Short communication

Importance of canine distemper virus (CDV) infection in free-ranging Iberian lynxes (Lynx pardinus)

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A B S T R A C T

Canine distemper virus (CDV) is a morbillivirus that is the etiological agent of one of the most important viral diseases affecting canids and an expanding range of other carnivores. Using real-time RT-PCR, CDV RNA was detected in organs of an Iberian lynx (Lynx pardinus) found dead in the Doñana National Park, Southwestern Andalusia, Spain. This finding may be of great importance for the conservation of the species; at present the Iberian lynx is the most critically endangered wild felid. The aim of the present study was to elucidate the significance of CDV for the Iberian lynx population. High viral loads were evident in the dead lynx, suggesting an etiological involvement of CDV in its death. When carnivores from the same region were analyzed by CDV RT-PCR, a stone marten (Martes foina) was positive. Phylogenetic analyses demonstrated high identity of the two detected CDVs and a close relationship to the European dog lineage of CDV. Antibodies to CDV were detected in 14.8% of 88 tested free-ranging Iberian lynxes. The sample seroprevalence was significantly higher in lynxes from the Doñana Natural Space (22.9%) than Sierra Morena (5%). The stone marten and a red fox (Vulpes vulpes) also tested seropositive. In conclusion, CDV is present in the Iberian lynx population, especially in the Doñana region, with sporadic cases of disease. To reduce the infectious pressure of CDV on this endangered population, a mass dog vaccination should be considered.

1. Introduction

The Iberian lynx (Lynx pardinus) is considered the most endangered felid species in the world (Nowell, 2002; IUCN, 2007). Less than 200 individuals are estimated to remain, confined to two isolated subpopulations in the southwest of Spain, the Doñana Natural Space and the Andújar-Cardeña mountains in Sierra Morena (Simón et al., 2009). An important prerequisite for long-term protection of the Iberian lynx population is the prevention of the introduction of new infectious diseases in the population.

CDV is a single-stranded RNA virus belonging to the genus Morbillivirus (Barrett, 1999). It is the etiological agent of one of the most important viral diseases of wild and domestic carnivores (Pardo et al., 2005). Interspecies transmission frequently occurs, often leading to devastating epizootics in highly susceptible or immunologically naïve populations, such as in the Serengeti lions (Panthera leo) (Roelke-Parker et al., 1996). In Spain, CDV was frequently identified as a cause of death in dogs (Canis

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Table 1
Samples analyzed by real-time RT-PCR and serology.

	CDV RNA		CDV antibodies		
	positive/tested		positive/tested		Prevalence (95% CI)
	Blood	Feces	Serum	Plasma	
Iberian lynxes sampled between November 2003 and October 2007					
Iberian lynx (Lynx pardinus)	1/82	1/50	13/88		14.8% (8.1–23.9)
Carnivores sampled in 2002 and 2003					
Red fox (Vulpes vulpes)	0/23		1/6[a]		16.7%
Eurasian badger (Meles meles)	0/1				
Stone marten (Martes foina)	1/1		1/1		100%
Eurasian otter (Lutra lutra)	0/2		0/2		0%
Egyptian Mongoose (Herpestes ichneumon)	0/2				
Genet (Genetta genetta)	0/2		0/2	0/2	0%
Wildcat (Felis silvestris)	0/2				
Other animals sampled in 2002 and 2003					
Wildboar (Sus scrofa)	0/1				

[a] Serum was not available from all 23 animals.

lupus familiaris), ferrets (Mustela putorius furo) and minks (Mustela lutreola) (Nieto et al., 1992) and the presence of CDV antigen was shown in other species including red foxes (Vulpes vulpes) (Lopez-Pena et al., 1994) and in genets (Genetta genetta) (Lopez-Pena et al., 2001). Additionally, serological evidence of CDV infection was reported in Spanish wolves (Canis lupus) (Sobrino et al., 2008) and domestic cats (Felis catus) (Millan et al., 2009).

