Animal Conservation

ZSL LIVING CONSERVATION

Animal Conservation. Print ISSN 1367-9430

Management measures to control a feline leukemia virus outbreak in the endangered Iberian lynx

G. López[1], M. López-Parra[1], L. Fernández[1], C. Martínez-Granados[2], F. Martínez[3], M. L. Meli[4], J. M. Gil-Sánchez[1], N. Viqueira[1], M. A. Díaz-Portero[1], R. Cadenas[5], H. Lutz[4], A. Vargas[2] & M. A. Simón[5]

1 Empresa de Gestión Medioambiental de la Junta de Andalucía (EGMASA), Johan Gutenberg, Seville
2 Espacio Natural de Doñana, El Acebuche, Matalascañas
3 Programa de Conservación Ex-Situ del lince ibérico, El Acebuche, Matalascañas
4 Clinical Laboratory of the Vetsuisse, Faculty of the University of Zurich, Zurich, Switzerland
5 Consejería de Medio Ambiente de la Junta de Andalucía, Manuel Siurot, Seville

Keywords
Iberian lynx; feline leukemia virus; outbreak; infectious diseases; felids.

Correspondence
Guillermo López, Empresa de Gestión Medioambiental de la Junta de Andalucía (EGMASA), Johan Gutenberg, 1. 41002, Seville.
Email: guiloza@gmail.com

Received 11 August 2008; accepted 6 January 2009

doi:10.1111/j.1469-1795.2009.00241.x

Abstract

The feline leukemia virus (FeLV) is a retrovirus that affects domestic cats all over the world. Its pathogenic effects generally include anemia, immunosuppression or tumors. Dissemination over populations is linked to cat sociality, because the virus is transmitted by direct contact. Although the domestic cat is its common host, FeLV infection has also been described in some wild felids. In the Iberian lynx *Lynx pardinus*, some sporadic FeLV infection cases have been reported since 1994, but an outbreak with the involvement of several animals has never been described until now. During spring 2007, an FeLV outbreak hit the Doñana (SW Spain) population. The infection rapidly spread throughout the densest subpopulation throughout Doñana. Infected animals showed very acute anemic disease, most of them dying in <6 months. To avoid FeLV dissemination, a control program was carried out that included removal of viremic lynxes, vaccination of negative individuals and reduction of the feral cat population. The program was implemented both in Doñana and in Sierra Morena populations. In Doñana, around 80% of the total lynx population and 90% of the outbreak focus subpopulation were evaluated. Seven out of the 12 infected individuals found died and two reverted to latency; the remaining viremic animals have been kept in captivity. The outbreak appears to have been successfully confined to the subpopulation where the virus appeared and no more cases have been found since August 2007. In the larger Sierra Morena population, 8% of the lynx population was surveyed. Thirty-four uninfected Iberian lynxes were vaccinated at least once. The FeLV prevalence was found to be 27% in the Doñana population and 0% in the Sierra Morena population.

Introduction

The Iberian lynx *Lynx pardinus* is the most endangered wild felid in the world, as it has been cataloged as 'critically endangered' by IUCN (IUCN, 2003). Endemic to the Iberian Peninsula (Ferrer & Negro, 2004), the Iberian lynx population size dramatically decreased during the 20th century (Rodríguez & Delibes, 1992), and currently only two isolated breeding populations survive in southern Spain (Guzmán *et al*., 2004). The diet of the Iberian lynx is composed mainly of wild rabbits *Oryctolagus cuniculus* (Delibes, 1980; Gil-Sánchez, Ballesteros-Duperon & Bueno-Segura, 2006). The species is highly solitary (Ferreras *et al*., 1997), although family groups have been detected over the years (Aldama & Delibes, 1991). Contact between individuals takes place mainly during the mating season (Ferreras *et al*., 1997), when, besides male–female contact, male–male contact competing for females also occurs (see López-Bao, Rodríguez & Ales, 2007), sometimes resulting in intraspecific fights. In addition, like most solitary carnivores, Iberian lynxes are commonly involved in interspecific fights. Contact with and killing of other carnivore species, such as domestic cats *Felis catus*, has been widely documented for the Iberian lynx (see Palomares & Caro, 1999).

The feline leukemia virus (FeLV) is a feline gammaretrovirus that infects cats worldwide under natural conditions (see Weijer, UytdeHaag & Osterhaus, 1987; Burmeister, 2001). It occurs in nature as a family of closely related viruses, every form of the virus causing different lesions in the host (Levy, 2008). FeLVs are categorized into three subtypes, FeLV-A, -B and -C, according to their superinfection interference properties (Sarma & Log, 1973; Jarrett & Russell, 1978). While FeLV-A is present in all FeLV-infected cats, only some of them are infected by

FeLV-B or -C. After infection, individuals can suffer persistent viremia, or develop an effective immune response that drives the virus into latency in the bone marrow (Pacitti, 1987; Hofmann-Lehmann *et al*., 1997). Reactivation of viremia can occur due to immunosuppression (Hofmann-Lehmann *et al*., 2008). Persistent viremic animals suffer from malignant and proliferative diseases including lymphomas and leukemia, as well as degenerative diseases including anemia, leading to death within months or years (Rezanka, Rojko & Neil, 1992). Dissemination of the virus takes place mainly through direct contact (Barr & Bowman, 2006).There is no curative treatment for FeLV-infected cats (Hartmann *et al*., 1999), although some drugs can improve quality of life and prolong life expectancy (Mari *et al*., 2004). Current molecular methods for detecting FeLV infection show high sensitivity and specificity, being capable of detecting one copy of provirus per reaction (Cattori *et al*., 2008; Tandon *et al*., 2008; Torres *et al*., 2008). Vaccination is the only available means to prevent the virus spread into a population, because it protects against persistent viremia (Hofmann-Lehmann *et al*., 2006, 2007).

