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Canine Distemper in an Isolated Population of Fishers (*Martes pennanti*) from California

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ABSTRACT: Four fishers (*Martes pennanti*) from an insular population in the southern Sierra Nevada Mountains, California, USA died as a consequence of an infection with canine distemper virus (CDV) in 2009. Three fishers were found in close temporal and spatial relationship; the fourth fisher died 4 mo later at a 70 km distance from the initial group. Gross lesions were restricted to hyperkeratosis of periocular skin and ulceration of footpads. All animals had necrotizing bronchitis and bronchiolitis with syncytia and intracytoplasmic inclusion bodies. Inclusion bodies were abundant in the epithelia of urinary bladder and epididymis but were infrequent in the renal pelvis and the female genital epithelia. No histopathologic or immunohistochemical evidence for virus spread to the central nervous system was found. One fisher had encephalitis caused by *Sarcocystis neurona* and another had severe head trauma as a consequence of predation. The H gene nucleotide sequence of the virus isolates from the first three fishers was identical and was 99.6% identical to the isolate from the fourth fisher. Phylogenetically, the isolates clustered with other North American isolates separate from classical European wildlife lineage strains. These data suggest that the European wildlife lineage might consist of two separate subgroups that are genetically distinct and endemic in different geographic regions. The source of infection as well as pertinent transmission routes remained unclear. This is the first report of CDV in fishers and underscores the significance of CDV as a pathogen of management concern.

Key words: California, canine distemper, epizootic, European wildlife lineage, fisher, hemagglutinin, *Martes pennanti*, mustelidae.

Fishers (*Martes pennanti*) are medium-sized mustelids within the genus *Martes* that inhabit mixed coniferous forests throughout North America (Powell, 1993). Currently, the fisher within the states of Washington, Oregon, and California is a candidate species for listing under the Endangered Species Act (United States Fish and Wildlife Service, 2004). Within California, the fisher population is both geographically and genetically isolated into two major groups, one in northwestern California and the other in the southern Sierra Nevada Mountains (Knaus et al., 2011). In addition, ongoing management efforts include reintroductions in the northern Sierra Nevada Mountains of California (Callas and Figura, 2008). One pathogen of management concern is canine distemper virus (CDV). Distemper has resulted in the decline or near-extirpation of small, isolated populations of various species (Woodroffe, 1999) and almost caused the extinction of both free-ranging and captive black-footed ferrets (*Mustela nigripes*, Williams et al., 1988). Given the susceptibility of mustelids to CDV, fishers are likely affected by this pathogen, but there are no reports on disease manifestations in this species (Gabriel et al., 2011). We describe the pathologic findings in four free-ranging fishers infected with CDV and discuss the phylogeny of virus isolates.

All fishers were part of a long-term demographic study and were monitored with mortality-signal–equipped VHF radio

TABLE 1. Serologic, histologic, and immunohistochemical (IHC) findings in four fishers infected with canine distemper virus (CDV), all of whom died within the southern Sierra Nevada Mountains in California, USA, 2009.

Parameter	Fisher no. 1	Fisher no. 2	Fisher no. 3	Fisher no. 4
Sex/age	Male/juvenile	Male/adult	Female/adult	Female/juvenile
Tissue preservation	Moderate	Good	Poor	Poor
Nutritional state	Emaciated	Fair	Good	Fair
CDV antibody titer				
Date live capture	6 November 2008	7 October 2008	12 September 2008	12 September 2009
Ig M	Negative	Negative	Negative	Positive (1:8)
Ig G	Negative	Negative	Negative	Positive (1:32)
Date found dead	22 April 2009	27 April 2009	4 Mai 2009	NA[a]
Ig M	Negative	Positive (1:32)	Positive (1:32)	
Ig G	Negative	Positive (1:64)	Positive (1:128)	
Microscopic morphologic diagnoses				
Lung	Mild, necrotizing bronchitis and bronchiolitis with syncytia (IHC CDV+)	Mild, necrotizing bronchitis and bronchiolitis with syncytia and inclusion bodies	Mild, necrotizing bronchitis and bronchiolitis with syncytia (IHC CDV+)	Mild, necrotizing bronchitis and bronchiolitis with syncytia (IHC CDV+)
Urothelium		Intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)	Intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)	Intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)
Genital epithelia	Epididymis: intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)	Epididymis: intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)	Uterus/vagina: rare intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)	Uterus/vagina: rare intraepithelial intracytoplasmic inclusion bodies (IHC CDV+)
Skin	Paws: severe ulcerative pododermatitis with syncytia and intralesional bacteria (IHC CDV+)	Paws: moderate, ulcerative dermatitis with intraepithelial, intracytoplasmic inclusion bodies and intraepithelial and intrafollicular fungi		
Spleen	Mild depletion; autolysis	Mild depletion	Mild depletion; autolysis	Mild depletion; autolysis
Brain	No lesions (IHC CDV-)	Cerebral cortex: moderate, multifocal, non-suppurative encephalitis with intralesional protozoa (IHC CDV-)	Severe trauma due to predation (IHC CDV-)	No lesions (IHC CDV-)
Cause of death	CDV infection	CDV infection and secondary protozoal encephalitis	Predation (possibly increased susceptibility due to CDV infection)	CDV infection in combination with anesthesia

