FECALLY TRANSMITTED VIRUSES ASSOCIATED WITH PACIFIC FISHERS (*MARTES PENNANTI*) IN NORTHWESTERN CALIFORNIA

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***Abstract*:** Western populations of fisher (*Martes pennanti)* recently were designated by the U.S. Fish and Wildlife Service to be a distinct population segment meriting listing under the federal Endangered Species Act. Disease was noted in the designation as a potential issue affecting fishers, and this study provides information on exposures of a fisher population in the Hoopa Valley Indian Reservation to pathogens in northwestern California. Of 31 fishers sampled between December 2004 and March 2005, 1 (3%) had been exposed previously to canine distemper virus (CDV) and 13 (42%) had been exposed to canine parvovirus (CPV). Although little is known about disease in fishers, both viruses can be transmitted via fecal contamination and other means, and CDV causes high rates of mortality in other mustelids. We recommend disinfecting traps and handling equipment between captures to minimize risks of spreading pathogens to fishers and other carnivores.

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Fishers (*Martes pennanti)* are midsize, forest-dwelling mustelids. The geographic distribution of the species historically included the boreal forests of southern Canada, the northern Rocky Mountains, northeastern and upper-midwestern United States, and southward through the Cascades and coastal mountains of the Pacific Coast, northern California, and the west side of the Sierra Nevada Mountains (Gibilisco 1994). However, forest management practices combined with the effects of over trapping during the early 1900s resulted in low population densities throughout Washington, Oregon, and California, and led to the isolation of the population in the southern Sierra Nevada

Mountains (U. S. Fish and Wildlife Service 2004). In 2004, populations in the Cascade Mountains of northwestern Washington extending south through California were designated as a "distinct population segment" (DPS) that merited listing under the federal Endangered Species Act (U. S. Fish and Wildlife Service 2004). In the listing, disease was considered to be 1 of 5 threats to fishers in the DPS and a conservation strategy was developed that included possible reintroduction of fishers along the Pacific Coast.

Relatively little is known about the diseases of fishers (Douglas and Strickland 1999, Powell et al. 2003), but some outcomes can be predicted by extrapolating from closely related species. Pathogens causing potentially severe disease in related mustelids include: rabies virus (Rupprecht et al. 2001); canine distemper virus (Williams 2001, Langlois 2005); parvoviruses (PVs) (Barker and Parrish 2001, Williams 2001, Langlois 2005); influenza viruses; corona viruses; *Brucella* spp. (the cause of brucellosis); *Yersinia pestis* (the cause of plague) (Williams et al. 1994); *Leptospira interrogans* (the cause of leptospirosis) (Powell et al. 2003); *Toxoplasma gondii* (the cause of toxoplasmosis) (Dietz et al. 1993, Frank 2001, Burns et al. 2003, Philippa et al. 2004); *Dioctophyma renale* (the giant kidney worm) (Powell et al. 2003); and

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Trichinella spiralis (the cause of trichinosis) (Dick and Leonard 1979, Dick et al. 1986, Powell at al. 2003). In addition, canine adenovirus (the cause of canine infectious hepatitis) has been associated with severe disease and death of striped skunks (*Mephitis mephitis*) (Karstad et al. 1975) as well as members of the Canidae.

Significant mortality has been caused by CDV and PVs in mustelids, such as the black-footed ferret (*Mustela nigripes*), mink (*M. vison*), and others (Barker and Parrish 2001, Williams 2001, Langlois 2005). The transmission of CDV is through respiratory droplets, contact with virus-laden discharges, or through fecal material among populations of wild and domesticated carnivores (Williams 2001, Langlois 2005). Species susceptible to CDV include the wolf (*Canis lupus*), coyote (*C. latrans*), domestic dog (*C. familiaris*), gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), coatis (*Nasua narica*), stone marten (*M. foina*), black-footed ferret, mink, polecat (*M. putorius*), domestic ferret (*M. furo*), European badger (*Meles meles*), weasel (*Mustela* spp.), striped skunk, African wild dog (*Lycaon pictus*), raccoon dog (*Nyctereutes procyonoides*), greater panda (*Ailuropoda melanoleuca*), lesser panda (*Ailurus fulgens*), and African lion (*Panthera leo*).

