



Serological survey of feline viral pathogens in free-living European wildcats (*Felis s. silvestris*) from Luxembourg

Mike Heddergott¹ · Sandra Steeb² · Natalia Osten-Sacken^{1,3} · Peter Steinbach⁴ · Simone Schneider^{1,5} · Jacques P. Pir¹ · Franz Müller^{2,6} · Lise-Marie Pigneur⁷ · Alain C. Frantz¹

Received: 17 May 2018 / Accepted: 27 July 2018 / Published online: 30 July 2018
© Springer-Verlag GmbH Austria, part of Springer Nature 2018

Abstract

European populations of free-living wildcats have been shown to be exposed to cat viruses. Luxembourg has a high degree of habitat fragmentation, and hybridisation rates between domestic cats and wildcats are high. We therefore assessed the seroprevalence of six viruses in 34 serum samples collected between 2001 and 2016 from wildcats in Luxembourg. The values for feline leukemia virus (FeLV; 52.9%) and feline coronavirus (FCoV; 47.1%) were amongst the highest reported for wildcats. We found evidence for the cumulative likelihood of exposure to FCoV affecting its seroprevalence. Routine monitoring of viral agents in this strictly protected species should be considered.

While ranging from the Iberian Peninsula to Eastern Europe, the current distribution of the European wildcat (*Felis s. silvestris* Schreber, 1777) is strongly fragmented as a result of extermination by humans since the 17th century. Due to strict legal protection – the wildcat is listed in Annexe IV of the EU Habitats and Species Directive – the species is

slowly recovering in Western and Central Europe [11, 15], where hybridisation with domestic cats nevertheless remains a serious conservation threat [11]. To optimise conservation efforts, regional and national authorities have therefore developed specific conservation plans for the species [4, 25]. Understanding the prevalence of diseases in the wildcat population can be important in this context [25]. Viral infections in particular can have a severe negative effect on populations of threatened species [20, 22].

The most important viruses of domestic cats (*Felis s. catus* Linnaeus, 1758) are feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline coronavirus (FCoV), feline calicivirus (FCV), feline herpesvirus (FHV) and feline parvovirus (FPV). FeLV is one of the most common causes of death in cats worldwide, including the highly endangered Iberian lynx (*Lynx pardinus* Temminck, 1827) [20]. Transmission generally occurs through close contact between animals, and frequent interaction puts cats and kittens at particular risk of infection [9, 18]. The cosmopolitan FIV induces an immunodeficiency syndrome and is transmitted mainly by bites and sometimes during mating, but can also be transmitted transplacentally, perinatally or galactogenically [10]. The orally transmitted, cosmopolitan FCoV sometimes causes enteritis and can induce fatal peritonitis [2]. Cats of all ages can become infected, with kittens becoming susceptible when the protection by maternal antibodies decreases [9, 18]. FCV and FHV both cause upper respiratory tract diseases in cats. FCV is transmitted by contact with saliva and other respiratory tract secretions

Handling Editor: Tim Skern.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00705-018-3972-x>) contains supplementary material, which is available to authorized users.

✉ Mike Heddergott
mike-heddergott@web.de

¹ Musée National d'Histoire Naturelle, 25, rue Münster, 2160 Luxembourg, Luxembourg

² Arbeitskreis Wildbiologie an der Justus-Liebig-Universität Gießen e.V., Leihgesterner Weg 217, 35392 Giessen, Germany

³ Fondation Fauna-Flora, 25, rue Münster, 2160 Luxembourg, Luxembourg

⁴ Faculty of Chemistry, University of Göttingen, Tammannstraße 4, 37077 Göttingen, Germany

⁵ Biological Station SICONA, 12, rue de Capellen, 8393 Olm, Luxembourg

⁶ Hauptstraße 22, 36129 Gersfeld, Germany

⁷ Laboratoire de génétique de la conservation (GeCoLAB), Université de Liège, quartier vallée 1, chemin de la Vallée 4, 4000 Liège, Belgium

[18]. Infection with FHV mainly results from direct mucosal contact with latently infected animals [9], putting kittens at particular risk of infection [14]. Finally, FPV causes panleukopenia, a disease that causes high mortality worldwide, especially among kittens after the decrease of maternal antibodies [28]. All secretions and excrements are infectious, and the pathogen's high resistance allows indirect transmission [8, 9, 18].