In 2005, an adult female Iberian lynx was found dead in Doñana National Park (DNP). Blood, lymph node and fecal samples were positive for CDV by real-time RT-PCR (Meli et al., 2009). The goal of this study was to assess the significance of CDV for the Iberian lynx population by determining the prevalence of CDV in lynxes and other carnivores of the same habitat and trace the source of the infection by molecularly characterizing the CDV strain of the Iberian lynx.

2. Materials and methods

2.1. Animals and materials collected

From late 2003 until October 2007, EDTA-anticoagulated blood, fecal and serum samples from 88 free-ranging Iberian lynxes were collected in both Doñana (n = 48) and Sierra Morena (n = 40) areas in the Southwest of Spain. Blood samples were collected from the Vena cephalica, the thoracic cavity or from the heart of animals that were found dead or caught and anesthetized during biological studies and management programs. In addition, blood samples were collected by cephalic or jugular venipuncture from 34 other wild animals (Table 1) that had been caught in box-traps and anesthetized with ketamine (Imalgène, Merial, Spain) and medetomidine (Domitor, Salud Animal-Pfizer, Spain).

2.2. CDV serology

Antibodies to CDV were detected by immunofluorescence assay (IFA) as described (Ramsauer et al., 2007). All sera were screened at a dilution of 1:40. Positive samples were titrated up to 1:320. IFA titers were shown to correlate well with virus neutralization results (Boller, 2006). The antigen slides were tested by real-time PCR and RT-PCR for absence of potentially contaminating viruses as described (Kummrow et al., 2005).

2.3. Total nucleic acids (TNA) isolation and cDNA synthesis

TNA were isolated from 200 ml of blood and feces samples using the MagNA Pure LC TNA Isolation Kit I (Roche Diagnostics, Switzerland). To monitor for cross-contamination, negative controls consisting of 200 ml phosphate-buffered saline were concomitantly prepared with each batch. As a positive control the tissue-culture-adapted CDV Onderstepoort vaccine strain (4th passage in Vero cells, gift of A. Zurbriggen, Department of Clinical Research and Veterinary Public Health, Switzerland, originally donated in 1975 to U. Kihm, Federal Institute of Virus Diseases and Immune Prophylaxis, Switzerland, by IFFA Mérieux, France) was used. For amplification and sequencing, the RNA, after enzymatic digestion of contaminating genomic DNA, was reverse-transcribed into cDNA using QuantiTect Reverse Transcriptase Kit (Qiagen, Switzerland).

2.4. Real-time assays

CDV was detected using a specific real-time RT-PCR assay based on the highly conserved phosphoprotein (P) gene as described (Meli et al., 2009). The assay detects CDV RNA in cell culture supernatant corresponding to 10^{-3} $TCID_{50}$. The TNA quality was assessed using a real-time PCR for the 18S rRNA gene as described (Boretti et al., 2009). Samples with a Ct Ç 30 were reextracted. With each PCR, an extraction- and a PCR-negative control were run to monitor for cross-contamination.

2.5. Amplification and sequencing of H and P genes

To characterize the CDV strains of the Iberian lynx and the stone marten, the complete hemagglutinin (H) gene (1824 bp), and the partial transcriptase-associated phosphoprotein (P) gene (633 bp) were sequenced as described