Although the host range is very broad, susceptibility varies among these species with high rates of mortality in the black-footed ferret, gray fox, raccoon, and lesser panda, and there is some level of resistance in the striped skunk and red fox. Other carnivores, including American black bears (*Ursus americanus*), develop antibodies when exposed to CDV but rarely suffer severe disease (Williams 2001). PVs are transmitted through a fecal–oral route in populations of felids, canids, procyonids, and mustelids, including mink, American marten (*M. americana)*, weasels, and domestic ferrets (Barker and Parrish 2001, Langlois 2005). Susceptibility of mustelids to these viruses varies and most likely depends on characteristics of specific strains of the viruses and population characteristics of the hosts. The most virulent PVs are most likely Aleutian disease of mink (ADV) and mink virus enteritis (MEV) for some mustelids (Barker and Parrish 2001, Langlois 2005), but the virulence of the different PV strains in fishers has yet to be reported.

The current study was undertaken to determine: (1) whether relevant disease agents are present within the fisher population in northwestern California; and (2) provide information relative to possible future management of fishers within the state. Although our program is a large study of multiple pathogens associated with a community of midsize carnivores, this paper contains information only on exposures of fishers to CDV and PV. Both viruses can be transmitted through contaminated feces, and CDV can be transmitted through saliva and oculonasal discharges. Therefore, these pathogens should be a concern for biologists and managers who use potentially contaminated equipment to trap, handle, or relocate fishers.

STUDY AREA

Sampling was conducted on the Hoopa Valley Indian Reservation (HVIR) in northeastern Humboldt County, California. The HVIR occupies approximately 362 km^2, and is bisected by the Trinity River in a south-to-north direction with elevations ranging from 76 to 1,170 m. The landscape is a heterogeneous mix of habitats, but Douglas-fir (*Pseudotsuga menziesii*) and tan oak (*Lithocarpus densiflorus*) forests predominate (Singer and Begg 1975). The HVIR is not isolated, and the fisher population we sampled is assumed to be a subset of the larger fisher population in northwestern California.

METHODS

Fishers were trapped in wire-mesh live traps (81 x 25 x 31 cm) (Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) and sampled between December 2004 and March 2005. Traps were attached to wooden nest boxes for security, to reduce environmental stressors, and to facilitate animal handling (Gabriel and Wengert 2005), and were baited with chicken. Body weight of trapped fishers was estimated to the nearest 0.2 kg. Fishers were handled using a handling cone with metal rods, and animals were anesthetized with

ketamine (20 mg/kg of body weight) and diazepam (<1 mg/kg of body weight) (Aubry and Raley 1996). Each anesthetized fisher was maintained in lateral recumbency to stimulate blood circulation and avoid adverse post-capture effects. A physical exam was performed to identify traumatic injuries and physical defects, and heart rate, capillary refill time, jaw tone, pupil position, and respiration rate were monitored to assess the effects of the anesthesia.

All fishers were ear tagged and injected with a passive integrated transponder (PIT) for future identification. Hair and tissue samples were collected for genetic identification and analysis of population genetics. Dentition was evaluated for field estimation of the age of each animal, and a first, upper premolar was removed for aging by cementum annuli (Matson's Laboratory, Milltown, Montana, USA). All fishers were released at their sites of capture after recovery from anesthesia. Approximately 1–3 ml of whole blood was collected by venapuncture and frozen in the anticoagulant ethylene diamine tetraacetic acid (EDTA) until shipped to the University of California, Davis, for further evaluation. Blood was thawed and centrifuged to separate the plasma from the cells. Plasma samples were then refrozen until ready for analysis and serially diluted in 10 mM phosphate–buffered saline (138 mM NaCl, 2.7 mM KCl).