European populations of free-ranging wildcats have been shown to be exposed to cat viruses [5, 7, 21]. Given the potential severity of the associated diseases, it is important to monitor their occurrence, especially in areas where frequent and close contact between free-ranging wildcats and domestic cats is likely. Luxembourg has a central geographic location in the largest continuous western European wildcat population, which extends from north-western France and southern Belgium to western and central Germany [16, 29]. The landscape of Luxembourg is highly fragmented, [13] and hybridisation rates between domestic cats and wildcats are relatively high compared to neighbouring regions in Germany [29, 30]. Given that higher contact rates are likely, a higher risk of viral transmission between the two subspecies in the country is also likely. We therefore aimed to assess the seroprevalence of the major cat viruses in the Luxembourg wildcat population and to identify factors that explain their prevalence.

Between 2001 and 2016, 34 road-killed wildcats were collected in Luxembourg and stored at -20°C . During dissection, we collected tissue samples for genetic analysis and 1–5 ml of blood from the heart or the chest cavity. Blood samples were centrifuged for 15 min at 1000g using an EBA 200 benchtop centrifuge (Hettich, Tuttlingen, Germany). The detection of FIV antibodies and FeLV antigens was done immediately, and the remaining sera were stored at -20°C until further analysis. The age of the wildcats was determined based on incremental growth lines in the enamel of a lower-jaw canine [1]. After demineralization with 5 % nitric acid (HNO_3), the teeth were cross-sectioned (width, 5 μm) with a rotary microtome (RM 2050, Leica Biosystems Nussloch GmbH, Germany) and stained with hematoxylin-eosin. The growth lines were counted under a B1-220A light microscope (Motic, Wetzlar, Germany) at $\times 40$ –100 magnification. Following Piechocki and Stiefel [24], animals were either classified as subadults (≤ 24 months; one growth line) or adults (≥ 25 months; two or more growth lines). The dataset consisted of 23 adults and 11 subadults (comprising 19 males and 15 females). All 34 cats were tested for hybridization with domestic cats and found to be genetically pure wildcats [29, 30].

FIV antibodies and FeLV antigens were identified using the SNAP[®] FIV/FeLV Combo Test (IDEXX Laboratories Inc., Westbrook, Maine, USA). Inactivated gag and env (gp40) antigens were used for the detection of FIV antibody.

Murine Anti-p27 antibodies were used to identify the FeLV p27 antigen. Antibodies against FCoV, FCV, FHV and FPV were identified by indirect immunofluorescence tests (IFAT) using various MegaFLUO[®] tests (company Megacor, Hörbranz, Austria): FCoV (MegaFLUO[®] FCoV), FCV (MegaFLUO[®] FCV), FHV (MegaFLUO[®] FHV) and FPV (MegaFLUO[®] PAN). Sera with a titer of 1:20 (FCV, FHV and FPV) and 1:25 (FCoV) or higher were considered positive, and those with doubtful results were re-tested. Each procedure incorporated positive and negative control sera according to the manufacturer's instructions.

We used the Wilson procedure with a correction for continuity to calculate the 95% confidence intervals of the proportion of seropositive animals [23]. For each virus, we performed a logistic regression in the program R v3.4.4 [12] to test for an effect of sex and age on the presence of antibodies.

Viral antibodies/antigens were detected in 28 wildcats (82.4%; Online Resource 1), with the six negative animals being female (five subadults, one adult). FeLV p antigens were detected in 18 wildcats (52.9%; 95% CI: 35.4–69.8). Antibodies against FPV were found in 10 (29.4%; 95% CI: 15.7–47.7), against FCoV in 16 (47.1%; 95% CI: 30.2–64.6), against FHV in 3 (8.8%; 95% CI: 2.3–24.8) and against FCV in 10 (29.4%; 95% CI: 15.7–47.7) of the 34 tested wildcats. Antibodies against FIV were not detected in any wildcat. Based on the results of the logistic regression, age had a significant effect on the presence of FCoV antibodies, with adults more likely to be seropositive (Table 1). Indeed, all but two of the 16 animals that were seropositive for this virus were adults. While males tended to be more likely to be seropositive for FeLV, we did not detect any other effect of sex or age on the seroprevalence of the other viruses (Table 1). While all serologically positive wildcats had low FPV, FCoV and FHV antibody titers, most animals that tested positive for FCV antibodies had high titers (1:160) (Table 2). While seven wildcats had contact with only one virus, more than one viral disease was detected in 21 animals (Table 3). Of these, 14 cats had contact with two agents (49.9 %), six with three (21.4 %) and one with four (3.7 %).

