Disease threats to the endangered Iberian lynx (*Lynx pardinus*)

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Abstract

The Iberian lynx, (*Lynx pardinus*), is the most endangered felid in the world. To determine whether sympatric carnivores are reservoirs of pathogens posing a disease risk for the lynx, evidence of exposure to 17 viral, bacterial and protozoan agents was investigated in 176 carnivores comprising 26 free-living lynx, 53 domestic cats, 28 dogs, 33 red foxes (*Vulpes vulpes*), 24 Egyptian mongooses (*Herpestes ichneumon*), 10 common genets (*Genetta genetta*) and 2 Eurasian badgers (*Meles meles*) in the areas inhabited by the last two populations of Iberian lynx, both in Andalusia (South-Western Spain).

The results indicated that the lynx had low rates of contact with viral pathogens, with one seropositive finding each for feline leukemia virus, parvovirus and canine adenovirus-1, whereas contact with bacteria and protozoa appeared more frequent. Active infections with parvovirus, *Ehrlichia* spp., *Mycobacterium bovis*, *Leptospira interrogans* and *Cytauxzoon* spp. were confirmed. In contrast, 53% of the domestic cats were exposed to some infectious agent (prevalence range 4.5–11.4%). Antibodies to canine distemper virus and parvovirus were frequently found in dogs (32% and 42%, respectively) and foxes (30% and 12%). Past or present infections with parvovirus, *Ehrlichia* spp., *Chlamydophila* spp., *M. bovis*, *Salmonella enterica*, *L. interrogans*, *Toxoplasma gondii*, and *Neospora caninum* were also detected in these and other species surveyed.

Questionnaires to owners revealed that 14% of the dogs but none of the cats had been vaccinated, and no cat had been neutered. Based on the apparent absence of acquired immunity of the lynx against infectious agents, the frequent detection of agents among sympatric carnivores, and the reported lack of immunocompetence of the Iberian lynx, a disease outbreak among the local abundant carnivores may pose a serious disease risk for lynx conservation.
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Introduction

Epidemics can be a serious conservation threat for free-living populations of endangered species (Thorne and Williams, 1988; Roelke-Parker et al., 1996) and can cause mortality, reduce host fitness and/or alter dispersal and movement patterns of infected animals (Scott, 1988). As any of these outcomes may have dire consequences for the persistence of small populations, monitoring the prevalence of disease should be a priority in conservation (Scott, 1988).

Menaced species are less likely to sustain infectious agents because intra-species interactions are infrequent,

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and the epidemiology of a disease is driven not by intra-species pathogen transmission but by transmission from reservoir species that are able to maintain a relatively high pathogen population (Power and Mitchell, 2004). For example, high-density domestic dog populations are a more likely source of canine distemper virus (CDV) for infected African carnivores (Cleaveland et al., 2000). More-over, because most individuals in an endangered popula-tion are seldom exposed to a pathogen, there is little acquired immunity, so when an epidemic occurs it tends to infect a large proportion of the population and mortality levels may be high (McCallum and Dobson, 1995).

The Iberian lynx (*Lynx pardinus*) is the most endangered felid in the world (Nowell and Jackson, 1996) with only about 160–200 individuals remaining (Palomares et al., 2003; Guzmán et al., 2004). In spite of the serious conse-quences that an epidemic might have on the two remaining lynx populations, very little is known about diseases affect-ing the species. There is little evidence of contact with microparasites other than *Toxoplasma gondii* (Peña et al., 2006; Roelke et al., 2008). However, the lynx is susceptible to bovine tuberculosis (TB) (Pérez et al., 2001; Aranaz et al., 2004), and is known to host the protozoal blood par-asite *Cytauxzoon* spp. (Luaces et al., 2005). Roelke et al. (2008) speculated that the lack of evidence of exposure of Iberian lynx to pathogens such as feline immunodeficiency virus (FIV), feline leukemia virus (FeLV) or CDV might make the lynx vulnerable to outbreaks of these diseases in the future. The lynx has reportedly experienced demo-graphic bottlenecks and associated losses of genetic diver-sity that may exacerbate such vulnerability (Johnson

et al., 2004). Inbreeding was recently proposed as a cause of the loss of effectiveness of the immune system in the lynx (Peña et al., 2006).

The Iberian lynx coexists with other abundant wild and domestic carnivore species that may potentially act as res-ervoirs of disease. Many urban areas are close to or within areas inhabited by the lynx, so favouring disease transmis-sion between domestic animals and wildlife (Bradley and Altizer, 2007; Whiteman et al., 2007). However, there is no information about infectious diseases affecting other carnivores in these areas other than TB (Martín-Atance et al., 2006). Sympatric carnivores act as reservoirs of hel-minth and arthropod species parasitizing the Iberian lynx (Millán and Casanova, 2007; Millán et al., 2007b).

Our aims in this study were to describe the prevalence of selected disease agents in the Iberian lynx and sympatric carnivore species, and to determine whether these carni-vores and/or other species might act as reservoir hosts.