[a] NA = not available; animal died during the initial capture.

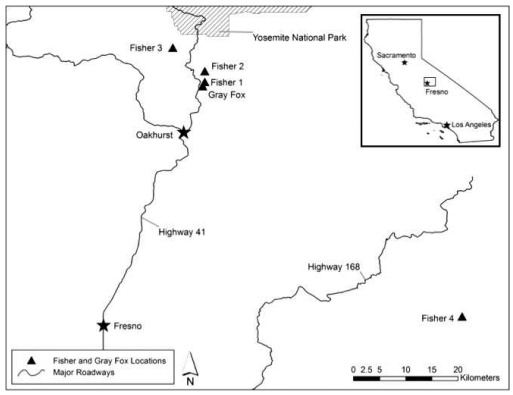

FIGURE 1. Map of locations of dead fishers (*Martes pennanti*) infected with canine distemper virus and a suspected distemper-infected gray fox (*Urocyon cinereoargenteus*) within the southern Sierra Nevada Mountains in California, USA, 2009.

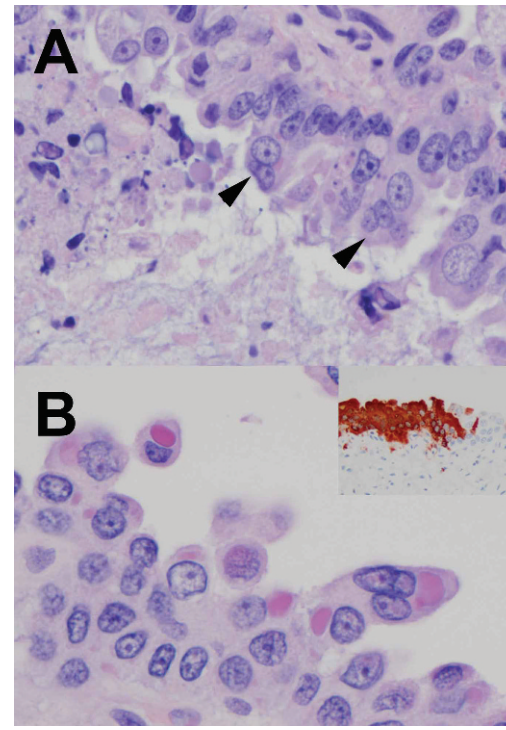

FIGURE 2. Histopathologic findings from fisher (*Martes pennanti*) no. 2 that died as a consequence of canine distemper virus (CDV) infection within the Sierra Nevada Mountains in California, USA, 2009. (A) H&E-stained section of lung. The bronchiolar epithelium is hyperplastic and has abundant syncytia (arrowheads). The lumen is filled with sloughed epithelial cells and necrotic debris. Few cells have intracytoplasmic inclusion bodies. (B) H&E-stained stained section of urinary bladder. The epithelium contains abundant intracytoplasmic inclusion bodies with syncytia. Inset: Immunohistochemistry for CDV antigen reveals strong, segmental epithelial reactivity.