In the present study, we confirm the presence of several viral infectious diseases in Luxembourg wildcats. The seroprevalence for FCoV of 47.1% in the present study is, to the best of our knowledge, the highest value reported in wildcats thus far [5, 17, 27], and only one study with a comparable sample size reported higher prevalence values for FeLV (26 of 34 animals in a French population [76.5%]; [17]). The seroprevalence of FHV of 8.8% is, with one exception (3% in a French population; [17]), the lowest prevalence reported thus far [3, 5, 6, 17, 21, 27]. The prevalence values for FCV and FPV antibodies were in the range of results reported from other studies [3, 5, 6, 17, 21, 27]. In line with other studies, we did not detect any antibodies for FIV [3, 5, 17,

Table 1 Results of logistic regression identifying predictors for the presence of viruses in European wildcats (*Felis s. silvestris*)

Virus	Coefficients	Estimate	s.e.	z-value	p-value
FeLV	(Intercept)	-0.3704	0.6153	-0.602	0.5472
	Sex–males	1.4030	0.7515	1.867	0.0619
	Age–subadults	-0.8915	0.8026	-1.111	0.2667
FPV	(Intercept)	-0.9643	0.6652	-1.450	0.1470
	Sex–males	0.2222	0.7725	0.288	0.7740
	Age–subadults	-0.1203	0.8239	-0.146	0.8840
FCoV	(Intercept)	-0.0988	0.6223	-0.159	0.8738
	Sex–males	0.9198	0.7763	1.185	0.2361
	Age–subadults	-1.8901	0.9070	-2.084	0.0372
FHV	(Intercept)	-1.8301	0.9089	-2.014	0.0441
	Sex–males	-1.0331	1.2902	-0.801	0.4233
	Age–subadults	-0.1067	1.3085	-0.082	0.9350
FCV	(Intercept)	-1.6256	0.8072	-2.014	0.0440
	Sex–males	1.4883	0.8984	1.657	0.0976
	Age–subadults	-0.7292	0.9345	-0.780	0.4352

FeLV = feline leukemia virus; FCV = feline calicivirus; FHV = feline herpesvirus; FCoV = feline coronavirus; FPV = feline parvovirus. The logistic regression coefficient gives the change in the log odds of seroprevalence when considering males and subadults relative to females and adults, respectively

Table 2 Viruses identified in serologically positive European wildcats (*Felis s. silvestris*)

Virus	Titer	Number of wildcats
FPV	1:40	8
	1:160	2
FCoV	1:25	12
	1:100	4
FHV	1:20	2
	1:80	1
FCV	1:20	3
	1:80	2
	1:160	5

FCV = feline calicivirus; FHV = feline herpesvirus; FCoV = feline coronavirus; FPV = feline parvovirus

19, 21, 26, 27]. To date, antibodies against FIV in wildcats were only detected in three out of 38 (8%) individuals in a large population of stray domestic cats in central France [7]. The authors presume that the recolonization of the area by wildcats and the associated territorial conflicts with domestic cats were the cause of the FIV infection.

Despite FCoV being able to infect cats of all ages, our results suggest that adult animals are more likely to have come into contact with the virus. This implies that the cumulative likelihood of exposure plays a role in the

Table 3 Number of European wildcats (*Felis s. silvestris*) that tested seropositive for multiple viruses

Virus	Number of wildcats
Wildcats seropositive for two viral diseases	14
FeLV + FCoV	5
FeLV + FPV	4
FPV + FHV	1
FPV + FCoV	1
FCoV + FCV	3
Wildcats seropositive for three viral diseases	6
FeLV + FCoV + FCV	3
FeLV + FCoV + FHV	1
FPV + FCoV + FCV	2
Wildcats seropositive for four viral diseases	1
FeLV + FPV + FHV + FCV	1
Total	21

FeLV = feline leukemia virus; FCV = feline calicivirus; FHV = feline herpesvirus; FCoV = feline coronavirus; FPV = feline parvovirus

seroprevalence of this virus. However, Watt et al. [31] described fatalities in young captive wildcats infected with FCoV. We therefore cannot exclude the possibility that the relative absence of antibodies in subadults resulted from a high mortality rate in young animals. Larger sample sizes are needed to gain a better understanding of the factors that contribute the prevalence of the viral antibodies in wildcats.

