged from 48 to 768 for eluted samples and 16 for serum. One animal tested seropositive for CPV-2 (2%) with an antibody titer of 94. Antibodies to CAV were not detected. Because 27 samples were cytotoxic for the CAV neutralization test, these negative results are based on a sample size of 37 (23 serum samples and 14 Nobutu strip samples). Two of the CDV-seropositive animals were males and two were females; the two males were aged at 2 and 3 yr, one female was aged at 1 yr, and the age of the other female was unknown. The CPV-2-positive animal was a yearling male. None of the seropositive animals were caught or harvested in immediate proximity to any of the local villages.

To our knowledge, this survey provides the first reported evidence for occurrence of CDV and CPV-2 in wild wolverines. The prevalence of antibodies to CDV in this population was lower or similar to what has been found in grizzly bears (14%) and gray wolves (2–8%) in similar environments (Deem et al., 2000). It is also similar to CDV antibody prevalence reported for a population of North American river otters (*Lontra canadensis*) (4.7%) from eastern New York (Kimber et al., 2000).

The failure to find any animals seropositive for CAV, as well as our low seroprevalence of CPV-2, agrees with a serologic survey of Alaska grizzly bears (Chomel et al., 1998). In that study, bears from southern Alaska had substantially higher seroprevalence of CAV-1 than bears from interior Alaska where our wolverine samples were sampled. Antibodies to both CPV-2 and CAV-1 are common in populations of Alaska wolves (Zarnke et al., 2004). Antibodies to CPV-2 have been reported from 31% of European brown bears (Madic et al., 1993) and 16% of American black bears (Dunbar et al., 1998), indicating the ability of the virus to infect noncanid species.

These serologic results may underestimate the actual rate of infection in the wolverine population. For example, a high mortality rate associated with these viruses in this species would leave few seropositive

animals in the population. Antibody prevalence may also be underestimated by low test sensitivity or through loss of antibody titers over time. Although the technique of using filter strips to collect blood for serologic analysis routinely has been used to detect antibodies to several pathogens in a wide range of species (Ahlm et al., 1997; Mueller-Anneling et al., 2000), we did not validate the technique specifically for the serologic techniques applied to wolverines in this study.

Our results suggest that both CDV and CPV-2 were present in this wolverine population and antibody prevalence to CDV and CPV-2 appeared similar or somewhat lower than reported in other medium and large carnivores in North America. We suggest that future efforts should be made to investigate potential pathologic effects of CDV and CPV-2 in wolverines to better understand serologic results and to determine whether these viruses can potentially limit or regulate these populations.

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