Antibodies that bound to CDV, CPV, and FPV antigen on microscope slides were detected by indirect immunofluorescence assays (IFAs). Antigen for the CDV IFA was from kidney cells collected from CDV-infected domestic ferrets (American Bioresearch, Milton, Tennessee, USA). Positive controls were serum samples from domestic dogs previously shown to have high standardized titers, and negative controls were from dogs maintained at a specific-pathogen-free (SPF) colony. Positive results were defined as those reacting at a dilution of ≥1:8. Antigen slides for the CPV and FPV IFAs were purchased from a commercial source (Veterinary Medical Research and Development, Pullman, Washington, USA). Positive controls were blood samples from dogs or cats (*Felis domesticus*) previously shown to have high standardized titers to CPV or FPV. Negative controls were from SPF dogs or cats, respectively. Positive results were defined as those reacting with characteristic localization of bright-green fluorescence at a dilution of ≥1:25. Laboratory work was done between April 2005 and May 2006.

A *t*-test was used to compare the mean age of male and female fishers. Differences in prevalence of exposure of male vs. female or young vs. adult fishers were assessed with 2-tailed Fisher's exact tests of association. We used Cohen's kappa statistic, based on results tabulated as either positive or negative, to evaluate agreement between serologic tests using FPV and CPV as an antigen.

RESULTS

A full complement of data concerning age and disease-exposures was collected from 31 fishers comprising 13 females and 18 males. The mean age of males (2.6 years) did not differ from the mean age of females (2.3 years) (P = 0.372). Evaluation of plasma samples yielded positive reactions from 1 fisher (3%) to CDV, 13 fishers (42%) to CPV, and 11 fishers (36%) to FPV. The animal exposed to CDV was a 3-year-old female, and the test resulted in a weak reaction, indicating either a minimal immune response, a long time since infection, or a fairly rapid loss of IgG antibodies.

All fishers that tested positive for FPV were also positive for CPV (kappa = 0.865), but differentiation between the infecting viruses was not possible using the IFA. We used PV to designate exposure to PVs for which specific identification was unknown. Amplification using the polymerase chain reaction (PCR) and sequencing of DNA from fisher scats collected at HVIR subsequently found that the viruses in all positive scat samples were CPV (J. E. Foley, University of California, Davis, unpublished data). Exposures to PV did not differ between the sexes (P = 1.000) as 5 (39%) female and 8 (44%) male fishers tested positive for PV. The percentage of animals exposed, however, was much greater in animals ≥2 years of age—12 of 17 fishers ≥2 years and 1 of 14 juvenile and yearling fishers were seropositive for PV (P = 0.001) (Fig. 1).