In the majority of cases, cat viruses are transmitted by close contact between animals. The fact that the landscape of Luxembourg is highly fragmented and hybridisation rates between domestic cats and wildcats are relatively high did not translate into seroprevalence values that were generally higher than those reported in other studies. Indeed, the prevalence of feline viruses in wildcats in Scotland, where there is extensive hybridisation of animals, was lower than the values reported here. On the other hand, Račnik et al. [26] explained the absence of antibodies to FeLV and FIV in 15 Slovenian wildcats by the lack of contact with domestic cats. Generally, it is not clear (except in the case of FIV) whether contact with the viruses necessarily originates from contact with domestic cats, or whether infections are self-sustaining in the wildcat populations [7].

Wildcat populations are currently not being regularly screened for infectious viral diseases, despite their potential impact. However, the results presented here and in the literature indicate that wildcats are exposed to many relevant viral agents. In accordance with Annex IV of the EU Habitats and Species Directive, routine monitoring of the seroprevalence of viral agents in this strictly protected species should be considered.

Acknowledgements We would like to thank G. Biver, F. Daems, L. Dondelinger, C. Felten, M. Gaston, D. Grosbusch, J. M. Guinet, S. Hermes, F. Hoss, M. Jans, J. Junck, M. Kugener, X. Mestdagh, M. Moes, G. M. Ronkaz, R. Schauls, G. Schmidt, F. Schoos, M. Schulz, F. Sowa, R. Stranen and F. Wolff for providing us with wildcat carcasses.

Funding NOS was supported by a Grant from the Luxembourg National Research Fund (AFR 7484378).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All animals had been road-killed and were collected with the authorisation of the Luxembourg Ministry of Sustainable Development and Infrastructures (Ref.: 70646 GW/sc).