Materials and methods

Study areas

The study was carried out in the areas inhabited by the last two populations of Iberian lynx, both in Andalusia (South-Western Spain, Fig. 1). Sierra Morena (38°13′ N, 4°10′W; 1125 km^2; 120–140 lynx) is a hilly area with private hunting estates within protected areas. There are many villages in and around the lynx areas. Ranch houses are frequent, and numerous chalets (Viñas), some permanently inhabited, are scattered in the South-Eastern part of the area. Doñana (37°0′N, 6°30′W; 2000 km^2; 30–50 lynx) includes the Doñana National Park (DNP) and Doñana Natural Park (Dnp). It borders along 4 km with the village of Mata-lascañas, which receives some 100,000 holidaymakers each summer. There
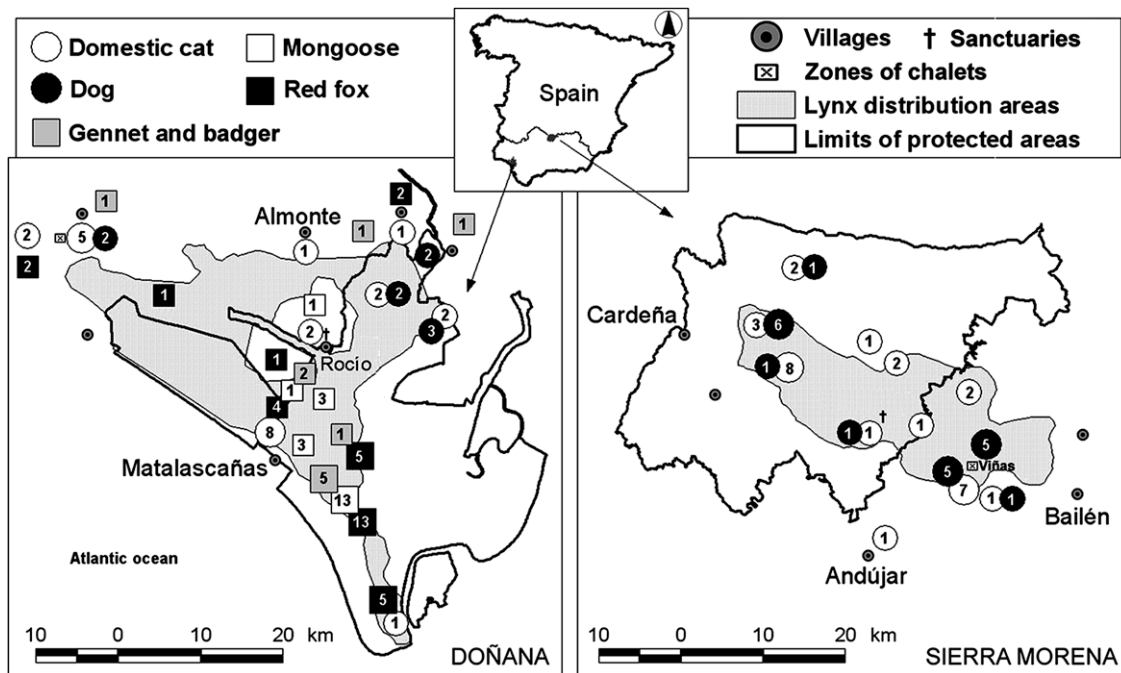

Fig. 1. Map of the study areas where Iberian lynx and other sympatric carnivores were studied for exposure against infectious and protozoan pathogens. Approximate lynx distributions areas are adapted from Guzmán et al. (2004). Locations of samples with exact known origin are shown with symbols representing the different species surveyed. Sample size is given into the symbols.

are other villages in and around lynx areas, and abundant chalets are also located close to the West side of DNP.

Dogs and cats are widespread in both lynx areas. Shepherding and guard dogs and free-roaming cats are found in private residences and ranch houses. Hunting dogs, including large dog packs used for big game ('rehalas'), often move across the Sierra Morena hunting estates and into Dnp during the hunting season. Feral dogs and cats can be frequently seen in both areas, including within DNP. Wild carnivores, especially the red fox (Vulpes vulpes) and the Egyptian mongoose (Herpestes ichneumon), but also the common genet (Genetta genetta), and the Eurasian badger (Meles meles) are common in both areas (see Palomo and Gisbert, 2002, and references therein for estimated densities, mainly for the Doñana area).

Sampling and laboratory analyses

From June 2004 to June 2006, we sampled 176 carnivores, including 26 free-living lynx, 53 cats, and 28 dogs (in both areas), and also (but only in Doñana) 33 foxes, 24 mongooses, 10 genets, and 2 badgers. We examined animals dead (mostly road-killed or hunted) or alive (Table 1).

The wild carnivores and feral cats were caught in box-traps, and anaesthetised with a combination of ketamine (Imalgène, Merial) with medetomidine (Domtor, Salud Animal-Pfizer) before sampling. We did not sedate dogs and only blood was taken. Blood was obtained by cephalic or jugular venepuncture, collected in serum separator tubes and allowed to clot, and then centrifuged at 50 g for 15 min. The serum was removed and frozen at −20 °C. One millilitre of whole blood was placed in lithium-heparin-coated tubes. Swab samples using specific media for viruses, Chlamydia and bacteria were taken from the oro-pharyngeal cavity, ocular conjunctiva and anus. Thin smears from tonsil scrapings were prepared on glass slides for immunofluorescence analysis, fixed by immersion in acetone for 10 min and frozen at −40 °C. Urine and faeces were obtained whenever possible, kept in sterile tubes, and frozen at −20 °C.