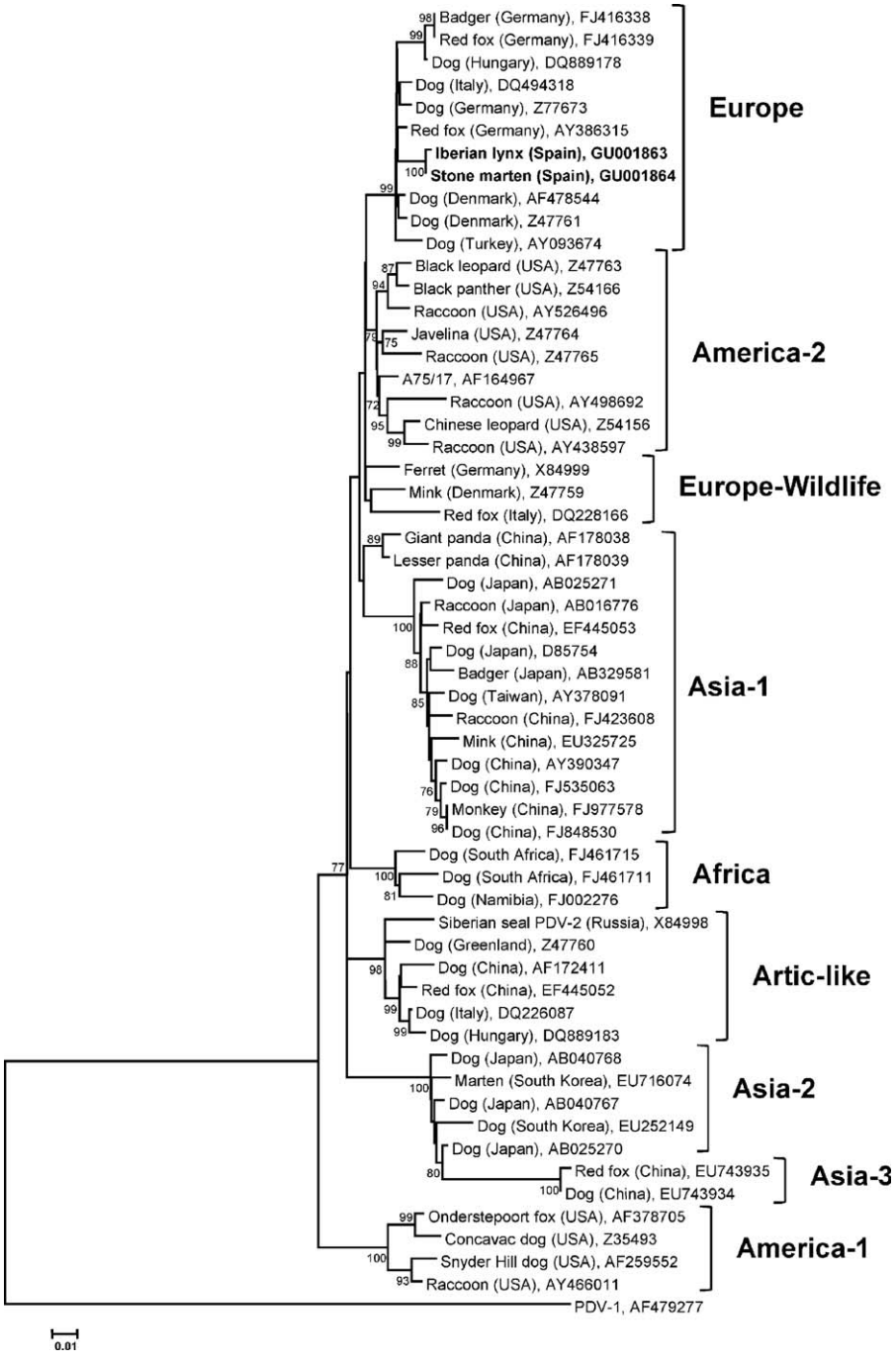

Fig. 1. Phylogenetic tree of CDV strains based on complete H gene sequences. Bootstrap consensus tree demonstrating the close evolutionary homology of the Iberian lynx and stone marten CDV isolates to the European lineage, forming a geographical clustering. Sequence alignments were performed using ClustalW. The tree was calculated using MEGA4 software (neighbor-joining algorithm, Kimura-2 parameter model). The numbers at the nodes were generated from 1000 bootstrap resamplings, values <70 are not shown. The bar represents the mean number of differences per 100 sites. Strain AF178038 (giant panda isolate) is the resultant of a genetic recombination between an ''Asia-1'' and a ''European wildlife'' strain (Han et al., 2008).

(Mochizuki et al., 1999; Pardo et al., 2005). PCR products were cloned into the pCRII vector using the TOPO TA cloning® Kit (Invitrogen). Sequencing was performed using the BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems, Switzerland). Products were analyzed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura et al., 2007). The sequences were deposited in the GenBank database (accession numbers: GU001863–GU001866).

