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Short communication

Canine distemper virus may affect European wild cat populations in Central Spain

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ABSTRACT

The main objective of this brief communication is to inform about the exposure to certain pathogens of interest for mesocarnivores in wildcats (*Felis silvestris silvestris*) that inhabit a human-domestic-wild ecotone located in a Natural Park (Serranía de Cuenca, Central Spain). Blood and mucosal swabs (nasal, conjunctival and rectal) samples were collected from nine alive animals to detect canine distemper virus (CDV), parvovirus (CPV/FPV), feline leukaemia virus (FeLV), feline coronavirus (FCoV), feline immunodeficiency virus (FIV), *Leptospira interrogans, Chlamydia felis, Ehrlichia canis, Toxoplasma gondii,* and *Neospora caninum.* ELISA, immunochromatograpy, microscopy agglutination test and PCR assays were used. The results show the first worldwide detection of exposure of wildcats to L. *interrogans* (3 positive/9 analysed) and the first detection of exposure to CVD (7/9), of carriers of *C. felis* (2/9) and of fecal spreading of CPV-FPV (2/9) in wildcats in Spain. Exposure to *T. gondii* and CPV-FPV was detected in 5 of the 9 wildcats analysed, and to FelV in 4 of 9. No FIV, FCoV, *Ehrlichia canis* and *Neospora caninum* were detected. The results reveal the circulation of pathogens among the wildcat population studied, but more vigilance is needed for an accurate assessment of the impact of these pathogens on the health status of this population.

From a health perspective, the existence of a human-domestic-wild interface is critical in the transmission of pathogens between species and the emergence of diseases (Hassell et al., 2017). These interfaces imply the appearance of ecotones, transition zones between two adjacent ecological systems, where processes are concentrated and intensified (Despommier et al., 2007; Bradley and Altizer 2007), as well as the appearance of new complex multiple host species (multi-host, hereafter) scenarios. The study area, the Parque Natural Serranía de Cuenca (SCNP), is a good multi-host example, with the coexistence of the three elements of the human-domestic-wildlife interface. Its bioclimatic conditions favor for one of the most effective and oldest systems of extensive breeding of ruminants in Spain (Merino sheep, goats and cattle, most of which are migratory). These foster the existence of pastures. The abundant presence of domestic ruminants and the related human activity means that animals that accompany humans (mainly cats and dogs) are present, and some of these become free-roaming. The domestic herds mentioned above share habitat with wild ruminants, Iberian ibex (*Capra pyrenaica*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), European mouflon (*Ovis aries musimon*), roe deer (*Capreolus capreolus*), and with well-established wild mesocarnivores populations, of which one of the most elusive and near threatened is the wildcat (*Felis silvestris*) (López-Martín et al. 2007). Wild cat populations are decreasing everywhere, so they are fully protected in many European range states and are listed in the EU Habitats and Species Directive (Annex IV), the Bern Convention (Appendix II) and CITES (Appendix II) (Nowell et al. 2010).

Feral cats, *Felis silvestris catus*, are currently one of the most common free-roaming domestic animals in Mediterranean Europe. The coexistence of wild cat and feral cat populations may affect the distribution of feline pathogens, due to the effective transmission routes of the main pathogens and the large home ranges of these domestic free-roaming feral carnivores. The presence of these amplifies the abundance of pathogens in the extra-host environment through direct contamination, as happens in the case of viruses with high environmental resistance (Steinel et al., 2001), feline coronavirus (FCoV) and parvovirus (CPV-FPV), or in the case of oocysts of the feline protozoan *Toxoplasma gondii*, responsible for

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Table 1
Frequency of pathogens in wild cats in the Serranía de Cuenca Natural Park (SCNP, Spain) found by detection of antibodies, antigens or DNA.

Age and sex of wildcats	No	CDV Ab	CDV Ag	CPV FPV Ab	CPV FPV Ag	*Lept Ab*	*ChlaAb*	*C. felis* DNA	FeLV Ab	FeLV Ag	*Tg*	FCoV Ab	FCoV Ag	FiV	*Ehrl*	*Nc*
Total Females	5	3	0	1	1	3	0	1	1	0	3	0	0	0	0	0
Young females	2	0	0	0	0	2[1]	0	1	0	0	1	0	0	0	0	0
Adult females	3	3	0	1	1	1[2]	0	0	1	0	2	0	0	0	0	0
Total Males	4	4	0	4	1	0	0	1	3	0	2	0	0	0	0	0
Total number of animals exposed	9	7	0	5	2	3	0	2	4	0	5	0	0	0	0	0
Frequency (%)		78	0	56	22	33	0	22	44	0	56	0	0	0	0	0