Dead animals were subjected to detailed post-mortem examination. Samples of the main tissues and blood from cardiac puncture were obtained and frozen at −80 °C. Some feral cats and foxes, captured during predator control programmes in DNP, were anaesthetised, routinely sampled, humanely euthanised with sodium thiopental (B. Braun Medical), and necropsied. Evidence of active or past contact with 17 agents was studied using a variety of methods (Table 2). Vaccinated dogs were not included with respect to the agent(s) against which they were vaccinated.

Questionnaires to domestic animal owners

We asked owners to complete a questionnaire asking for the total number of domestic animals on their property, any vaccination programmes carried out, and details of diseases suffered by their animals. The aim was to collect information about the health care that dogs and cats receive from their owners in the study areas. We also recorded the occurrence of sterilisation of cats in order to get an indication of the relative turnover of the population.

Statistics

For statistical analyses, lynx were separated into two age classes: (1) young (including juveniles living in the natal area, and subadults during the modal natal dispersal period), and (2) adults (>2 years old). The other species were divided in two age classes (juveniles and adults). Statistical analyses were performed with Statistica 6.0 (StatSoft, Inc.). Prevalence differences between areas and ages were tested using Fisher's exact test.

Results

With the exception of feline herpesvirus (FHV)-1 and Borrelia burgdorferi, evidence of current or past contact with all the studied agents were detected in at least one of the studied species (Fig. 2).

Contact with pathogens in the Iberian lynx

The Iberian lynx showed low rates of contact with viral agents (Fig. 2). Antibodies against parvovirus (PV), FeLV, and canine adenovirus-1 (CAV-1) were each detected in one lynx (4.5%, 95% confidence intervals = 0.2–20%) in Sierra Morena. Antigens from PV were detected in the faeces of the lynx with antibodies against the virus. Bacteria and protozoa were more frequently indicated than viruses. The highest seroprevalence was observed for T. gondii (80.7%, 61–92), followed by Leptospira interrogans (31.8%, 15–54; serovars Icterohemorragiae [N = 4]; Canicola [1], Ballum [1], Sejroë [1]), Neospora caninum (21%, 7.5–44), Ehrlichia spp. (13.6%, 3.8–34), and Chlamydophila spp. (4.5%, 0.2–20). Prevalence through direct detection was Mycobacterium bovis: 25%, 4.6–63; Cytauxzoon felis: 15%, 4.2–37; and Ehrlichia spp. and L. interrogans: in both cases 12.5%, 0.6–50. Infection by Mycobacterium bovis (two positives from three lynx sampled, one with a generalised infection) and Cytauxzoon spp. (3/9) were only detected in lynx from Sierra Morena, though differences between areas were not statistically significant. Excretion of T. gondii in lynx faeces was not confirmed.

Significant age-related differences were only found for T. gondii, with juvenile lynx having lower prevalence (33%) compared to older age groups (86%; Fisher's exact test, P = 0.024). One of the cases of TB was found in an individ-

Table 1
Sample size and origin of the animals sampled for diseases surveillance in the Iberian lynx distribution areas (Spain) during 2004–2006

	Sierra Morena			Doñana			Total
	In vivo	Post-mortem	Total	In vivo	Post-mortem	Total	
Iberian lynx Lynx pardinus	9	3	11	13	5	15	26
Domestic cat Felis catus	20	10	30	10	13	23	53
Dog Canis familiaris	19		19	9		9	28
Red fox Vulpes vulpes				17	21	33	33
Egyptian mongoose Herpestes ichneumon				21	9	24	24
Common genet Genetta genetta				7	3	10	10
Eurasian badger Meles meles					2	2	2

Some animals were surveyed alive and later subjected to post-mortem examination.

Table 2
Summary of methodologies used to detect evidence of contact with disease agents in carnivores in the Iberian lynx distribution areas during 2004–2006, showing samples analysed, threshold for serology (if quantitative), sequences used for polymerase chain reaction (PCR), conjugates used for immunofluorescence, and media used for culture