2.6. Statistical analysis

Data were analyzed in Excel 2007 (Microsoft SP2, USA) and Analyse-it Clinical Laboratory Version 2.20 (Analyse-it Software, Ltd., UK). For the observed prevalence, 95% confidence intervals (CI) were calculated using R (Foundation for Statistical Computing, Austria). The frequency of infection between groups was compared using a two-tailed Fisher's exact test.

3. Results

3.1. CDV serology

Antibodies to CDV were found in 14.8% (95% CI: 8.1–23.9) of the 88 lynxes tested (Table 1). The sample prevalence was significantly higher ($p_F = 0.0318$) in animals from the Doñana (22.9%, 95% CI: 12–37.3) compared to the Sierra Morena area (5%, 95% CI: 0.6–16.9). Antibodies to CDV were detected in one of the six serum samples from red foxes and in a stone marten (Table 1).

3.2. Detection of CDV by real-time RT-PCR in lynxes and other wild carnivores

One out of 82 tested free-ranging Iberian lynxes was PCR-positive in blood and feces (Table 1). The positive animal was the Iberian lynx that was found dead in 2005; it had high viral loads both in blood and fecal samples (Meli et al., 2009). The CDV-positive lynx was negative for all other feline pathogens tested. The TNA samples from 23 red foxes and 11 other mammals (Table 1), that had been confirmed to contain sufficient amounts of amplifiable nucleic acids, were tested for CDV by real-time RT-PCR: 1 stone marten was positive (Table 1).

3.3. Characterization of CDV H and P gene sequences

The sequences of the H gene and P gene from the lynx and from the marten were 100% identical. Phylogenetic analysis of the H gene demonstrated ~98% identity of the retrieved isolates with a cluster of European CDV dog isolates (Fig. 1). They formed a cluster separate from the sequences identified as ''Europe-wildlife'' (Martella et al., 2006) and CDV isolates from Southern Africa, the United States, and Japan. The sequence identity to the Onderstepoort vaccine strain was 92%.

4. Discussion

In the current study, we molecularly characterized a CDV strain from an infected Iberian lynx found dead in Doñana National Park. Histological examination could not be performed and no postmortem evaluation of macroscopic lesions was possible due to advanced autolysis state of the carcass. However, based on the high viral loads in all materials, CDV infection was assumed to be etiologically involved in the animal's death. Although the occurrence of CDV is well documented in many red fox populations of the Iberian peninsula (Lopez-Pena et al., 1994; Sobrino et al., 2008; Millan et al., 2009; Santos et al., 2009), no CDV RNA was detected in 23 red foxes sampled in our study, and only 1 individual was CDV seropositive. One of the four samples collected from mustelids was CDV-positive; suggesting that a sizable portion of the mustelid population could be CDV-infected. This assumption is in agreement with the results of many studies on CDV in mustelids (Ulbrich, 1972; Palmer et al., 1983; Steinhagel and Nebel, 1985; Woolf et al., 1986; Hewicker et al., 1990; van Moll et al., 1995; Sekulin et al., 2010). To obtain information about the origin and the relationship to other strains the entire H gene and a part of the P gene from the CDV of the lynx and the stone marten were sequenced. For phylogenetic analysis the H gene was used as it shows the highest antigenic variation in morbilliviruses (Harder et al., 1996; Mochizuki et al., 1999; Pardo et al., 2005; Martella et al., 2006). The H genes of lynx and marten were identical and showed a very close relationship to the European dog lineage of CDV, however they formed a cluster separate from the sequences identified as ''Europe-wildlife'' (Martella et al., 2006) as well as from CDV isolates from other continents. This finding supports the hypothesis that both animals were exposed to the same European strain of CDV originating most probably from the local canine and/or carnivore population.