Although the natural FeLV host is the domestic cat, the FeLV infection has also been reported in wild felids (Fromont *et al*., 2000; Sleeman *et al*., 2001; reviewed in Filoni & Catão-Dias, 2005), usually originating from domestic cats (Sleeman *et al*., 2001; Brown *et al*., 2006). Interestingly, FeLV does not seem to represent an important health risk for most wild felid populations (Fromont *et al*., 2000; Filoni *et al*., 2003, 2006). To our knowledge, only the Florida panther *Puma concolor coryi* has suffered an FeLV outbreak (Cunningham *et al*., 2006). In the Iberian lynx, after the evaluation of more than 150 individuals, only some sporadic FeLV-latent infected cases with no viremia had been found up to late 2006 (Luaces *et al*., 2008; Roelke *et al*., 2008). Hence, lynx exposure to FeLV is thought to have been constant during recent decades.

The Iberian lynx population in Doñana area functions with a metapopulation system as it is composed of several subpopulations in a source–sink system (Gaona, Ferreras & Delibes, 1998). The *Coto del Rey* subpopulation (CRS) is the densest of the entire Doñana Iberian lynx population (CRS mean value: 2.4 individuals/1000 ha; others: 0.4 individuals/1000 ha), and it has acted as the main source subpopulation during the last decade (Palomares *et al*., 1996; Ferreras *et al*., 1997). The others (Abalario-Vera and Aznalcázar) have been considered as sinks. In December 2006, the CRS was composed of 10 adult breeding individuals (five males:five females) and six yearlings (three males:three females). Because of the high prey abundance and the good quality of habitat structure (Palomares *et al*., 2000), the CRS presents the smallest lynx home ranges of the entire metapopulation (CRS: kernel 50% mean surface 267.67 ± 82.25 ha, $n = 5$; rest: 1126.56 ± 245.31 ha, $n = 7$). The mating system in the Iberian lynx can be monogamous or polygynous (Ferreras *et al*., 1997). The CRS high carrying capacity allowed a monogamous mating system at the beginning of the mating season 2006–2007. Such a high density is thought to increase intraspecific contact. Moreover, at the beginning of the mating season 2006–2007, seven supplementary feeding stations were operational in the CRS as part of a research project, and 12 drinking points were placed as part of a conservation management project. Feeding stations are square enclosures, with sides of 3 or 6 m and a 1.5-m-high surrounding fence, where domestic rabbits are deposited three times a week. Drinking points are 500 mL containers linked to a 20 L tank. These structures are believed to increase, with respect to natural conditions, the chances of contact between individuals.

During the mating and copulation season 2006–2007, an FeLV outbreak occurred in the CRS, in the Doñana area Iberian lynx population (M. Meli, unpubl. data). In December 2006, just before the mating season, an adult male from the CRS was found to be positive for FeLV viremia in a routine sanitary evaluation performed within the framework of a research project. The rest of the individuals evaluated in the CRS tested negative for the virus. All animals were released. Subsequent sequencing of the virus showed that the most probable source of infection to the lynx was a domestic cat (M. Meli, unpubl. data). A large feral cat population inhabits the urban outskirts of Doñana. Previous data about the FeLV in wild felids did not lead the managers to suspect that the presence of that positive animal in the population would represent a great risk. Trying not to disturb the mating process, a passive surveillance of the infected individual was chosen. On 13 March 2007, a 3-year-old male living in the southern CRS was found dead. The necropsy revealed FeLV viremia, although the final cause of death was a generalized infection by *Plesiomonas shigelloides*. On 17 March, an 11-year-old male inhabiting the north part of CRS was found dead because of a generalized infection by *Streptococcus canis*. FeLV viremia was also detected by molecular methods. Although the FeLV control program was started as soon as April, two more FeLV viremic adult males died before they could be trapped: the first one on April 24 (unidentified cause) and the second one on 8 May (generalized infection by *S. canis*).

An expert commission planned an FeLV Control Program for the Iberian Lynx (FCP). The commission comprised of individuals and representatives from each institution with competence in the conservation of the Iberian lynx and a group of advisory national and international experts in FeLV and wild felids, in the Iberian lynx and in wildlife diseases (Table 1). The goals of the FCP were: (1) to control the FeLV focus, in order to stop the spread of the virus over the whole Doñana Iberian lynx population; (2) to remove the virus from the population; (3) to minimize the possibility of occurrence of a new outbreak. The FCP implemented the following guidelines: (1) removal from the wild of FeLV viremic Iberian lynxes; (2) FeLV vaccination of all naïve lynxes; (3) modification of all management structures to minimize the contact between individuals; (4) reduction of the feral cat population. Because they may not be infective, and because of the scarcity of individuals, we decided that latently infected individuals should be allowed to remain in the field under an intense monitoring. Although the FCP was focused on the Doñana lynx population, it was

Table 1 Names and affiliations of the members of the expert commission that planned the FCP that allowed to control the FeLV outbreak in the Doñana Iberian lynx *Lynx pardinus* population

Name	Affiliation
Alvaro Muñoz	Andalusian Analysis Laboratory
Antonio Leiva	Iberian lynx conservation LIFE project
Antonio Sanz	Regional Environment Ministry
Arturo Menor	Regional Environment Ministry
Astrid Vargas	Iberian lynx *Ex-Situ* Conservation Program
Christian Gortázar	Cinegetic Resources Research Institute
Dolores Cobo	Doñana Natural Space
Eloy Revilla	Doñana Biological Station research institute
Eva Rojas	Iberian lynx conservation LIFE project
Fernando Martínez	Iberian lynx *Ex-Situ* Conservation Program
Francisco Palomares	Doñana Biological Station research institute
Francisco Quirós	Doñana Natural Space Direction
Gema Ruiz	Iberian lynx conservation LIFE project
Gerardo Valenzuela	Iberian lynx conservation LIFE project
Guillermo López	Iberian lynx conservation LIFE project
Hans Lutz	Veterynary Faculty, Zürich University
Irene Zorrilla	Andalusian Analysis Laboratory
Isabel Molina	Rescue centers Direction
Jorge Velarde	Los Villares Rescue Center
José Antonio Godoy	Doñana Biological Station research institute
José María Gil	Iberian lynx conservation LIFE project
José Vicente López	Doñana Biological Station research institute
Juan Bosco Neches	Iberian lynx conservation LIFE project
Juan Carlos Rubio	Doñana Natural Space Direction
Juan José Areces	National Environment Ministry
Magdalena Vara	Iberian lynx conservation LIFE project
Marcos López	Iberian lynx conservation LIFE project
María José Pérez	Iberian lynx *Ex-Situ* Conservation Program
Marina Meli	Veterynary Faculty, Zürich University
Mark Cunningham	Florida Fish and Wildlife Commission
Melody Roelke	National Cancer Institute
Miguel Díaz	Iberian lynx conservation LIFE project
Miguel Ángel Pineda	Regional Environment Ministry
Miguel Delibes	Doñana Biological Station research institute
Rafael Cadenas	Iberian lynx conservation LIFE project
Sandra Bañuls	Doñana Natural Space