Agent[a]	Sample[b]	Assay[c]	Detects[d]	Name of commercial kits, positive cut-off (serology), sequences (PCR), conjugates (FA), and media (culture)	Manufacturers (in parenthesis) and/or references
FCV	Serum	cELISA	c	F1008-AB02 Feline Calicivirus ELISA	Eurovet Veterinaria
FHV-1	Serum	cELISA	c	F107-AB02 Feline Herpes ELISA	Eurovet Veterinaria
	Oro-pharyngeal swab	FA	ai	Feline serum anti-FHV-1 210-41-FHV/FVR	Eurovet Veterinaria VMRD
FCoV	Serum	cELISA	c	Ingezim Corona Felino	Ingenasa
	Enteric mucosa, mesenteric LN, faeces	FA	ai	Feline serum anti-FCoV Anti-felineIgG FITC conjugate	Ingenasa VMRD
		RIC	ai	FCoVMegacor (FB995)	VMRD
FeLV	Serum	cELISA	c	Ingezim FeLV	Ingenasa
	Serum	ELISA-d	ai	Ingezim FeLV-Das	Ingenasa
FIV	Serum	cELISA	c	Ingezim FIV	Ingenasa
PV	Serum	cELISA	c	Ingezim CPV	Ingenasa
	Intestine, intestinal content, faeces	PCR	ai	BAD 1017A VPF (5'-GAT GGC ACC TCC GGC AAA GA-3') VPR (5'-TTT CTA GGT GCT AGT TGA G-3')	Eurokit S.L. Reed et al. (1988)
		ELISA-d	ai	Ingezim CPV/FPV-Das	Ingenasa
		IPo	ai	Rabbit IgG anti-feline panleukopenia virus 29989 ImmunoPure biotinylated protein A. 21123 ImmunoPure Avidin, Horseradish peroxidase conjugate	Pierce Chemical Co., Palacín (1984), Rakich et al. (1986)
		FA	ai	Feline serum anti-FPV Anti-feline IgG FITC conjugate	Ingenasa VMRD
CDV	Serum	cELISA	c	Ingezim Moquillo	Ingenasa
	Tonsils, retro-pharyngeal and/or submandibular LN	FA	ai	Canine serum anti-CDV Anti-canine IgG FITC conjugate	Ingenasa VMRD
		PCR	ai	Ingene CDV	Ingenasa
CAV-1	Serum	cELISA	c	Canine hepatitis IgG ELISA	Eurovet Veterinaria
	Tonsils, retro-pharyngeal and/or submandibular LN	FA	ai	Canine serum anti-CAV-1 Anti-canine IgG FITC conjugate	Eurovet Veterinaria VMRD
EH	Serum	cELISA	c	Ingezim *Ehrlichia*	Ingenasa
	Spleen	FA	ai	Canine serum positive to *Ehrlichia canis*. Ref-211-P-EC Rabbit anti-dog IgG Fc fragment antibody, FITC conjugated	VMRD Rockland Immunochemicals
CH	Serum	cELISA	c	*Chlamydia* antibodies	Eurovet Veterinaria
	Conjunctival swab	RIC	ai	Clearview *Chlamydia* MF	Unipath
LI	Serum	MAL	c	1:100	Faine (1982)
	Kidney and/or urine	Staining	ai	Argentic stain	León-Vizcaíno et al. (1987)
		IPo + IFA	ai	Ig Rabbit anti *Leptospira interrogans* serovar Goat Ig anti-rabbit IgG: GAR/Ig/FITC or /IPo	Nordic Immunological Laboratories, León-Vizcaíno et al. (1987)
		Culture	ai	EMJH medium	Difco Laboratories Baranton and Postic (1989)
BB	Tonsil smears	FA	ai	Serum anti *Borrelia burgdorferi* Anti-canine IgG FITC	Institut Pasteur Production VMRD, Baranton and Postic (1989)
SE	Multiorgan	Culture	ai	Selenite-cystine and Rappaport-Vassiliadis + Salmonella-Shigella agar and brilliant green agar	Millán et al., 2004
MB	Serum	cELISA	c	Protein MBP70; ⩾30%	Martín-Atance et al., 2006
	Multiorgan	Culture	ai	Middlebrook's 7H11 and Lowenstein-Jensen media	Corner and Nicolacopoulos (1988), Gallager and Horwill (1977)
		PCR	ai	TB1-F (5'-GAA CAA TCC GCA GTT GAC AA-3') TB1-R (5'-AGC ACG CTG TCA ATC ATG TA-3')	Böddinghauss et al. (1990), Liébana et al. (1996)
TG	Serum	MAT	c	1:25	Dubey and Desmonts (1987)
	Faeces	FA	ai	Toxo-Troll 'F' 75 411	BioMérieux Industry

Table 2 (*continued*)

Agent[a]	Sample[b]	Assay[c]	Detects[d]	Name of commercial kits, positive cut-off (serology), sequences (PCR), conjugates (FA), and media (culture)	Manufacturers (in parenthesis) and/or references
NC	Serum	ELISA + IFA	c	>40%	VMRD
CF	Entire blood	PCR	ai	7549 (5́-GTCAGGATCCTGGGTTGATCCTGCCAG-3́) 7548 (5́-GACTGAATTCGACTTCTCCTTCCTTTAAG-3́)	Reichard et al. (2005), Millán et al. (2007a)

[a] FCV, feline calicivirus; FHV-1, feline herpesvirus-1; FCoV, feline coronavirus; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; PV, feline parvovirus subgroup virus; CDV, canine distemper virus; CAV-1, canine adenovirus-1; EH, *Ehrlichia* spp.; CH, *Chlamydophila* spp.; LI, *Leptospira interrogans* (serovars Australis, Autumnalis, Ballum, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterogaemorrhagiae, Pomona, Saxkoebing, Sejroë, Tarassovi); BB, *Borrelia burgdorferi*; SE, *Salmonella enterica*; MB, *Mycobacterium bovis*; TG, *Toxoplasma gondii*; NC, *Neospora caninum*; CF, *Cytauxzoon felis*.