No: Number of animals sampled. Ab: Antibodies. Ag: Antigens. CDV: Canine distemper virus. CPV-FPV: Parvoviruses. *Lept*: *Leptospira interrogans. Chla: Chlamydia* spp. *C. felis: Chlamydia felis.* FeLV: Feline leukemia virus. *Tg: Toxoplasma gondii.* FCoV: Feline coronavirus. FIV: Feline immunodeficiency virus. *Ehrl: Ehrlichia canis. Nc Neospora caninum.*

one of the most frequent zoonoses in the world, whose existence in the border areas of ecotones has been associated with the presence of hybrids or wildcats in these areas (Gotteland et al., 2014).

The presence of feral cats favours the increase of interspecific contacts, which have a direct effect on exposure to pathogens (Hollings et al., 2013). Moreover, it also produces phenomena of genetic introgression when breeding with populations of wild cats (McFarlane et al., 2012). The agonistic contacts between males may increase the transmission of pathogens whose most important route of elimination is through saliva (Fromont et al., 2000); on the other hand, the feline leukaemia virus (FeLV), transmitted through coital transmission, can be maintained in a feline population by vertical epigenetic transmission, without the need for contact with carriers.

Finally, the predatory habits of felines increase the possibility of transmission of pathogens carried by their prey. *Leptospira interrogans*, which causes one of the most frequent zoonoses in the world and has rodents and other small mammals as its most important maintenance reservoir in outdoor farms (García et al., 2013); indirectly, human presence in the landscape increases the probability of infection by fostering rodent populations.

The main objective of this study is to ascertain the exposure of wildcats in the SCNP to pathogens. The study detected antibodies, antigens or DNA (where possible) of 10 pathogens in samples of feral cats, so establishing the presence of exposure and carrier animals. Bearing in mind that the study area is a human-domestic-wild ecotone, in which a large number of species share a habitat, we would expect a high rate of exposure to multi-host pathogens, cosmopolitans, or that are closely related to human-linked animals.

An opportunistic sample of 9 wildcats (5 females and 4 males; 7 adults-over one-year-old- and 2 juveniles) were trapped alive in the SCNP (1°51′2°03Ẃ, and 40°12′40°28Ń) between 2011 and 2013. The sample size used in this study was proportional to the population density theoretically estimated for the SNCP. Assuming that the European wildcat population has remained stable in recent years in Central Spain (Monterroso et al., 2009) and that the minimum home range is 1.95 km^2 per animal, we estimated a maximum theoretical population of 128 wildcats for the SCNP. Assuming an expected mean seroprevalence of 25% (±3%) and a 95% confidence level, the minimum sample size is between 9 and 11 wildcats. The wildcats were trapped with fixed traps, anaesthetized with a mixture of ketamine hydrochloride (Imalgene©; Merial, Lyon, France) and xylazine (Rompún©, Bayer, Spain), tagged for recapture and released after re-animation at the same place of capture. Blood samples taken from the radial vein, faeces and mucosal (conjunctival, nasal and rectal swabs, Amies W/O CH Copan Innovation®, Italy) were collected. A complete biometric and clinical examination was made of each animal to evaluate its physical and health status. The animals were identified as wild cats on the basis of their phenotype (coat colour and pattern of the stripes) (Daniels et al., 1999), and not on the basis of subsequent genetic analysis. They were classified as adults or juveniles on the basis of tooth wear. We established two non-exclusive expected variables: (i) the animal presents antibodies against any of the target pathogens, which means host exposure and (ii) the animal presents antigens or DNA from any of the target pathogens, which means host carrier status. Results regarding antibody detection are expressed as evidence of exposure rather than as present or past infection (Biek et al., 2006). Since the wildcat is an elusive species, we have a small sample size. For this reason, and applying the precautionary principle, the results have been expressed as detection frequencies, and not as prevalence. Non-parametric statistical tests (chi2 test) were used to measure the independence between the variables. Details of all pathogens tested are shown in the attached supplementary material file, together with the samples used for direct or indirect detection and the methodologies and procedures used, along with a brief description of these. Further information on the methodology is described in Candela et al (2017).

The results show an exposure to canine distemper virus (CVD) in 7 of the 9 wild cats. All animals exposed to CDV were adults, although none could be confirmed as carriers of this virus. 5 of the 9 cats showed exposure to parvovirus (CPV/FPV), and the presence of carriers was confirmed by the discovery of elimination of CPV/FPV by faeces in 2 seropositive animals. Exposure to FelV was detected in 4 of the 9 wildcats, but they were not carriers of this virus, nor was there any exposure or carrier of FIV.