The occurrence of CDV antibodies in Iberian lynxes was not completely unexpected. Eurasian lynx (Lynx lynx) and Canada lynx (Lynx canadensis) had been reported to be seropositive (Biek et al., 2002; Schmidt-Posthaus et al., 2002), and recently a CDV-associated pathology was reported in free-living Canada lynxes and bobcats (Lynx rufus) (Daoust et al., 2009). On the other hand, a recent study on Iberian lynxes from the Doñana region and other carnivores sampled between 2004 and 2006 failed to detect CDV antibodies (Millan et al., 2009). Therefore, the discovery of a dead, highly CDV RNA-positive lynx is remarkable. The absence of CDV antibodies and antigen in the tested badger (Meles meles), otters (Lutra lutra), genets, wildcats (Felis silvestris) and Egyptian mongoose (Herpestes ichneumon) suggested that these carnivores were not important vectors for CDV to the Iberian lynxes. Nevertheless, the small number of samples analyzed did not allow a statistically relevant statement.

The transmission of morbilliviruses between different species is well known and includes the devastating CDV outbreak in Serengeti lions originating from dogs, the CDV infection of seals in the Baikal Lake and in the Caspian Sea, and CDV transmission to cats, raccoons and lesser pandas (Kotani et al., 1989; Roelke-Parker et al., 1996; Kennedy et al., 2000; Ikeda et al., 2001; Lednicky et al., 2004). In the Doñana Natural Space there are many species that can act as possible CDV reservoirs. In addition, there are villages in close proximity of the lynx areas. It is known from reports of researchers in Doñana that many domestic dogs and cats have access to areas that are inhabited by the lynxes. Domestic cats may also be infected by CDV although usually clinically unapparent (Boller, 2006). Local veterinarians have observed an increase in the frequency of respiratory and/or neurologic manifestations in dogs (Dr. A. Vargas, personal communication). A mass CDV vaccination program for the local dog population could diminish the infectious pressure of circulating CDV in the wildlife,

thus reducing the risk of an outbreak. Since vaccination of domestic dogs has been adopted in the Serengeti National Park, no new CDV outbreaks have been observed in lions or in hyenas (Cleaveland et al., 2000). Nevertheless, it also should be considered that a dog vaccination program may lead to a seronegative, naïve lynx population due to the low incidence of viral contact, thus resulting in a markedly increased susceptibility of the lynxes to CDV infection. Therefore, ideally, oral CDV vaccination of lynxes with a recombinant vaccine should be considered.

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References

Barrett, T., 1999. Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. Vet. Microbiol. 69, 3–13.

Biek, R., Zarnke, R.L., Gillin, C., Wild, M., Squires, J.R., Poss, M., 2002. Serologic survey for viral and bacterial infections in western populations of Canada lynx (Lynx canadensis). J. Wildl. Dis. 38, 840–845.

Boller, C., 2006. Canine Distemper Virus Seroprevalence in Domestic Cats and Identification of the Feline Signaling Lymphocyte Activation Molecule. University of Zürich, Zürich.

Boretti, F.S., Perreten, A., Meli, M.L., Cattori, V., Willi, B., Wengi, N., Hornok, S., Honegger, H., Hegglin, D., Woelfel, R., Reusch, C.E., Lutz, H., Hofmann-Lehmann, R., 2009. Molecular investigations of Rickettsia helvetica infection in dogs, foxes, humans, and Ixodes ticks. Appl. Environ. Microbiol. 75, 3230–3237.

Cleaveland, S., Appel, M.G., Chalmers, W.S., Chillingworth, C., Kaare, M., Dye, C., 2000. Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. Vet. Microbiol. 72, 217–227.

Daoust, P.Y., McBurney, S.R., Godson, D.L., van de Bildt, M.W., Osterhaus, A.D., 2009. Canine distemper virus-associated encephalitis in free-living lynx (Lynx canadensis) and bobcats (Lynx rufus) of eastern Canada. J. Wildl. Dis. 45, 611–624.

Han, G.Z., Liu, X.P., Li, S.S., 2008. Cross-species recombination in the haemagglutinin gene of canine distemper virus. Virus Res. 136, 198–201.

Harder, T.C., Kenter, M., Vos, H., Siebelink, K., Huisman, W., van Amerongen, G., Orvell, C., Barrett, T., Appel, M.J., Osterhaus, A.D., 1996. Canine distemper virus from diseased large felids: biological properties and phylogenetic relationships. J. Gen. Virol. 77 (Pt 3), 397–405.