FeLV, feline leukemia virus; FCP, FeLV Control Program for the Iberian Lynx.

also applied to a sample of the Sierra Morena lynx population (see Fig. 1).

The aim of this paper is to show the management measures implemented for control and to present the results obtained with this management. About 80% of the entire Doñana lynx population was checked in 8 months. The outbreak could be controlled before the subsequent breeding season, but the risk of occurrence of a new outbreak persists.

Methods

Fieldwork

In Doñana, the Iberian lynx captures began in the CRS and were then sequentially applied to the other subpopulations. Meanwhile, trapping was also carried out in Sierra Morena. All Iberian lynxes were captured using double-entrance, electro-welded-mesh box traps ($2 \times 0.5 \times 0.5$ m), with the exception of one animal, which was trapped by means of a remote-controlled teleinjection system (see Ryser *et al.*, 2005). Traps were baited with a live domestic rabbit fixed to the trap by a harness. The effort to capture lynxes was 2350 trap day^{-1} during the capture period of the FCP. A maximum of 20 traps in Doñana and six in Sierra Morena were used at the same time. Traps were checked three to five times a day, depending on the age of the cubs living in the area: when cubs were <5 months old, traps were reviewed five times a day (to avoid prolonged retention in the traps); after the fifth month, intervals between checks were gradually increased to a period of 8 h. Also, in the summertime, traps were closed for 12 h day^{-1} because of the hot weather. Trap review teams were always composed of at least two people trained in lynx handling. Whenever a lynx was captured, it was transferred to a stainless-steel compression cage ($1 \times 0.5 \times 0.25$ m), in which the lynx was transported to a clinic.

Domestic cats were trapped using both guillotine and American-system, single-entrance, electro-welded-mesh box traps. Capture efforts were mainly focused on lynx distribution areas close to human populations. Traps were baited with dead chickens and sardines. A total of 15 and 10 traps were used at the same time in Doñana and Sierra Morena, respectively, working 5 days a week. After a physical examination of the cats, we took 10 mL of blood from the jugular vein. Three drops of blood were used to run the fast FeLV antigen ELISA test (IDEXX® Snap test).

The risk of infection to lynxes posed by supplementary feeding stations and artificial drinking points, as main structures thought to increase the contact rate between individuals, was also evaluated. Regarding feeding stations, it was decided that only one rabbit should be released at a time to avoid different lynxes feeding on the same prey. In addition, weekly cleaning and disinfection of drinking points was implemented.

Laboratory methods

Once in the clinics, all lynxes were subject to a routine standardized handling and sanitary evaluation protocol. As the sanitary evaluation includes blood extraction by means of vein puncture, two drops of blood were used to run a fast antigen FeLV ELISA test (IDEXX® Snap test), which detects viremia (p27 antigen). Using this test, the results are produced in 10 min; thus, it was a useful tool to make quick decisions. The antigen ELISA test is a very high sensitivity test to detect viremia (Lutz, Pedersen & Theilen, 1983), although it cannot detect latent infections.

Subsequently, ELISA and real-time PCRs were performed in the clinical laboratory of the Vetsuisse faculty in Zürich. Real-time PCR is a very sensitive diagnostic method that detects both viremic and latently infected animals (Tandon *et al.*, 2005; M. Meli *et al.* unpubl. data). The

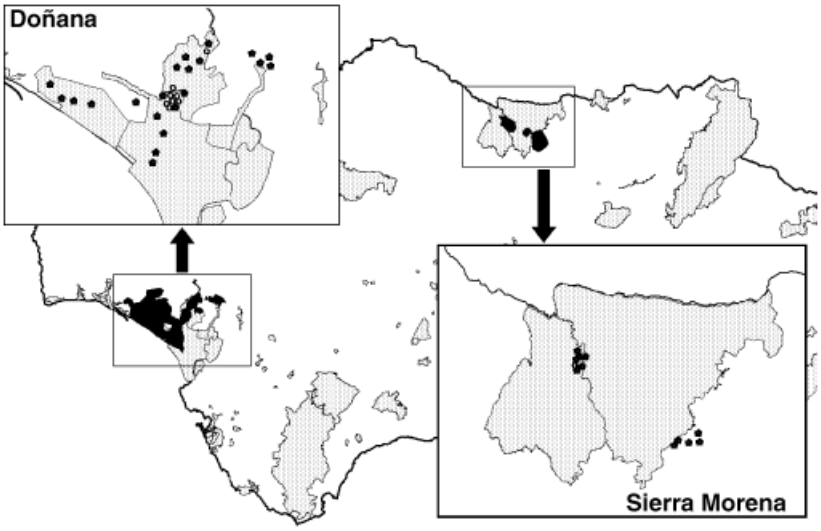

Figure 1 Map of the study site showing the protected areas in Andalusia (dotted areas), and the current Iberian lynx *Lynx pardinus* distribution (in black). More detailed maps of Doñana and Sierra Morena show all lynx captures within the FeLV Control Program for the Iberian Lynx (negative individuals: pentagons; positive individuals: open circles).

sandwich ELISA detects the p27 antigen in the serum (Lutz *et al.*, 1983; M. Meli *et al.* unpubl. data).