collars. Fisher no. 4 was lethargic at its initial capture and died during anesthesia. All other fishers were recovered by mortality signal. The signalment, location, date, and proximate cause of death are given in Table 1 and Figure 1. Serology was done on blood samples taken at the initial capture or at the time of necropsy, as described (Riley et al., 2004). Samples for histopathology were fixed in 10% buffered formalin and processed by routine methods. Immunohistochemistry for CDV antigen was done using a primary mouse monoclonal anti-CDV antibody which was a kind gift of Professor M. Vandevelde, Vetsuisse Faculty Bern, Switzerland. Canine distemper virus was isolated from spleen, lung, and kidney of all four fishers as described by Ledbetter et al. (2009), except that the virus was grown on VeroSlam cells and virus was identified by direct fluorescent antibody technique. The hemagglutinin (H) gene was amplified by established methods (Lan et al., 2006) and nucleotide and deduced amino acid sequences were submitted to GenBank (fishers no. 1–4: JN836734–JN836737). Phylogenetic and molecular evolutionary analyses were conducted using MEGA vers. 4 (Tamura et al., 2007). Cerebral protozoa in fisher no. 2 were identified by nested PCR analysis targeting the internal transcribed spacer region (ITS-1) and the B1 gene using established primers and methods (Grigg and Boothroyd, 2001; Rejmanek et al., 2009). The resulting sequence was submitted to GenBank (JN581383).

Detailed serologic, pathologic, and immunohistochemical findings are given in Table 1. The pathologic findings were consistent with previous reports in other

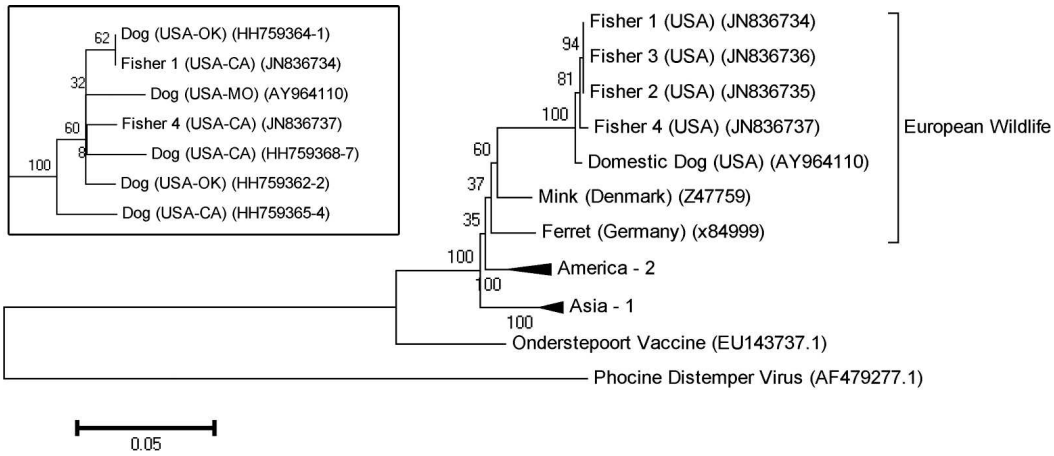

FIGURE 3. Phylogenetic tree for full-length H gene sequences of representative morbilliviruses with emphasis on European wildlife isolates. Inset: Phylogenetic tree for a 594-nucleotide segment of the H gene of European wildlife lineage isolates from North America. Numbers at the roots are the number of bootstrap iterations (out of 100) that support the nodes. Numbers in parentheses are the GenBank accession numbers for the reference and sample sequences.

mustelids (Fox et al., 1998). In brief, CDV-related gross lesions consisting of hyperkeratosis and alopecia of facial skin, as well as hyperkeratosis and ulceration of footpads, were found. Histopathologic lesions were centered on various epithelia and were absent in the central nervous system. The most consistent finding in all individuals was degeneration and necrosis of bronchiolar and bronchial epithelium with syncytia and rare intracytoplasmic and eosinophilic inclusion bodies (Fig. 2a) with varying degrees of interstitial pneumonia. Inclusion bodies with or without syncytia were abundant in the urothelium of the urinary bladder (Fig. 2b) and the epididymis but were infrequent in the renal pelvis and the female genital epithelia. Immunoreactivity for CDV was seen in pulmonary, urogenital, and cutaneous epithelia (Table 1). Antigen could also be demonstrated in epithelia that contained rare or no inclusion bodies. Brain tissue from all animals lacked reactivity for CDV antibodies. Fisher no. 2 had multifocal, nonsuppurative inflammation of the cerebral cortex with intralesional protozoa morphologically compatible with *Sarcocystis* spp. Sequencing of a 512-base pair (bp) ITS-1 band yielded a nucleotide sequence that was 99–100% similar to published *Sarcocystis neurona* sequences. *Sarcocystis neurona* infection has been implicated as the primary cause of death in one fisher (Gerhold et al., 2005), and the existence of an unrecognized *Sarcocystis* sp. has been suspected in multiple fishers from the eastern portion of their range (Gerhold et al., 2005; Larkin et al., 2011). In three animals, the proximate cause of death was determined to be encephalitis, predation, and complications during anesthesia, respectively. However, all three fishers had clinical CDV disease at the time of death as indicated by the necropsy findings. This suggests that CDV infection was the primary disease process predisposing to a secondary insult. An underlying CDV infection should therefore be considered as a differential diagnosis, even in cases with an allegedly obvious cause of death such as predation or road kill.