Hewicker, M., Damsch, S., Trautwein, G., 1990. Detection of canine distemper viral antigen in formalin-fixed and paraffin-embedded tissue of a fitch (Mustela putorius), using an immunoperoxidase technique. Dtsch. Tierarztl. Wochenschr. 97, 85–88.

Ikeda, Y., Nakamura, K., Miyazawa, T., Chen, M.C., Kuo, T.F., Lin, J.A., Mikami, T., Kai, C., Takahashi, E., 2001. Seroprevalence of canine distemper virus in cats. Clin. Diagn. Lab. Immunol. 8, 641–644.

IUCN, 2007. IUCN Red List of Threatened Species. http://www.iucnredlist.org/search/details.php/12520/summ.

Kennedy, S., Kuiken, T., Jepson, P.D., Deaville, R., Forsyth, M., Barrett, T., van de Bildt, M.W., Osterhaus, A.D., Eybatov, T., Duck, C., Kydyrmanov, A., Mitrofanov, I., Wilson, S., 2000. Mass die-off of Caspian seals caused by canine distemper virus. Emerg. Infect. Dis. 6, 637–639.

Kotani, T., Jyo, M., Odagiri, Y., Sakakibara, Y., Horiuchi, T., 1989. Canine distemper virus infection in lesser pandas (Ailurus fulgens). Nippon Juigaku Zasshi 51, 1263–1266.

Kummrow, M., Meli, M.L., Haessig, M., Goenczi, E., Poland, A., Pedersen, N.C., Hofmann-Lehmann, R., Lutz, H., 2005. Feline coronavirus serotypes 1 and 2: seroprevalence and association with disease in Switzerland. Clin. Diagn. Lab. Immunol. 12, 1209–1215.

Lednicky, J.A., Dubach, J., Kinsel, M.J., Meehan, T.P., Bocchetta, M., Hungerford, L.L., Sarich, N.A., Witecki, K.E., Braid, M.D., Pedrak, C., Houde, C.M., 2004. Genetically distant American Canine distemper virus lineages have recently caused epizootics with somewhat different characteristics in raccoons living around a large suburban zoo in the USA. Virol. J. 1, 2.

Lopez-Pena, M., Quiroga, M.I., Vazquez, S., Nieto, J.M., 1994. Detection of canine distemper viral antigen in foxes (Vulpes vulpes) in northwestern Spain. J. Wildl. Dis. 30, 95–98.

Lopez-Pena, M., Vazquez, S., Aleman, N., Lopez-Beceiro, A., Munoz, F., Pereira, J.L., Nieto, J.M., 2001. Canine distemper in a genet (Gennetta gennetta), associated with endogenous lipid pneumonia. J. Comp. Pathol. 124, 207–211.

Martella, V., Cirone, F., Elia, G., Lorusso, E., Decaro, N., Campolo, M., Desario, C., Lucente, M.S., Bellacicco, A.L., Blixenkrone-Moller, M., Carmichael, L.E., Buonavoglia, C., 2006. Heterogeneity within the hemagglutinin genes of canine distemper virus (CDV) strains detected in Italy. Vet. Microbiol. 116, 301–309.

Meli, M.L., Cattori, V., Martínez, F., López, G., Vargas, A., Simón, M.A., Zorrilla, I., Munoz, A., Palomares, F., López-Bao, J.V., Pastor, J., Tandon, R., Willi, B., Hofmann-Lehmann, R., Lutz, H., 2009. Feline leukemia virus and other pathogens as important threats to the survival of the critically endangered Iberian lynx (Lynx pardinus). PLoS One 4, e4744.

Millan, J., Candela, M.G., Palomares, F., Cubero, M.J., Rodriguez, A., Barral, M., de la Fuente, J., Almeria, S., Leó n-Vizcaino, L., 2009. Disease threats to the endangered Iberian lynx (Lynx pardinus). Vet. J. 182, 114–124.