[b] LN, lymph node.

[c] ELISA, enzyme-linked immunosorbent assay (c, competitive; d, direct); FA, direct immunofluorescence; IFA, indirect immunofluorescence assay; IPo, immunoperoxidase; LA, latex aglutination; MAL, microaglutination-lisis; MAT, modified agglutination test; RIC, rapid immunochromatography.

[d] ai, active infection; c, contact with the agent.

ual of about 4 months of age. *Cytauxzoon felis* was only detected in three young males.

Contact with pathogens in sympatric carnivores

We detected 14/17 agents in cats, dogs and foxes (Fig. 2). The only difference between study areas was observed for the seroprevalence of feline PV subgroup in dogs, with a higher prevalence in Doñana (78%) than in Sierra Morena (23%; Fisher's exact $P = 0.012$).

We found evidence of contact with at least one pathogen in 53% of the surveyed domestic cats (seroprevalence range 4.5–11.4%). We also found that 26% of necropsied cats had an active infection with PV, FeLV, CDV, feline coronavirus (FCoV), *Ehrlichia* spp. or *Chlamydophila* spp. Two cats belonging to the same ranch in Sierra Morena presented titres to CDV (the dog sampled in this ranch was seronegative). Antibodies to CDV and PV were frequent in dogs (32%, 17–52; and 42%, 22–60, respectively) and foxes (30%, 12–54; and 12%, 2.1–35). We detected CDV in one fox and one domestic cat culled in DNP.

Ehrlichia spp. was present in all the species studied except the badger. The seroprevalence of *L. interrogans* spp. was ⩾10% in these species, and was detected by culture in the domestic cat and mongoose (overall, we detected the serovars Icterohemorragiae [$n = 24$], Canicola [6], Ballum [4], Sejroë [3], Australis [1]). Positive cultures for *Salmonella enterica* were obtained from the fox, genet, and mongoose. Titres to *Mycobacterium* spp. were found in the two studied badgers, one mongoose and one fox. *Mycobacterium bovis* was isolated from organs samples in two foxes. *Cytauxzoon* spp. was not detected in any of the analysed blood samples from the domestic cat, mongoose or genet. The seroprevalence of *T. gondii* was >50% in all species, and *T. gondii* oocysts were only detected in the faeces of cats (17%). Antibodies to *N. caninum* were found in the dog, cat and mongoose.

The required sample size for age-related statistical analyses was only obtained for cats and foxes. Evidence of contact with FeLV and *L. interrogans* were more frequently found in juveniles (13% and 43%, respectively) than in adult cats (3% and 12%; Fisher's exact $P = 0.03$ in both cases). All cats shedding *T. gondii* oocysts were adults. In foxes, contact with CDV, CAV-1, PV, and *M. bovis* was only apparent in adults. However, significant differences were only found for CDV (adults: 43%; juveniles: 0%; Fisher's exact $P = 0.02$).

Questionnaires to owners

Twenty-five owners responded to the questionnaire, representing a total population of 29 dogs and 75 cats. Seven owners (16%) vaccinated their animals against rabies (13 dogs [45%] and one cat [1.3%]). Only three owners (12%) vaccinated their dogs against other agents. Three dogs (10%) were vaccinated against PV, and one (3.4%) had received a pentavalent vaccine but before the owner had moved to the study area. No cat had been given any vaccine. Only one case of disease in one domestic cat was mentioned by an owner (conjunctivitis). No cat had been neutered on any property.

Discussion

Observed prevalences

The survey showed that Iberian lynx do not have frequent contact with viral agents, which agrees with a previous serosurvey (Roelke et al., 2008), and the findings in other lynx species (see, for example, Ryser-Degiorgis et al., 2005). The solitary social system of the Iberian lynx (Ferreras et al., 1997) may decrease the risk of intra-species transmission of viral agents, and lynx probably come in contact with these agents mainly during inter-species contacts. An alternative explanation for the observed low seroprevalence may be the low rates of survival of lynx infected by viruses.