CDV was isolated from all four fishers and RT-PCR amplified 1,805 bp of readable sequence of the H gene. Nucleotide sequences of CDV isolated from fishers no. 1–3 were identical and differed in 4 nucleotides (one amino acid) from fisher no. 4 (99.6% nucleotide identity). Isolates were most similar to sequences

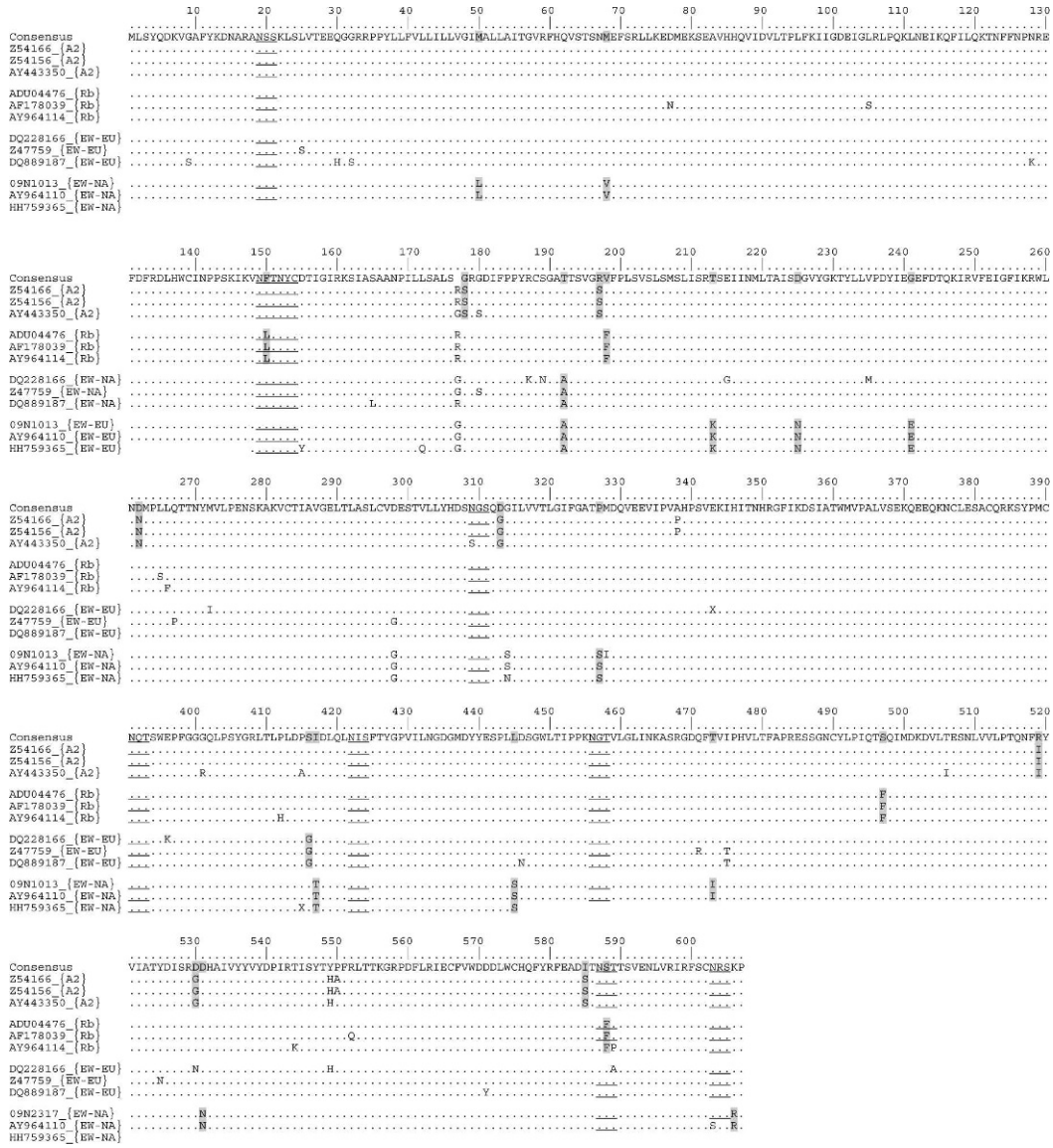

FIGURE 4. Alignment of H-gene amino acid sequences of representative strains of America-2 (A2), Rockborn-like (Rb), European wildlife – Europe (EW-EU) and European wildlife – North America (EW-NA) canine distemper virus isolates. Underlining indicates potential N-linked glycosylation sites (N-X-S/T or N-X-C). Grey shading marks amino acids that differentiate the respective lineage from other lineages. Z54166: Black panther/USA; Z54156: Chinese leopard/USA; AY443350: Raccoon/USA; ADU04476: Rockborn strain; AF178039: Lesser Panda/CHN; AY964114: Dog/USA; DQ228166: Red fox/ITA; Z47759: Mink/DNK; DQ889187: Dog/HUN; JN836734: Fisher/USA; AY964110: Dog/USA; HH759365: Dog/USA.

available from GenBank of six domestic dogs from North America (Fig. 3). These isolates (EW-NA) formed a distinct cluster within the same clade but were separate from classical European wildlife lineage strains (EW-EU). The nucleotide sequence differences translated into 12 distinct amino acid substitutions between EW-NA and EW-EU (Fig. 4). These data suggest that the European wildlife lineage might consist of two separate subgroups that are genetically distinct and endemic

in different geographic regions. However, the assessment of phylogenetic relationships was hampered by incomplete sequence data for several of the EW-NA isolates. Additional isolates and more comprehensive sequence data are needed to substantiate this notion.

Fishers no. 1–3 died in close temporal and spatial proximity and their virus isolates had identical H gene sequences. Fisher no. 4 died 4 mo later approximately 70 km from the first group and differed in 0.4% of the H gene nucleotide sequence. No other fisher mortalities from simultaneously monitored individuals in the project areas were noted in the intervening