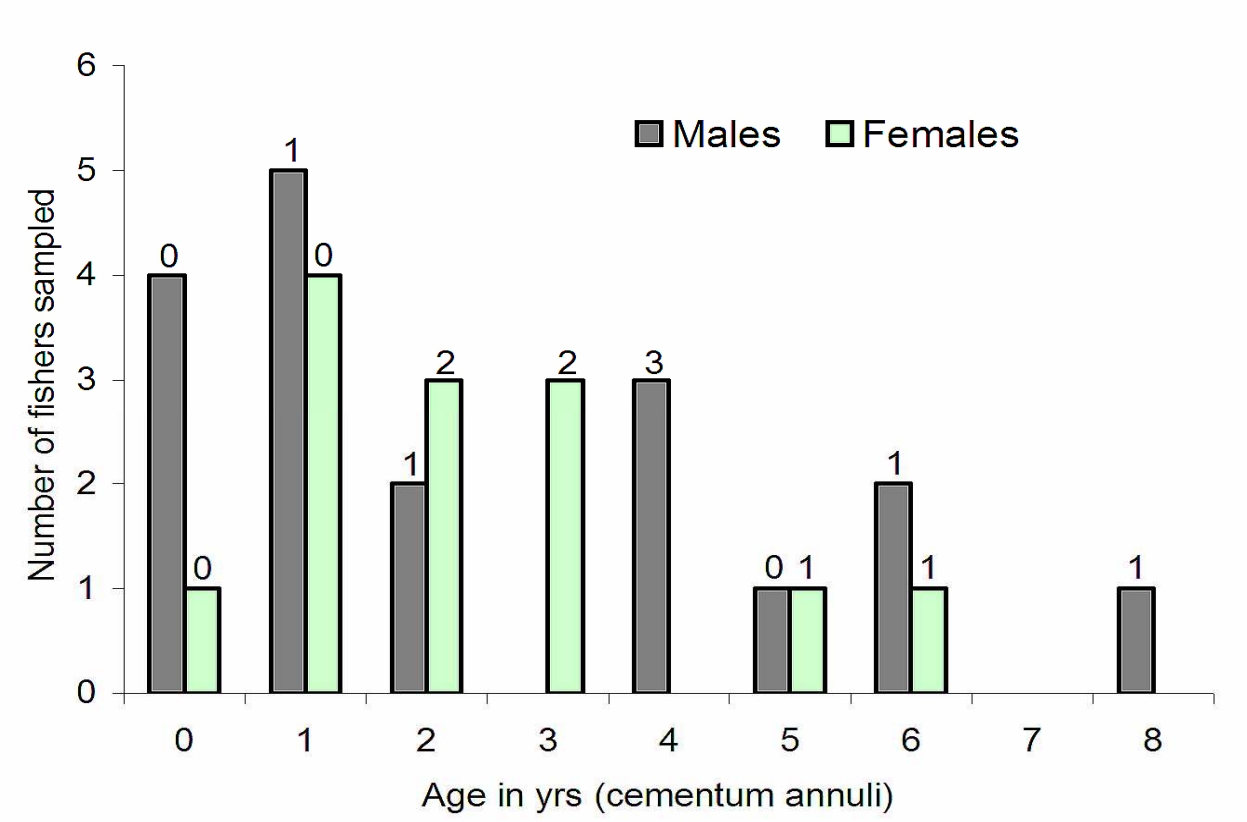

Fig. 1. Numbers of male and female fishers (*Martes pennanti*) trapped, aged by cementum annuli, and sampled at Hoopa Valley Indian Reservation between Dec 2004 and Mar 2005. Numbers above columns are the number of fishers in each age and sex category with positive antibodies to canine parvovirus.

DISCUSSION

We documented the presence of pathogens CDV and PV that could cause disease in fisherpopulations in northwestern California. Our amplification of CPV DNA from fisher scats strongly suggests that exposures of fishers to PV in our samples resulted from infections of CPV. The CDV virus is known to cause severe disease in mustelids, canids, procyonids, and some felids (Deem et al. 2000). All species of mustelids appear susceptible to clinical distemper (Deem et al. 2000), and severe disease has been reported in badgers, otters, mink, and black-footed ferrets (Deem et al. 2000, Williams 2001, Langlois 2005). Mustelids infected with CDV suffer respiratory disease, immunosuppression, neurological disease, and death (Deem et al. 2000, Williams 2001, Langlois 2005). Outward signs of distemper in carnivores vary with the strain, host species, age, and immune status of the host, but most show signs of respiratory disease, discharge from the eyes and nose that can become purulent in severe cases, coughing, vomiting, diarrhea, fever, and depression. Mustelids, including fishers, that survive infections of CDV may suffer lingering effects from damage to organs long after the virus is eliminated from the host (Williams 2001).

Lack of antibody response generally indicates that individuals have not been exposed previously to a pathogen, that the exposure is too recent to have stimulated a detectable immune response, or that exposed animals died before sampling. Therefore, low prevalence of response to CDV in populations of carnivores might indicate either low levels of exposure

(Delahay and Frolich 2000, Courtenay et al. 2001, Hanni et al. 2003) or high rates of mortality before sampling. We know that CDV is present on the HVIR because 24% of 80 black bears were exposed to CDV before 2001 (R. N. Brown, Humboldt State University, unpublished data). Moreover, unvaccinated dogs are common on the HVIR (R. N. Brown, Humboldt State University, unpublished data), and local veterinarians anecdotally recognize that CDV occurs in domestic dog populations throughout Humboldt County. The effects of CDV infections in fishers have not been reported, but it seems prudent to assume that infected fishers suffer severe disease, as do other mustelids studied (Deem et al. 2000). If most fishers exposed to CDV die of acute disease, few living animals would show evidence of previous exposure. Additional work with CDV in fishers is needed to allow clear interpretation of our results.