In contrast, the prevalence of viral agents in sympatric carnivores was higher. The domestic cat's social behaviour and high population size probably explain the detection of
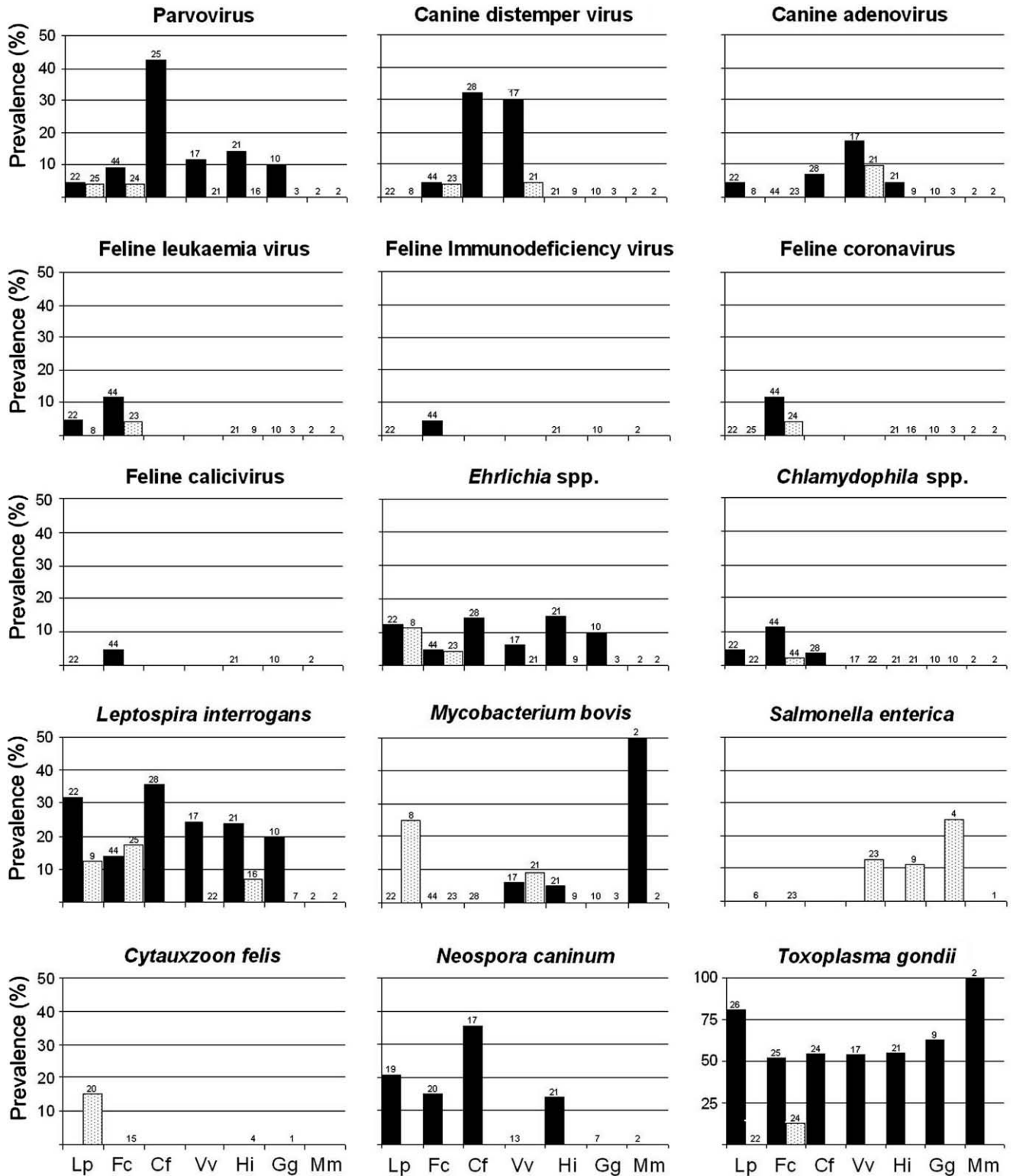
Fig. 2. Results of the survey for pathogens in Iberian lynx (Lp), domestic cat (Fc), dog (Cf), red fox (Vv), Egyptian mongoose (Hi), common genet (Gg) and Eurasian badger (Mm) in lynx areas. Black columns: evidences of contact with the agent; white columns: detection of active infections. Sample size is shown in the top of the column (absence of data means that no animal was analysed). See Table 2 and text for further details.

11 of the studied pathogens in this species. The seroprevalence of FeLV may be higher than indicated, since during the pathogenesis of feline leukemia, a proportion of infected cats remain latent, yielding ELISA negative results

(Cotter, 1998). Seroprevalence of FeLV was higher among young cats, indicating that cats may be infected by their mothers and/or that many cats may die at a young age and few infected animals reach an adult age. Either way,

the results showed that FeLV is actively circulating among the cat population within lynx areas.

In the case of FIV, due to the pathogenesis of the virus, the presence of antibodies implied that the seropositive individual was infected and thus a source of infection for other animals (Sellon, 1998). We also found that PV was broadly distributed in the carnivore population. The tests we ran did not allow us to determine the strain of PV, canine or feline. In fact, current taxonomy defines canine PV (CPV) and feline panleukopenia virus as a single taxonomic entity (Tattersall, 2006) and CPV was found to be responsible for about 5–10% of feline parvovirosis in cats in Germany (Truyen, 1999). Steinel et al. (2000) found CPV-2b sequences in five captive cheetahs (*Acinonyx jubatus*), and concluded that the observed distribution of feline PV antigenic types in captive large cats suggested a transmission from domestic dogs. The overall carnivore population studied here might well share this virus with the lynx. On the other hand, titres to CDV were found in a remarkable proportion of dogs and foxes. The higher seroprevalence we detected in adult foxes when compared to juveniles indicates that the agent is probably enzootic in the fox population in Doñana. Interestingly, we detected two seropositive cats, and the genome of CDV in a third one, which offers new evidence that interspecific transmission of CDV occurs (Harder and Osterhaus, 1997).