Destination of the animals

All lynxes found to be viremic in the antigen ELISA snap-test were moved to a rescue center, where a complete checking of all viremic animals was performed every 2 months. When a viremic individual became non-viremic (latent infection), it was returned to the wild.

All lynxes found to be non-viremic were vaccinated and released. A recombinant canarypox-vectored Merial® PureVax FeLV® vaccine (kindly donated by Dr J. C. Thibault, Merial, Lyon, France) was chosen because it was shown to be the most protective vaccine against FeLV in domestic cats (Hofmann-Lehmann *et al.*, 2006). As maximum immunization success is achieved through a two-dose protocol, a booster vaccination was given to as many animals as it was possible to re-trap.

FeLV viremic domestic cats were euthanized using sodium pentobarbital (Dolethal®). FeLV-negative feral cats were transported to an animal protection society, where they remained waiting for an owner. Cats that already had owners were vaccinated using PureVax RCPCH-FeLV® before they were returned to their owners.

Statistical analysis

A χ^2 analysis was performed to explore differences in FeLV incidence between sexes in the 13 lynxes inhabiting CRS when the disease was first detected. Those individuals were the only ones supposed to have contact with the virus.

Results

The FCP ran for more than 8 months. Given that most of the population had been evaluated, and that no positive lynxes had been found in 4 months, the lynx capture part of the program was stopped when the mating season began in order not to disturb breeding. In summary, 34 Iberian lynxes from the Doñana population (around 83% of the total) were handled and evaluated for absence of FeLV infection. In the CRS, 20 individuals (91% of the total subpopulation) were captured at least once. In Abalario-Vera and Aznalcázar subpopulations, 10 (55%) and four individuals (67%), respectively, were handled. A total of eight captured animals (44% of CRS) tested positive for FeLV (seven viremic and one latently infected), all in the CRS (Table 2). These latter animals, together with the four dead males, accounted for an FeLV prevalence value of 55% in the CRS and of 27% in the Doñana lynx population (60 and 35%, respectively, considering only the handled animals whose FeLV status was known). No positives were found outside the CRS. The last positive animal was found on 1 August 2007 (see Fig. 2). A sex bias towards males was found in the incidence of FeLV infection in the 13 lynxes inhabiting CRS when the first FeLV case was detected (χ^2 value = 6.24; d.f. = 1; $P = 0.01$). In the Sierra Morena lynx population, 12 lynxes (8% of the total Sierra Morena population) were captured and tested for FeLV. No viremic individuals were found in the sample of this population, although one individual showed FeLV latent infection.

A total of 22 lynxes belonging to the Doñana population were found negative and were vaccinated; 10 of them (45% of those vaccinated) were also given a booster vaccination (Table 2). In the Sierra Morena population, all 12 lynxes checked were vaccinated once, but it was possible to revaccinate only two of them (16%).

One adult female that was found to be viremic in early June was found to be latently infected, and non-viremic in early December 2007. She was released again in the center in her original home range, which had apparently not been occupied by any other individual. One month later she mated with a male and gave birth to a cub in the subsequent breeding season of 2008.

Agreement between the antigen ELISA and the real-time PCR results detecting viremia was 100%. Two lynxes were

Table 2 Lynx individuals captured during the FCP both in Doñana (in white) and in Sierra Morena (in gray)

Name	Capture	Population	Longitude	Latitude	Sex	Age	Ag ELISA	q-PCR	Vaccination	Booster
Viciosa	9 May 2007	DON	37°07′	06°26′	F	7	–	+	No	–
Inesperado	21 May 2007	DON	37°14′	06°19′	M	3	+	+	No	–
Teo	21 May 2007	DON	37°14′	06°19′	F	5	–	–	Yes	Yes
Rayuela	5 June 2007	DON	37°08′	06°26′	F	4	+	+	No	–
Daphne	8 June 2007	DON	37°08′	06°26′	F	0	+	+	No	–
Dalia	8 June 2007	DON	37°08′	06°26′	F	0	+	+	No	–
Durillo	13 June 2007	DON	37°08′	06°25′	M	0	–	–	Yes	Yes
Cicuta	21 June 2007	DON	37°07′	06°25′	M	1	+	+	No	–
Coca	22 June 2007	DON	37°07′	06°26′	M	1	+	+	No	–
Aliso	3 July 2007	DON	37°05′	06°28′	F	3	–	–	Yes	No
Calima	6 July 2007	SMO	38°17′	04°12′	F	1	–	–	Yes	No
Cándalo	13 July 2007	SMO	38°17′	04°12′	M	4	–	+	No	–
Jeme	25 July 2007	SMO	38°09′	03°56′	M	3	–	–	Yes	Yes
Cacao	1 August 2007	DON	37°08′	06°24′	M	1	+	+	No	–
Caberú	1 August 2007	SMO	38°09′	03°56′	M	1	–	–	Yes	No
Veintitrés	2 August 2007	SMO	38°09′	03°56′	M	1	–	–	Yes	No
Viana	15 August 2007	DON	37°11′	06°23′	F	5	–	–	Yes	Yes
Dardo	16 August 2007	DON	37°11′	06°23′	M	0	–	–	Yes	Yes
Dedalera	16 August 2007	DON	37°11′	06°23′	F	0	–	–	Yes	Yes
Bonares	21 August 2007	DON	36°58′	06°28′	F	2	–	–	Yes	No
Clavo	22 August 2007	DON	36°58′	06°28′	M	1	–	–	Yes	Yes
Jabata	22 August 2007	DON	37°01′	06°27′	F	5	–	–	Yes	Yes
Boliche	24 August 2007	DON	37°05′	06°28′	M	2	–	–	Yes	Yes
Salado	29 August 2007	DON	37°14′	06°14′	M	4	–	–	Yes	Yes
Dulce	4 September 2007	DON	37°16′	06°13′	F	0	–	–	Yes	Yes
Damán	7 September 2007	DON	37°16′	06°13′	M	0	–	–	Yes	No
Daro	17 September 2007	DON	37°07′	06°25′	F	0	–	–	Yes	No
Wari	19 September 2007	DON	37°07′	06°25′	F	8	–	–	Yes	No
Duquesa	20 September 2007	DON	37°16′	06°13′	F	0	–	–	Yes	No
Yeguas	3 October 2007	SMO	38°18′	04°15′	M	4	–	–	Yes	No
Piruétano	4 October 2007	SMO	38°18′	04°15′	M	8	–	–	Yes	No
Baya	5 October 2007	SMO	38°18′	04°15′	M	2	–	–	Yes	Yes
Cristal	5 October 2007	SMO	38°18′	04°15′	F	1	–	–	Yes	No
Drupa	26 October 2007	DON	37°11′	06°23′	F	0	–	–	Yes	No
Dumbo	21 November 2007	DON	37°08′	06°45′	M	0	–	–	Yes	No
Mata	21 November 2007	DON	37°08′	06°45′	F	5	–	–	Yes	No
Bocacha	27 November 2007	DON	38°08′	06°46′	M	2	–	–	Yes	No
Bruma	28 November 2007	DON	38°08′	06°46′	F	2	–	–	Yes	No
Lula	5 December 2007	DON	37°09′	06°33′	F	>5	–	–	Yes	No
Bornizo	10 December 2007	SMO	38°07′	03°59′	M	4	–	–	Yes	No
Civeta	13 December 2007	SMO	38°07′	03°59′	M	1	–	–	Yes	No
Charqueña	18 January 2008	SMO	38°18′	04°15′	F	1	–	–	Yes	No