Exposure to *L. interrogans* was detected in 3 of the 9 wildcats. Antibodies against Ballum serovar were detected in the serum of an adult female, and against Icterohaemorrhagiae serovar in the two young wildcats. *C. felis* DNA was detected from conjunctival, nasal and faecal swabs in 2 of the 9 wildcats, although we did not find animals with antibodies against *Chlamydia* LPS antigen. Exposure to *T. gondii* was established in 5 of the wild cats.

Although the captured wildcats were clinically asymptomatic, all but one young female showed evidence of contact with at least one of the pathogens studied. The maximum number of different pathogens detected in a single animal was 4, in an adult female carrying CPV/FPV, which also presented antibodies against CPV/FPV, *T. gondii*, FeLV and CDV. Finally, there was no evidence of exposure or animals carrying FCoV, *Erhlichia canis* or *Neospora caninum* in any of the wildcats analysed. All results are summarized in Table 1.

This study contributes to the knowledge of the infections circulating in wildcats, which has received little attention where free-living individuals are concerned. All but one of the wildcats showed evidence of contact with at least one of the pathogens studied, and the maximum number of pathogens detected in an animal was four. We detected cosmopolitan pathogens, such as CDV and CPV-FPV, whose spectrum of potential hosts is constantly growing; pathogens of sanitary importance for felines, such as FelV; and pathogens of public health interest, such as *Leptospira* and *T. gondii*. Even at the risk of being anecdotal, due to the small number of wildcats captured, the work carried out to collect these few samples was considerable, which increases the value of the data obtained, and we are convinced that any contribution to the knowledge of pathogens in wild feline populations can be a step forward in their conservation.

Parvoviruses are considered endemic in most domestic and wild carnivore populations, probably due to their effective indirect transmission routes through environmental contamination and their resistance for weeks, and even months, in the extra-animal environment (Steinel et al., 2001). Thanks to this study, the faecal spread of parvovirus (CPV/FPV) has been detected in wildcats, which together with the exposure found in our study (5 of 6 seropositive animals), indicates circulation of this virus in wildcats in SCNP. Present findings provide the first detection of wildcat as host of CPV/FPV in the Iberian Peninsula, although the presence of parvovirus carriers in other wild carnivore populations studied in Portugal is notable, reaching 79% of the red foxes (*Vulpes vulpes*) and 60% of the martens (*Martes foina*) (Duarte et al., 2013). There are studies in wildcats that do not detect parvovirus carriers (Duarte et al., 2012; León et al., 2017), but these do not analyse previous exposure to the pathogen by detecting antibodies either, so they may present an incomplete image of the distribution of this virus. In our sample we have found a significantly higher proportion of antibodies against parvovirus than that detected by Millán and Rodríguez (2009), which could indicate a greater distribution in the SCNP due to potential hosts, such as free-roaming domestic cats. This pathogen can have a substantial impact on population dynamics due to the high sensitivity shown by offspring during lactation, leading to an acute enteric disease crisis (Steinel et al., 2001).

Regarding the canine distemper virus (CDV), our results are the first to detect exposure to this virus in wildcats in Spain. The high rate of seropositive animals in the sample (78%), which is significantly higher than that found in the same species in Portugal (Duarte et al., 2012), and also higher than that found in lynx (*Linx pardinus*) in Spain (Meli et al., 2009), may indicate that the cosmopolitan virus circulates easily in the area studied. Our results support that indirect detection of antibodies is a valuable tool for detecting virus circulation in communities with multiple hosts, and that studies based exclusively on the detection of carriers may be underestimating their presence (León et al., 2017).

Both retroviruses, FeLV and FIV, are considered potentially dangerous for felines. This has been observed in captive wildcats (Hosie et al., 1989) and Iberian lynx (Meli et al., 2009), where FeLV is associated with high mortality outbreaks, while also significantly reducing the physical condition of wildcats (Fromont et al., 2000). In most studies conducted in Europe (Fromont et al., 2000; Duarte et al., 2012), it is suggested that FeLV is widely distributed. The epigenetic transmission of feline leukaemia virus increases its probability of permanence in low-density populations, by reducing the need for transmission by direct contact, probably influencing the frequency of distribution of feline retroviruses. This study shows a high exposure to FeLV, although lower than that found in other studies in France, Central Europe or Spain (Artois and Remond, 1994; Leutenegger et al., 1999; Millán and Rodríguez, 2009). Unlike the high proportion of carriers found by León et al. (2017), we were unable to detect wildcats as carriers of the FeLV gp-70 antigen. The pathogenicity of circulating strains and mortality events due to infection are currently unknown, making it difficult to estimate the pathological potential of FeLV in SCNP. Despite the high prevalence of FIV in free-ranging wildcats in Africa and North America, studies carried out in Europe do not detect seropositive wildcats (with the exception of the three detected by Fromont et al. (2000) in France), which coincides with our results.