period. The temporal and spatial distribution of mortalities, as well as the similarity of the virus isolates, suggests two spillover events from one or multiple other sympatric species. The source of infection for the fishers in this study is unknown. Two isolates from the 1990s belonged to the America-2 lineage (Harder et al., 1995, 1996). A significant population decline in Santa Catalina island foxes in 1999–2000 was attributed to a CDV epizootic but no H gene sequence was reported (Timm et al., 2009). The only two recent CDV isolates were from domestic dogs and belonged to the EW-NA lineage (Kapil et al., 2008; Kapil, 2010). These isolates had about 99% nucleotide identity with the fisher isolates of this study. However, given the lack of recent Californian wildlife isolates, the high nucleotide similarity does not, per se, point to the domestic dog as the source for this outbreak. In North America, sylvatic species such as raccoons (*Procyon lotor*), black bears (*Ursus americanus*), and wild canids have been implicated as reservoir hosts in addition to the domestic dog. More comprehensive studies are needed to elucidate the epidemiology of CDV in pertinent habitats. These data will help establish protective measures for insular fisher populations in the American west.

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LITERATURE CITED

Callas, R. L., and P. Figura. 2008. Translocation plan for the reintroduction of fishers (*Martes pennanti*) to lands owned by Sierra Pacific Industries in the northern Sierra Nevada of California. California Department of Fish and Game, Sacramento, California, 80 pp.

Fox, J. G., R. C. Pearson, and J. R. Gorham. 1998. Viral diseases. *In* Biology and diseases of the ferret, J. G. Fox (ed.). Lippincott Williams and Wilkins, Baltimore, Maryland, pp. 355–374.

Gabriel, M. W., G. M. Wengert, and R. N. Brown. 2012. Biology and conservation of martens, sables, and fishers: A new synthesis. *In* Pathogens and parasites of *Martes* species: Management and conservation implications, K. B. Aubry (ed.). Cornell University Press, Ithaca, New York, pp. 138–185.