Bacteria and protozoa were frequent in lynx and the other species. The direct and indirect analyses conducted in the present study evidenced a wide distribution of *L. interrogans*. This spirochete is often found in carnivores, both wild and domestic (Richardson and Gauthier, 2003). The more frequent serovars were those that have rodents as a reservoir. The reservoir of serovar Canicola is the dog, and this serovar was already reported in wild canids (Khan et al., 1991) but, as far as we know, was not in any other wild carnivore. On the other hand, *S. enterica* presented prevalences in the range of these reported in wild carnivores in Northern Spain (Millán et al., 2004). The most probable source of *Salmonella* and *Leptospira* is infected water, scraps or rodents.

During the present survey, *M. bovis* was cultured from organ samples from two lynx. Bovine TB was already known to affect the Iberian lynx (see, for example, Pérez et al., 2001), which is probably an incidental host (Martín-Atance et al., 2006). In addition, evidence of exposure was found in three species of scavenger carnivores (fox, badger and mongoose) in Doñana. Wild ungulates display high prevalences of TB in both areas (Aranaz et al., 2004; Vicente et al., 2006), which makes them the most likely maintenance host. In fact, wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) are believed to be TB reservoirs in Mediterranean Spain (Gortazar et al., 2006). The route of infection for the lynx is unknown, although (at low proportions) ungulates are part of the lynx diet (Delibes, 1980; Gil-Sánchez et al., 2006). Other potential sources of infection that must be investigated are contaminated water or soil. Since felines are very susceptible to infection by *M.

bovis*, repeated contacts with the agent are probably not necessary for the lynx to develop fatal infections. The finding of a juvenile lynx with a generalised infection supports this hypothesis.

Evidence of contact with *Ehrlichia* spp. was often found, including in three lynx. There is scarce information about *Ehrlichia* spp. in wild felids although Filoni et al. (2006) found antibodies against the agent in 1/18 pumas (*Puma concolor*). *Ehrlichia canis* and *E. platys* have been described in dog and foxes in Europe (Neer, 1998; Fishman et al., 2004) but, in Spain, although reactivity against agents implicated in feline ehrlichiosis have been reported in cats (see, for example, Ortuño et al., 2005) the presence of *E. canis* has not so far been confirmed.

Antibodies to *Chlamydophila* spp. were also detected in one lynx. It is not known whether *C. felis* or other Chlamydiae (e.g. *C. psittaci* or *C. abortus*) were detected in our survey. Few attempts have been made to detect this agent in wild felids; Paul-Murphy et al. (1994), for example, failed to detect it in pumas.

The seroprevalence of *T. gondii* in lynx was higher than that reported by Roelke et al. (2008), probably because the technique we used was more sensitive. High seroprevalences of *T. gondii* are commonly observed in free-living lynx species (Ryser-Degiorgis et al., 2006). Older lynx presented higher seroprevalences than juveniles, which may indicate that adult animals have an increasing probability of being exposed to *T. gondii* during their life-time, as has been observed in other lynx species (e.g. Ryser-Degiorgis et al., 2006). We did not detect oocysts in lynx faecal samples. Although it is accepted that all species of felids can act as a definitive hosts for *T. gondii*, Iberian lynx have not been shown to excrete oocysts demonstrating their role as a definitive host. Infection of the Iberian lynx probably occurs after exposure to infected prey, most probably rabbits (Almería et al., 2004). Lynx may also become infected transplacentally, through milk or by ingestion of food or water contaminated by oocysts from cat faeces (see Roelke et al., 2008). In fact, 17% of cats sharing a habitat with lynx showed oocysts in their faeces, which could explain the high seroprevalence of *T. gondii* infection observed in all the analysed species.

Antibodies to *N. caninum* are reported here for the first time in both the Iberian lynx and the mongoose. Recently, Sedlak and Bartova (2006) reported antibodies in the captive Eurasian lynx (*Lynx lynx*). Canids are the only proven definitive hosts of *N. caninum* (Gondim, 2006) and the highest seroprevalence of *N. caninum* antibodies in the present study was observed in dogs, which may be of epidemiological significance as the seropositive dogs might well have shed oocysts. We did not observe antibodies in the foxes we examined, although the presence of parasite DNA of *N. caninum* in the fox has been reported in Spain (Almería et al., 2002).

Cytauxzoon spp. was recently found infecting the Iberian lynx (Luaces et al., 2005) and, in agreement with that work, we only found *Cytauxzoon* spp. in lynx from Sierra Morena.

Possible causes for the absence of the agent in Doñana included the absence of the intermediate host or the possibility of extinction of the protozoa in that area (see Millán et al., 2007a for further details). No domestic cat was found to be infected, but due to the acute course of cytauxzoonosis in cats (Kier and Greene, 1998), the probability of finding an infected individual is very low. The mongoose and genet were not found to be parasitised, probably due to the high specificity of this organism for felids.