The feline PV subgroup includes viruses that vary in pathogenicity and virulence for a range of carnivores, including mustelids. Fishers at the HVIR had a relatively high (42%) exposure to the parvoviral antigen. Sequencing of the viral DNA amplified from the HVIR fishers has revealed the presence of only CPV and not FPV, MEV, or ADV, which are known to cause disease in mink and ferrets (Barker and Parrish 2001) and that might be more likely to cause disease in fishers. In captive studies, CPV replicated in mink and ferret cells, which explains the generation of specific antibodies, but did not cause severe disease. In striped skunks, CPV failed to replicate and caused minimal antibody response (Barker and Parrish 2001). The effects of CPV in infected fishers may be similar to infections of mink and ferrets, but the variation in signs observed in related species makes such a prediction difficult. Outward signs of PV enteritis might include diarrhea with blood or mucus, fever, and dehydration. As with distemper, disease caused by CPV would be expected to be most severe in young animals.

The prevalence of exposures of fishers to CPV on the HVIR was greater in adult than young animals, but our amplification of DNA from feces sampled from adult fishers indicates that there were current infections during our sampling period. There are several possible explanations for this pattern. Older fishers may be exposed longer than younger fishers, as would occur if exposure was from a single source, such as domestic dogs or gray fox, to which younger fishers might not have been exposed. Alternatively, disease caused by CPV may be more severe in young animals, and older fishers may have subclinical infections, as do adult mink and domestic ferrets. The disease caused by PV infection is related to characteristics of both the host and the infecting virus strain, and further studies are needed to predict the outcome of CPV infections in fishers in northern California. The high level of agreement (87%) between serologic tests using FPV and CPV as antigens indicates cross reaction between tests. Such agreement indicates that serologic tests are inadequate to differentiate between viruses, and that molecular studies of viral DNA are needed to identify PVs infecting fishers.

The source of CDV and CPV in HVIR fishers is unknown, and gray foxes or other carnivores may be local reservoirs for these viruses. Domestic dogs, however, are another virus source; unleashed dogs are common in areas near houses and uncommon in the backcountry of the HVIR (J. M. Higley, Hoopa Tribal Forestry, unpublished data). If dogs are a virus source, fishers living near humans are likely to be exposed to the virus more often than fishers with home ranges farther from human habitation. Proximity to humans has been correlated with CPV infections in spotted hyenas (*Crocuta crocuta*) in the Masai Mara of Kenya and gray foxes living near parks in central California (Riley et al. 2004). Molecular analysis of more fecal samples from tagged fishers will allow spatial analysis to evaluate such a trend. Both CDV and CPV have the potential to cause immunosuppression, especially of young animals, and to work synergistically with other pathogens to increase morbidity or mortality in a susceptible population. Although our sample of 31 individuals is small, we believe this to be the first report associating these viruses with fishers in the western DPS.

MANAGEMENT IMPLICATIONS

Our results do not indicate that fishers develop severe disease from exposures to CPV or CDV. We found few reports of exposures of fishers to pathogens in the literature, and no one has reported laboratory studies of the effects of these pathogens in fishers. Therefore, we must infer risks from diseases known from related species. Because other species of mustelids are susceptible to CDV, usually with high expected rates of mortality, we should assume that fishers are similarly susceptible.

Respiratory aerosols, oculonasal discharges, and fecal contamination spread CDV, but the virus does not survive long in the environment. In contrast, PVs persist for prolonged periods of time in the environment (Kaaden et al. 1990, Barker and Parrish 2001). Although control will be difficult, there are options to limit risks from these viruses to fishers. Exposures of CDV in black-footed ferrets, for example, are managed by vaccination and by risk monitoring. Because both CDV and CPV are transmitted through a fecal-oral route, fecal contamination of traps and handling cones are a potential source of infections to animals subsequently exposed to the same equipment. To minimize exposure risks to trapped fishers, we recommend that equipment be cleaned after processing each fisher. All visible fecal material should be removed, surfaces should be wiped clean, and equipment should be generously sprayed with a disinfectant rated to kill PVs or with a solution of 10% bleach. Although this will not likely eliminate the potential for researcher-facilitated transmission of these viruses, it should significantly minimize disease transmission risks.

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