Gerhold, R. W., E. W. Howerth, and D. S. Lindsay. 2005. *Sarcocystis neurona*-associated meningoencephalitis and description of intramuscular sarcocysts in a fisher (*Martes pennanti*). Journal of Wildlife Diseases 41: 224–230.

Grigg, M. E., and J. C. Boothroyd. 2001. Rapid identification of virulent type I strains of the protozoan pathogen *Toxoplasma gondii* by PCR-restriction fragment length polymorphism analysis at the B1 gene. Journal of Clinical Microbiology 39: 398–400.

Harder, T. C., M. Kenter, M. J. Appel, M. E. Roelke-Parker, T. Barrett, and A. D. Osterhaus. 1995. Phylogenetic evidence of canine distemper virus in Serengeti's lions. Vaccine 13: 521–523.

———, ———, H. Vos, K. Siebelink, W. Huisman, G. Van Amerongen, C. Orvell, T. Barrett, M. J. Appell, and A. D. Osterhaus. 1996. Canine distemper virus from diseased large felids: Biological properties and phylogenetic relationships. Journal of General Virology 77 (Pt 3): 397–405.

Kapil, S 2010. Immunogenic compositions, vaccines and diagnostics based on canine distemper viruses circulating in North American dogs. WO/2010/088552.

———, R. W. Allison, L. Johnston III, B. L. Murray, S. Holland, J. Meinkoth, and B.

Johnson. 2008. Canine distemper virus strains circulating among North American dogs. Clinical and Vaccine Immunology 15: 707–712.

Knaus, B. J., R. Cronn, A. Liston, K. Pilgrim, and M. K. Schwartz. 2011. Mitochondrial genome sequences illuminate maternal lineages of conservation concern in a rare carnivore. BMC Ecology 11: 10.

Lan, N. T., R. Yamaguchi, A. Inomata, Y. Furuya, K. Uchida, S. Sugano, and S. Tateyama. 2006. Comparative analyses of canine distemper viral isolates from clinical cases of canine distemper in vaccinated dogs. Veterinary Microbiology 115: 32–42.

Larkin, J. L., M. Gabriel, R. W. Gerhold, M. J. Yabsley, J. C. Wester, J. G. Humphreys, R. Beckstead, and J. P. Dubey. 2011. Prevalence to *Toxoplasma gondii* and *Sarcocystis* spp. in a reintroduced fisher (*Martes pennanti*) population in Pennsylvania. Journal of Parasitology 97: 425–429.

Ledbetter, E. C., S. G. Kim, and E. J. Dubovi. 2009. Outbreak of ocular disease associated with naturally-acquired canine herpesvirus-1 infection in a closed domestic dog colony. Veterinary Ophthalmology 12: 242–247.

Powell, R. A. 1993. The fisher: Life history, ecology, and behavior, 2nd Edition. University of Minnesota Press, Minneapolis, Minnesota, 237 pp.

Rejmanek, D., E. VanWormer, M. A. Miller, J. A. Mazet, A. E. Nichelason, A. C. Melli, A. E. Packham, D. A. Jessup, and P. A. Conrad. 2009. Prevalence and risk factors associated with *Sarcocystis neurona* infections in opossums (*Didelphis virginiana*) from central California. Veterinary Parasitology 166: 8–14.

Riley, S. P. D., J. E. Foley, and B. B. Chomel. 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. Journal of Wildlife Diseases 40: 11–22.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Timm, S. F., L. Munson, B. A. Summers, K. A. Terio, E. J. Dubovi, C. E. Rupprecht, S. Kapil, and D. K. Garcelon. 2009. A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (*Urocyon littoralis catalinae*). Journal of Wildlife Diseases 45: 333–343.

United States Fish and Wildlife Service. 2004. 50 CFR, Part 17, Endangered and threatened wildlife and plants; 12 month findings for a petition to list the West Coast distinct population segment of the fisher (*Martes pennanti*); proposed rule. Federal Register 69: 18770–18792.

Williams, E., E. Thorne, M. Appel, and D. Belitsky. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases 24: 385.

Woodroffe, R. 1999. Managing disease threats to wild mammals. Animal Conservation 2: 185–193.