L. interrogans infection has been detected in different natural areas of Spain in free-roaming cats (Millán et al., 2009a), and in large wild carnivores such as the wolf (Millán et al., 2014), but our findings show the first detection anywhere in the world of exposure of wildcats to different serovars of this spirochete. We detected an immune response to the Ballum serovars in an adult female and to Icterohaemorrhagiae in the two juveniles studied. The serovars detected in this study have rodents as their main reservoir (Andre-Fontaine 2006), suggesting that *L. interrogans* could be a food borne infection in wildcats. The limited number of samples prevents conclusive results; however, detection of infection in juvenile wildcats may indicate early contact with the pathogen, as was observed in dogs and cats (Millan et al., 2009a).

C. felis infection is widespread in cats, especially in free-roaming cats (Yan et al., 2000), forming part of the feline respiratory syndrome that causes acute and chronic conjunctivitis (Sykes, 2005). We detected *C. felis* DNA in two of the analysed wildcats, and as far as we know, it is the first time in the world that carriers of *C. felis* have been detected in this wild species. At the time of sampling, positive cats were excreting *C. felis* through their mucous membranes, since DNA of the pathogen was detected in the tested swabs; however, it was seronegative, i.e. the antibody response to chlamydia was not detected. The status of silent carrier, clinically inapparent, but persistent excretor of the pathogen, is frequently used by other Chlamydia species as a persistence mechanism in the host population (Nietfeld, 2001). This phenomenon could explain our results, since the state of latency may imply absence of immune response, i.e. seronegative animals.

T. gondii is a worldwide protozoan that infects humans and warm-blooded animals, making it very important for public and animal health, as it can cause abortions or congenital infections in its intermediate hosts (Tenter et al., 2000). Cats, specifically domestic cats, release millions of oocysts into the environment shortly after primary infection (Dubey and Beattie, 1988), and this is considered a risk factor for wildcats. In ecosystems with ecotone areas and the presence of free-roaming domestic cats, the prevalence of *T. gondii* is positively correlated with the density of domestic ruminant farms (Afonso et al., 2010), and may reach 100% (Millán et al., 2009b). The seroprevalence against *T. gondii* detected in this study is intermediate between that detected in wild cats (50%, Sobrino et al., 2007) and that detected in lynx (62.8%, Garcıa-Bocanegra et al., 2010). In any case, common detection of exposure or infection suggests circulation of this protozoan in wildcat populations.

Although tempting, it is inappropriate to frame our data in conclusions relating to broad concepts about the dynamics of the pathogens mentioned above. However, on the premise that ecotones contribute to the intensification and concentration of processes relating to the distribution and transmission of pathogens (Despommier et al., 2006), some interesting points to discuss and to examine more deeply in relation with mesocarnivores would be: i) the impact of these pathogens on the health status of this wildcat population, ii) to evaluate the presence of free domestic stray cats as a risk factor for the appearance of diseases in wild cats, iii) whether the detection in wild carnivores of pathogens with multiple potential hosts, including ruminants, should alert to spill-over or spill-back phenomena, and (iv) detection of zoonotic pathogens in wild carnivores as a complement to increasingly accurate epidemiological maps of under-diagnosed diseases.

We can conclude that wildcat population of the SCNP has contact with a varied group of pathogens, some of them typically feline (*T. gondii*, FelV, *C. felis*), and others with a marked cosmopolitan or multi-host features (CDV, CPV/FPV, *L. interrogans*). In our study area, wildlife coexists with mountain livestock and human-linked animals (cats, dogs and rodents), and the presence of pathogens such as *T. gondii, Coxiella burnetii* and *Chlamydia abortus* have been reported in both domestic and wild ruminants in this area (Martín-Atance, 2009). Elsewhere, the SCNP wildcat population has been identified as carrying *C. burnetii* (Candela et al., 2017), a pathogen whose main hosts are ruminants. For this reason, studies of the presence of pathogens are of greatest interest, not only in domestic animals or wild ruminants, whose samples are accessible, but also in wild populations that are difficult to access, such as wild mesocarnivores.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mambio.2019.04.006.

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