References

1. Ansorge H (1995) Notizen zur Altersbestimmung nach Wachstumslinien an Säugetierschädeln. In: Stubbe M, Stubbe A, Heidecke D (eds) Methoden feldökol. Säugetierforsch. Wiss Beitr Univ Halle, Halle, pp 95–102
2. Addie DD, Schaap IA, Nicolson L, Jarrett O (2003) Persistence and transmission of natural type I feline coronavirus infection. *J Gen Virol* 84:2735–2744
3. Artois M, Remond M (1994) Viral diseases as a threat to free-living wild cats (*Felis silvestris*) in continental Europe. *Vet Rec* 134:651–652
4. Birkenbach K, Klar N (2009) Aktionsplan zum Schutz der Europäischen Wildkatze in Deutschland—Schutzkonzept für eine Zielart des überregionalen Waldverbands. *Naturschutz und Landschaftsplanung* 41:325–332
5. Daniels MJ, Golder MC, Jarrett O, MacDonald DW (1999) Feline viruses in wildcats from Scotland. *J Wildl Dis* 35:121–124
6. Duarte A, Fernandes M, Santos N, Tavares L (2012) Virological survey in free-ranging wildcats (*Felis silvestris*) and feral domestic cats in Portugal. *Vet Microbiol* 158:400–404
7. Fromont E, Sager A, Leger F, Bourgemestre F, Jouquelet E, Stahl P, Pontier D, Artois M (2000) Prevalence and pathogenicity of retroviruses in wildcats in France. *Vet Rec* 146:317–319
8. Greene CE, Scott FW (1990) Feline Panleukopenia. In: Greene CE (ed) Infectious diseases of the dog and cat. W. B. Saunders Company, Philadelphia, pp 291–299
9. Hartmann K (2003) Virale Infektionskrankheiten. In: Kraft W, Dürr U (Hrsg) Katzenkrankheiten, 5 Auflage. Verlag M.&H. Schaper, Alfeld-Hannover, pp 157–252
10. Hartmann K (2011) Clinical aspects of feline immunodeficiency and feline leukemia virus infection. *Vet Immunol Immunopathol* 143:190–201
11. Herrmann M, Kitchener A, Meinig H, Stubbe M, Fernandes M, Conroy J, Giannatos G, Herrero J, Kranz A, Olszanska A (2007) *Felis silvestris*. The IUCN red list of threatened species 2007: e.T60354712A112955994. Downloaded on 11 April 2018
12. Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. *J Comput Graph Stat* 5:299–314
13. Jaeger JAG, Madrinan LF (2011) Landscape fragmentation in Europe. Joint EEA-FOEN report, European Environmental Agency, Publications Office of the European Union, Luxembourg (ISSN: 1725-9177)
14. Kolbl S (1998) Diagnostik der Hunde- und Katzenvirose. Enke Verlag, Stuttgart
15. Klar N, Fernández N, Kramer-Schadt S, Herrmann M, Trinzen M, Büttner I, Niemitz C (2008) Habitat selection models for European wildcat conservation. *Biol Conserv* 141:308–319
16. Léger F, Stahl P, Ruelle S, Wilhelm JL (2008) La répartition du chat forestier en France: évolutions récentes. *Faune Sauvage* 280:24–39
17. Leutenegger C, Hofmann-Lehmann R, Riols C, Liberek M, Worel G, Lups P, Fehr D, Hartmann M, Weilenmann P, Lutz H (1999) Viral infections in free-living populations of the European wildcat. *J Wildl Dis* 35:678–686
18. Lutz H (2005) Virusinfektionen. In: Horzinek M, Schmidt V, Lutz H (eds) Krankheiten der Katze. Enke Verlag, Stuttgart, pp 107–156
19. McOrist S, Boid R, Jones TW, Easterbee N, Hubbard AL, Jarrett O (1991) Some viral and protozoal diseases in the European wildcat (*Felis silvestris*). *J Wildl Dis* 27:693–696
20. Meli ML, Cattori V, Martínez F, López G, Vargas A, Simón MA, Zorrilla I, Muñoz A, Palomares F, López-Bao JV, 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
21. Millán J, Rodríguez A (2009) A serological survey of common feline pathogens in free-living European wildcats (*Felis silvestris*) in Central Spain. *Eur J Wildl Res* 55:285–291
22. Murray DL, Kapke CA, Evermann JF, Fuller TK (1999) Infectious disease and the conservation of free-ranging carnivores. *Anim Conserv* 2:241–254
23. Newcombe RG (1998) Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 17:857–872
24. Piechocki R, Stiefel A (1988) Über die Altersstruktur der Verluste der Wildkatze (*Felis s. silvestris* Schreber 1777). *Hercynia N F* 25:235–258
25. Poirson C, Dutilleul S (2014) Plan régional de restauration du chat forestier (*Felis silvestris*) et de la martre des pins (*Martes martes*) en Nord—Pas de Calais. Coordination Mammalogie du Nord de la France, pour le Conseil Régional Nord-Pas de Calais, p 161
26. Račnik J, Skribinšek T, Potočnik H, Kljun F, Kos I, Tozon N (2008) Viral infections in wild-living European wildcats in Slovenia. *Eur J Wildl Res* 54:767–770
27. Steeb S (2015) Postmortale Untersuchungen an der Europäischen Wildkatze (*Felis silvestris silvestris*) (Schreber, 1777). PhD thesis, Justus Liebig Universität, Fachbereich Veterinärmedizin, Gießen, VVB Lauferweiler Verlag Gießen, p 230
28. Steinel A, Parrish CR, Bloom ME, Truyen U (2001) Parvovirus infections in wild carnivores. *J Wildl Dis* 37:594–607
29. Steyer K, Kraus RHS, Mölich T, Anders O, Cocchiaro B, Frosch C, Geib A, Götz M, Herrmann M, Hupe K, Kohnen A, Krüger M, Müller F, Pir JP, Reiners TE, Roch S, Schade U, Schiefenhövel P, Siemund M, Simon O, Steeb S, Streif S, Streit B, Thein J, Tiesmeyer A, Trinzen M, Vogel B, Nowak C (2016) Large-scale genetic census of an elusive carnivore, the European wildcat (*Felis s. silvestris*). *Conserv Genet* 17:1183–1199
30. Steyer K, Tiesmeyer A, Muñoz-Fuentes V, Nowak C (2018) Low rates of hybridization between European wildcats and domestic cats in a human-dominated landscape. *Ecol Evol* 8:2290–2304
31. Watt NJ, Mac Intyre NJ, McOrist S (1993) An extended outbreak of infections peritonitis in a closed colony of European wildcats (*Felis silvestris*). *J Comp Pathol* 108:73–79