Every line represent an individual with capture date, population (Doñana: DON; Sierra Morena: SMO), location (with longitude and latitude), sex, age (in years), result of the FeLV p27 antigen ELISA, result of the real-time PCR, vaccination and booster vaccination when applicable.
FCP, FeLV Control Program for the Iberian Lynx.

found to be antigen-ELISA negative and real-time PCR positive, which was considered to be indicative of a potential presence of latent infection. The rest of the antigen-ELISA-negative results were also real-time PCR negative.

Since the beginning of the FCP, in May 2007, up to April 2008, a total of 61 domestic cats have been trapped in Doñana (59 inside the Abalario-Vera lynx subpopulation area and two in the CRS) and 16 in Sierra Morena (Table 3). In Doñana, five cats were found to be positive for FeLV viremia (four in Abalario-Vera and one in the CRS). Thus, an overall prevalence of 8% of FeLV (5% in Abalario-Vera and 50% in the CRS) and 5% of FIV was found in Doñana. In Sierra Morena, all 16 domestic cats captured were found to be negative to the FeLV antigen, and they were all vaccinated once. The FeLV monitoring of domestic cats has continued in the Doñana population.

Discussion

Although the occurrence of FeLV in the Doñana Iberian lynx population is not a novel event, a severe outbreak had never been detected before 2007. In Sierra Morena, since

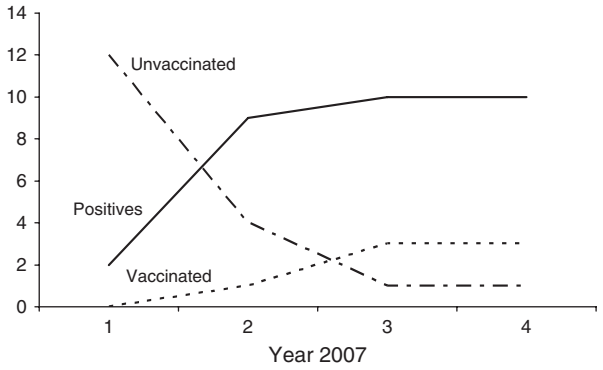

Figure 2 Aggregate values of feline leukemia virus (FeLV) positives, naive-found and vaccinated and unvaccinated Iberian lynxes *Lynx pardinus* in the Coto del Rey subpopulation during 2007, by quarters.

2004 and after the sanitary evaluation of nearly 90 lynxes, FeLV viremia has never been detected, and latent infection was found in only a single individual. Dissemination of the virus over the CRS population in early 2007 was much faster than expected for a species living in solitary, because only one adult male out of the 12 infected individuals found tested positive before the mating season 2006–2007. The possible causes proposed to explain this spreading pattern include: (1) the unexpectedly high pathogenicity of the FeLV strain infecting the lynxes (although it has not been detected in cats); (2) the timing of infection reaching the lynx population (just before the breeding season, when more inter-individual contact is known to occur); (3) lynx density of the affected subpopulation (the highest in the entire Doñana population). As the largest and densest lynx subpopulation in the entire Doñana population, the CRS must present a maximum intraspecific contact rate, especially during the breeding season. Moreover, artificial conservation management structures placed inside the lynx's home ranges, such as feeding stations and drinking points, may contribute toward gathering of the animals at the same spots, and may thereby further increase the chances of lynx-to-lynx contact. Although not definitive, corrective measures applied to these structures are thought to have helped stop the dissemination of the virus through the lynx population. However, considering the potential risk these structures pose for the population, we suggest that they should only be used when strictly necessary, and should always take into account all the possible safety measures. The idea that the FeLV strain affecting Iberian lynxes in Doñana was highly pathogenic is supported by the unusually fast course of infection that animals presented. While FeLV infection usually lasts years until death occurs (Miyazawa, 2002; Barr & Bowman, 2006), six out of the 12 lynxes found infected died <6 months after infection. The cause of death of all the lynxes seemed to be an opportunistic infection probably aided by an FeLV-mediated immunosuppression. Despite the fast course, no neoplastic signs were detected. The hyperpathogenicity could be intrinsic to the virus, or even extrinsic, due to the high susceptibility of individuals. This latter factor could be due to the low genetic diversity existing in the inbred Doñana Iberian lynx subpopulation (J. A. Godoy, unpubl. data). In this context, a deeper study of the virus strain(s) involved and the clinical signs of infected lynxes is needed to better understand the cause of death.