Pathological significance of the detected agents

Very little is known about the pathological significance of the studied agents for the Iberian lynx. In most cases, only inferences from studies in other hosts can be made. For example, FeLV has been demonstrated to be fatal in the bobcat (*Lynx rufus*) (Sleeman et al., 2001), and it is considered a potential emerging disease in large cats (Kennedy-Stoskopf, 1999). Infection with FIV causes feline acquired immunodeficiency disease syndrome (AIDS), which is characterised by a loss of immunological capability, allowing subsequent opportunistic infections, and death from micro-organisms that would not normally harm the animal. However, so far there has been no verifiable disease association for any of the wild felid species for which FIV strains have been found (Troyer et al., 2005).

Mortality due to FPL has been observed in the bobcat (Wassmer et al., 1988) and cougar (Roelke et al., 1993). Some isolates of FCoV are known to induce feline infectious peritonitis (FIP), while others cause very mild to fatal enteritis (Addie and Jarrett, 1998). FIP is the single most important infectious cause of death in young cats (Fehr et al., 1996), and FCoV have been isolated from captive cheetahs that died during an epizootic (Pfeifer et al., 1983). In fact, only TB has been reported to be lethal for the Iberian lynx (Pérez et al., 2001); this chronic disease may not cause substantial mortality but may extract individuals from the population with the subsequent loss of population size and genetic variability. In contrast, whether or not *Cytauxzoon* spp. may cause disease in the Iberian lynx has still to be clarified (see Millán et al., 2007a). For other diseases (e.g. ehrlichiosis, chlamydiosis, neosporosis), little information is available regarding their potential pathogenicity in wild felids, and the relevance of the infections for lynx conservation needs to be elucidated. Since *Ehrlichia* spp. was widely distributed among the studied carnivores and produces a severe syndrome, it might well affect the lynx population dynamics.

It is generally believed that wild carnivores act as asymptomatic carriers of *Salmonella* (Millán et al., 2004) and *Leptospira* (Greene et al., 1998), but where there is immunosuppression by a concomitant agent (such as FIV), clinical cases may occur. *Toxoplasma gondii*, according to the high seroprevalence in adult lynx, may be enzootic in the lynx population, and is probably not a relevant cause of morbidity or mortality although fatal toxoplasmosis has been reported in bobcats (Dubey et al., 1987).

Are sympatric carnivores acting as reservoir of diseases for the Iberian lynx?

Often, the presence of infectious agents in other lynx species has been associated with the presence of the disease in domestic carnivores (Sleeman et al., 2001; Ryser-Degiorgis et al., 2006), and indeed dogs were considered to be the most likely source of infection of CDV for carnivores in Africa. Iberian lynx kill other carnivores (Palomares et al., 1996), which probably provides opportunities for direct transmission of pathogens such as FIV, FeLV or CDV. In addition, other agents can survive for a time in the environment (e.g. PV, FCoV, *Toxoplasma*, or *Neospora*), increasing the probability of reaching a new susceptible host.

Evidence of contact with canine agents such as CAV-1, *Leptospira* Canicola or *N. caninum*, the detection of PV or *L. interrogans* in most of the studied species, or the cases of CD in cats, indicates that inter-specific transmission is likely in lynx areas. However, to confirm this hypothesis, it will be necessary to fully identify and compare the strains of PV or FeLV, or the species of *Chlamydophila* spp. or *Ehrlichia* spp. present among carnivores in these areas. For example, if lynx are susceptible to CPV, the high prevalence of PV detected in the dog and fox may pose a risk for lynx conservation. It would also be necessary to clarify whether the positive result for CAV-1 is due to a cross-reaction or whether this canine virus is actually able to infect the lynx. In the case of *Cytauxzoon* spp., it has not so far been determined whether one or more strains or species infect the Iberian lynx and, in addition, the intermediate host has not been still identified in Spain (Millán et al., 2007a). On the other hand, our results indicate that other sympatric animals (and not only carnivores) such as wild ungulates (TB), rodents (*L. interrogans*) or rabbits (*T. gondii*) are probably also acting as maintenance host for pathogens, and this must be taken into consideration.

Management implications

Although vaccines are available against many of the canine agents that displayed high prevalences among the studied dogs, and also against feline pathogens, they appear to be seldom used. According to the results from the questionnaires, there were on average three cats per owner, and neutering was never used to control cat populations. This implies that new potential hosts are frequently available for agent maintenance, as reflected by the high seroprevalence of FeLV in young cats. The lack of vaccination and neutering of domestic animals will assist in creating a rich environment for disease agents.

Conclusions

Our results indicate that the Iberian lynx is far from safe from disease risks. With a high diversity of disease agents in the populations of sympatric carnivores, and the

apparent lack of acquired immunity and immunocompetence, we believe that a disease outbreak among the local abundant carnivores may pose a serious disease risk for lynx conservation. Management actions are necessary to avoid a potential disease outbreak among sympatric carnivores that might affect the two remaining Iberian lynx populations. A campaign to control domestic carnivore populations (by vaccination, euthanasia, and/or neutering) would reduce the incidence of circulating pathogens. In addition, we believe that diseases have to be taken into account when planning lynx translocations or reintroductions (Wild et al., 2006). Disease-related issues should be carefully considered in endeavouring to optimise the conservation efforts targeted at endangered species such as the Iberian lynx.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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