Mochizuki, M., Hashimoto, M., Hagiwara, S., Yoshida, Y., Ishiguro, S., 1999. Genotypes of canine distemper virus determined by analysis of the hemagglutinin genes of recent isolates from dogs in Japan. J. Clin. Microbiol. 37, 2936–2942.

Nieto, J.M., Quiroga, M.I., López, M., Antonio, R.F., 1992. Distemper in mink in the NW of Spain. Norwegian J. Agric. Sci. Suppl. 9, 398–404. Nowell, K., 2002. Revision of the Felidae Red List of Threatened Species. In: IUCN/SSC Cat Specialist Group, Authority, I.S.R.L.F. (http://lynx.uio.no/lynx/catsgportal/red-list/03_cats-and-red-list/nowell-cat_news_no37_2002.pdf).

Palmer, D., Ossent, P., Waldvogel, A., Weilenmann, R., 1983. Staupe-Enzephalitis beim Steinmarder in der Schweiz. Schweizer Archiv für Tierheilkunde 125, 529–536.

Pardo, I.D., Johnson, G.C., Kleiboeker, S.B., 2005. Phylogenetic characterization of canine distemper viruses detected in naturally infected dogs in North America. J. Clin. Microbiol. 43, 5009–5017.

Ramsauer, S., Bay, G., Meli, M., Hofmann-Lehmann, R., Lutz, H., 2007. Seroprevalence of selected infectious agents in a free-ranging, low-density lion population in the Central Kalahari Game Reserves in Botswana. Clin. Vacc. Immunol. 14, 808–810.

Roelke-Parker, M.E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S.J., Pospischil, A., Hofmann-Lehmann, R., Lutz, H., et al., 1996. A canine distemper virus epidemic in Serengeti lions (Panthera leo). Nature 379, 441–445.

Santos, N., Almendra, C., Tavares, L., 2009. Serologic survey for canine distemper virus and canine parvovirus in free-ranging wild carnivores from Portugal. J. Wildl. Dis. 45, 221–226.

Schmidt-Posthaus, H., Breitenmoser-Wursten, C., Posthaus, H., Bacciarini, L., Breitenmoser, U., 2002. Causes of mortality in reintroduced Eurasian lynx in Switzerland. J. Wildl. Dis. 38, 84–92.

Sekulin, K., Hafner-Marx, A., Kolodziejek, J., Janik, D., Schmidt, P., Nowotny, N., 2010. Emergence of canine distemper in Bavarian wildlife associated with a specific amino acid exchange in the haemagglutinin protein. Vet. J. (Epub ahead of print), doi:10.1016/j.tvjl.2009.1012.1029.

Simón, M.A., Cadenas, R., Gil-Sánchez, J.M., López-Parra, M., García, J., Ruiz, G., López, G., 2009. Conservation of the free-ranging Iberian lynx (Lynx pardinus) populations in Andalusia. In: Vargas, A., Breitenmoser, C., Breitenmoser, U. (Eds.), Iberian Lynx Ex-situ Conservation: An Interdisciplinary Approach. Fundación Biodiversidad, Madrid, Spain, pp. 42–55.

Sobrino, R., Arnal, M.C., Luco, D.F., Gortazar, C., 2008. Prevalence of antibodies against canine distemper virus and canine parvovirus among foxes and wolves from Spain. Vet. Microbiol. 126, 251–256.

Steinhagel, Nebel, 1985. Staupe beim Steinmarder in Schleswig-Holstein, ein Beitrag zur Epidemiologie der Staupe. Deutsche Tierärztliche Wochenschrift 92, 178–181.

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

Ulbrich, F., 1972. Staupevirusinfektionen bei wildlebenden Steinmarder. In: Internationales Symposium über Erkrankungen der Zootiere, pp. 327–329.

van Moll, P., Alldinger, S., Baumgartner, W., Adami, M., 1995. Distemper in wild carnivores: an epidemiological, histological and immunocytochemical study. Vet. Microbiol. 44, 193–199.

Woolf, A., Gremillion-Smith, C., Evans, R.H., 1986. Evidence of canine distemper virus infection in skunks negative for antibody against rabies virus. J. Am. Vet. Med. Assoc. 189, 1086–1088.