The sex distribution of the FeLV-infected Iberian lynxes found was biased; out of 12 total positives, eight (67%) were males. Previous studies of FeLV in the domestic cat have shown similar findings (Lutz *et al.*, 1990; Fuchs, Binzel & Lonsdorfer, 1994; Arjona *et al.*, 2000; Levy *et al.*, 2006). Laboratory studies, however, have failed to find a higher male sensitivity to FeLV (Hardy, 1980), and differences in prevalence have usually been attributed to the aggressive behavior of males (see Barr & Bowman, 2006). The data presented here, however, suggest that a higher male susceptibility to FeLV could exist in the Iberian lynx, because (1) five out of the six FeLV-related dead lynxes were males and (2) the two individuals that showed latency of the virus were females. In the Doñana Iberian lynx population, a sex ratio bias towards females in the adult stratum has been detected in the last decade (see Gaona *et al.*, 1998). Given these results, this lack of adult males might have been induced by the action of FeLV, as it is known to have been present in the lynx population since the early 1990s. Moreover, in the Doñana population of Iberian lynxes, a higher than normal sexual size dimorphism has been found (Pertoldi *et al.*, 2006) and, interestingly, one of the proposed effects of FeLV on populations is to increase sexual size dimorphism (Pontier *et al.*, 1998). In this scenario, FeLV could have been affecting the Doñana Iberian lynx population dynamics for a long time, and it could be a determinant factor hindering population growth. To answer these questions, more studies are needed.

The transmission of the FeLV to the Iberian lynx population is thought to have been caused by a domestic cat by means of lynx–cat aggression. Measures implemented within the FCP regarding feral cat population control (which were also extended to the feral dog population) are thought to be important to reduce the risk of new FeLV diffusion to the lynx population. To this end, despite the discontinuation of lynx handling, these measures are going to be continued into the future.

As FeLV latent infections can develop into viremia under situations of immunosuppression, the presence of two known latently infected female lynxes in the CRS may represent a risk of occurrence of a new outbreak in the future. Given this, both females will be trapped to evaluate absence of FeLV viremia every year; meanwhile, feces taken from their latrines are being evaluated weekly. Also, their cubs are being evaluated during the first month of their life to ensure that they are not infected. If the Doñana lynx population status reaches a level in which the removal of these individuals can be performed without fatal implications, elimination should be considered.

Possible scenarios for the disease in the future are (1) that the FeLV never occurs again in the Iberian lynx population; (2) that the FeLV reaches a low Iberian lynx density nucleus; (3) that the FeLV reaches a high Iberian lynx density

Table 3 Domestic cats handled during the FCP both in Doñana (DON) and Sierra Morena (SMO), including date of capture, sex, location and FeLV viremia status

Date	Sex	Population	Longitude	Latitude	FeLV status
26 June 2007	Unrecorded	DON	37°08′	06°29′	Negative
3 July 2007	Female	DON	37°10′	06°37′	Negative
9 July 2007	Unrecorded	DON	37°08′	06°32′	Negative
10 July 2007	Unrecorded	DON	37°10′	06°37′	Negative
10 July 2007	Unrecorded	DON	37°10′	06°37′	Negative
10 July 2007	Unrecorded	DON	36°59′	06°28′	Negative
10 July 2007	Unrecorded	DON	37°06′	06°30′	Negative
20 July 2007	Male	DON	37°10′	06°37′	Negative
10 August 2007	Male	DON	37°06′	06°30′	Negative
16 August 2007	Female	DON	37°00′	06°33′	Negative
10 September 2007	Female	DON	37°00′	06°33′	Negative
19 September 2007	Male	SMO	38°17′	04°12′	Negative
16 October 2007	Male	SMO	38°17′	04°12′	Negative
17 October 2007	Female	SMO	38°09′	03°58′	Negative
17 October 2007	Female	SMO	38°09′	03°58′	Negative
22 October 2007	Unrecorded	DON	37°08′	06°32′	Negative
23 October 2007	Unrecorded	DON	37°08′	06°31′	Negative
23 October 2007	Female	SMO	38°07′	03°58′	Negative
23 October 2007	Male	SMO	38°07′	03°58′	Negative
23 October 2007	Female	SMO	38°07′	03°58′	Negative
23 October 2007	Female	SMO	38°07′	03°58′	Negative
23 October 2007	Male	SMO	38°07′	03°58′	Negative
23 October 2007	Male	SMO	38°07′	03°58′	Negative
23 October 2007	Male	SMO	38°07′	03°58′	Negative
30 October 2007	Female	DON	37°00′	06°33′	Negative
31 October 2007	Male	DON	37°00′	06°33′	Negative
13 November 2007	Male	DON	37°00′	06°34′	Positive
13 November 2007	Female	SMO	38°07′	03°58′	Negative
13 November 2007	Female	SMO	38°07′	03°58′	Negative
14 November 2007	Male	DON	37°00′	06°34′	Negative
15 November 2007	Male	DON	37°00′	06°34′	Negative
15 November 2007	Female	DON	37°00′	06°34′	Negative
21 November 2007	Male	DON	37°00′	06°34′	Negative
21 November 2007	Female	DON	37°00′	06°34′	Negative
22 November 2007	Male	DON	37°00′	06°34′	Negative
22 November 2007	Male	DON	37°00′	06°34′	Positive
22 November 2007	Male	DON	37°00′	06°34′	Positive
28 November 2007	Male	DON	37°00′	06°34′	Negative
28 November 2007	Male	DON	37°00′	06°34′	Negative
29 November 2007	Male	DON	37°00′	06°34′	Negative
11 December 2007	Male	SMO	38°17′	04°12′	Negative
12 December 2007	Male	SMO	38°17′	04°12′	Negative
12 December 2007	Female	SMO	38°17′	04°12′	Negative
19 December 2007	Male	DON	37°00′	06°34′	Negative
16 January 2008	Male	DON	37°00′	06°34′	Negative
22 January 2008	Female	DON	37°00′	06°34′	Negative
31 January 2008	Male	DON	37°00′	06°34′	Negative
6 February 2008	Male	DON	37°03′	06°32′	Negative
7 February 2008	Female	DON	37°03′	06°32′	Negative
8 February 2008	Female	DON	37°08′	06°27′	Negative
8 February 2008	Female	DON	37°00′	06°34′	Negative
20 February 2008	Male	DON	37°07′	06°29′	Negative
22 February 2008	Male	DON	37°08′	06°27′	Negative
4 March 2008	Female	DON	37°07′	06°47′	Negative
4 March 2008	Female	DON	37°07′	06°47′	Negative
5 March 2008	Female	DON	37°07′	06°47′	Negative
5 March 2008	Male	DON	37°07′	06°47′	Negative

Table 3. Continued.

Date	Sex	Population	Longitude	Latitude	FeLV status
5 March 2008	Male	DON	37°07′	06°47′	Negative
5 March 2008	Male	DON	37°07′	06°47′	Negative
5 March 2008	Male	DON	37°07′	06°47′	Negative
26 March 2008	Male	DON	37°07′	06°47′	Negative
26 March 2008	Male	DON	37°07′	06°47′	Negative
26 March 2008	Female	DON	37°07′	06°47′	Negative
26 March 2008	Female	DON	37°07′	06°47′	Negative
26 March 2008	Female	DON	37°04′	06°32′	Negative
26 March 2008	Male	DON	37°07′	06°48′	Positive
2 April 2008	Female	DON	37°07′	06°47′	Negative
2 April 2008	Female	DON	37°07′	06°47′	Negative
2 April 2008	Female	DON	37°07′	06°47′	Negative
2 April 2008	Male	DON	37°07′	06°47′	Negative
2 April 2008	Female	DON	37°07′	06°47′	Negative
2 April 2008	Female	DON	37°07′	06°48′	Negative
2 April 2008	Male	DON	37°07′	06°48′	Negative
2 April 2008	Male	DON	37°07′	06°47′	Positive
2 April 2008	Male	DON	37°07′	06°47′	Negative
2 April 2008	Male	DON	37°07′	06°48′	Negative
15 April 2008	Male	DON	37°07′	06°47′	Negative

FeLV, feline leukemia virus; FCP, FeLV Control Program for the Iberian Lynx.

nucleus. In the second case, the disease is thought to be autolimiting, as the opportunities of spread are low. In the third case, however, the risk of a new outbreak is real. To prevent such situations, a surveillance program is being carried out by means of FeLV molecular analysis (q-PCR) of Iberian lynx scats collected through the whole population. This work will be continued in the next few years.

Acknowledgments

This research has been funded by the LIFE project LIFE-NAT 06/E/000209. Idexx® performed all combo FeLV/FIV tests (Snap test®). We are grateful to Dr J.C. Thibault for donating the Merial® (Purevax® FeLV) vaccine doses. I. Molina, J.C. Capuz, M.J. Pérez, C.I. León, K. Reyes, M.P. Ryser-Degiorgis, M. Roelke and J. Velarde collaborated with the sanitary handling of the lynxes. G. Ruiz, J.A. Báñez, R. Sanabria, D. Palacios, R.B. Millán, J.A. Tena, E. Rojas, C. Gutiérrez and the rest of the LIFE project members made lynx captures and handling possible. The expert commission and the Iberian Lynx Health Advisory Group led and facilitated the FCP. A. Ryser's hard work helped capture priority individuals. Most of the laboratory work was performed using the logistics of the Center for Clinical Studies at the Vetsuisse Faculty, University of Zürich. Thanks to a research project led by F. Palomares, the FeLV status of most CRS individuals before mating season 2006–2007 can be known. The *Los Villares* rescue center staff provided treatment and care to all the viremic animals removed from the field. Doñana Natural Space and Doñana Biological Station staff helped with fieldwork and detected two FeLV deaths.

References

Aldama, J.J. & Delibes, M. (1991). Observations of feeding groups in the Spanish lynx (*Felis pardina*) in the Doñana National Park, SW Spain. *Mammalia* **55**, 143–147.

Arjona, A., Escolar, E., Soto, I., Barquero, N., Martin, D. & Gómez-Lucía, E. (2000). Seroepidemiological survey of infection by feline leukemia virus and immunodeficiency virus in Madrid and correlation with some clinical aspects. *J. Clin. Microbiol.* **38**, 3448–3449.

Barr, C.S. & Bowman, D.D. (2006). *Canine and feline infectious diseases and parasitology*. Iowa: Blackwell Publishing.

Brown, M., Cunningham, M., Roca, A.L., Troyer, J., Johnson, W. & O'Brien, S. (2006). Genetic characterization of feline leukemia virus (FeLV) in the free-ranging Florida panther (*Felis cocncolor coryi*) population. Research Update. *8th International Feline Retrovirus Research Symposium*, National Cancer Institute, USA.

Burmeister, T. (2001). Oncogenic retroviruses in animals and humans. *Rev. Med. Virol.* **11**, 369–380.

Cattori, V., Pepin, A.C., Tandon, R., Riond, B., Meli, M.L., Willi, B., Lutz, H. & Hofmann-Lehmann, R. (2008). Real-time PCR investigation of feline leukemia virus proviral and viral RNA loads in leukocyte subsets. *Vet. Immunol. Immunopathol.* **123**, 124–128.

Cunningham, M., Brown, M., Terrell, S., Hayes, K., Blankenship, E., Johnson, W., Roca, A.L. & O'Brien, S. (2006)

Epizootiology and management of feline leukemia virus in free-ranging Florida panthers – research update. *8th International Feline Retrovirus Research Symposium*, National Cancer Institute, USA.

Delibes, M. (1980). Feeding ecology of the Spanish lynx in the Coto Doñana. *Acta Theriol.* **25**, 309–324.

Ferrer, M. & Negro, J.J. (2004). The near extinction of two large European predators: super specialists pay a price. *Conserv. Biol.* **18**, 344–349.

Ferreras, P., Beltrán, J.F., Aldama, J.J. & Delibes, M. (1997). Spatial organization and land tenure system of the endangered Iberian lynx (*Lynx pardinus*). *J. Zool. (Lond.)* **243**, 163–189.

Filoni, C., Adania, C.H., Durigon, E.L. & Catão-Dias, J.L. (2003). Serosurvey for feline leukemia virus and lentiviruses in captive small neotropic felids in São Paulo state, Brazil. *J. Zoo Wildl. Med.* **34**, 65–68.

Filoni, C. & Catão-Dias, J.L. (2005). Retrovirus infections (FeLV and FIV) in nondomestic felids: a review. *Clin. Vet.* **10**, 56–64.

Filoni, C., Catão-Dias, J.L., Bay, G., Durigon, E.L., Jorge, R.S., Lutz, H. & Hofmann-Lehmann, R. (2006). First evidence of feline herpesvirus, calicivirus, parvovirus, and Ehrlichia exposure in Brazilian free-ranging felids. *J. Wildl. Dis.* **42**, 470–477.

Fromont, E., Pontier, E., Sager, A., Jouquelet, E., Artois, M., Léger, F., Stah, P. & Bourguemestre, F. (2000). Prevalence and pathogenicity of retroviruses in wildcats in France. *Vet. Rec.* **146**, 317–319.

Fuchs, A., Binzel, L. & Lonsdorfer, M. (1994). Epidemiology of FeLV and FIV infection in the Federal Republic of Germany. *Tierarztl Prax* **22**, 273–277.

Gaona, P., Ferreras, P. & Delibes, M. (1998). Dynamics and viability of a metapopulation of the endangered Iberian lynx (*Lynx pardinus*). *Ecol. Monogr.* **68**, 349–370.

Gil-Sanchez, J.M., Ballesteros-Duperon, E. & Bueno-Segura, J.F. (2006). Feeding ecology of the Iberian lynx *Lynx pardinus* in eastern Sierra Morena (Southern Spain). *Acta Theriol.* **51**, 85–90.

Guzmán, J.N., García, F.J., Garrote, G., Pérez-de-Ayala, R. & Iglesias, M.C. (2004). *El Lince ibérico (Lynx pardinus) en España y Portugal. Censo-diagnóstico de sus poblaciones*. Madrid: DGCN, Ministerio de Medio Ambiente.

Hardy, W.D. Jr (1980). The virology, immunology and epidemiology of the feline leukemia virus. *Feline leukemia virus*: 33–78. Hardy, W.D. Jr (Ed.). New York, NY: Elsevier/North-Holland.

Hartmann, K., Block, A., Ferk, G., Beer, B., Vollmar, A. & Lutz, H. (1999). Treatment of feline leukemia virus (FeLV) infection. *Vet. Microbiol.* **69**, 111–113.

Hofmann-Lehmann, R., Cattori, V., Tandon, R., Boretti, F.S., Meli, M.L., Riond, B. & Lutz, H. (2008). How molecular methods change our views of FeLV infection and vaccination. *Vet. Immunol. Immunopathol.* **123**, 119–123.

Hofmann-Lehmann, R., Cattori, V., Tandon, R., Boretti, F.S., Meli, M.L., Riond, B., Pepin, A.C., Willi, B., Ossent, P. & Lutz, H. (2007). Vaccination against the feline leukemia virus: outcome and response categories and long-term follow-up. *Vaccine* **25**, 5531–5539.

Hofmann-Lehmann, R., Holznagel, E., Ossent, P. & Lutz, H. (1997). Parameters of disease progression in long-term experimental feline retrovirus (feline immunodeficiency virus and feline leukemia virus) infections: hematology, clinical chemistry, and lymphocyte subsets. *Clin. Diagn. Lab. Immunol.* **4**, 33–42.

Hofmann-Lehmann, R., Tandon, R., Boretti, F.S., Meli, M.L., Willi, B., Cattori, V., Gomes-Keller, M.A., Ossent, P., Golder, M.C., Flynn, J.N. & Lutz, H. (2006). Reassessment of feline leukemia virus (FeLV) vaccines with novel sensitive molecular assays. *Vaccine* **24**, 1087–1094.

IUCN (2003) *IUCN Red List of Threatened Species*. http://www.redlist.org

Jarrett, O. & Russell, P.H. (1978). Differential growth and transmission in cats of feline leukemia viruses of subgroups A and B. *Int. J. Cancer* **21**, 466–472.

Levy, J.K., Scott, H.M., Lachtara, J.L. & Crawford, P.C. (2006). Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. *J. Am. Vet. Med. Assoc.* **228**, 371–376.

Levy, L.S. (2008). Advances in understanding determinants in FeLV pathology. *Vet. Immunol. Immunopathol.* **123**, 14–22.

López-Bao, J.V., Rodríguez, A. & Ales, E. (2007). Field observation of two males following a female in the Iberian lynx (*Lynx pardinus*) during the mating season. *Mamm. Biol.* **73**(5), 404–406.

Luaces, I., Doménech, A., García-Montijano, M., Collado, V.M., Sánchez, C., Tejerizo, J.G., Galka, M., Fernández, P. & Gómez-Lucía, E. (2008). Detection of feline leukemia virus in the endangered Iberian lynx (*Lynx pardinus*). *J. Vet. Diagn. Invest.* **20**, 381–385.

Lutz, H., Lehmann, R., Winkler, G., Kottwitz, B., Dittmer, A., Wolfensberger, C. & Arnold, P. (1990). Feline immunodeficiency virus in Switzerland: clinical aspects and epidemiology in comparison with feline leukemia virus and coronaviruses. *Schweiz. Arch. Tierheilkd.* **132**, 217–225.

Lutz, H., Pedersen, N.C. & Theilen, G.H. (1983). Course of feline leukemia virus infection and its detection by enzyme-linked immunosorbent assay and monoclonal antibodies. *Am. J. Vet. Res.* **44**, 2054–2059.

Mari, K.D., Maynard, L., Sanquer, A., Lebreux, B. & Eun, H.M. (2004). Therapeutic effects of recombinant feline interferon-omega on feline leukemia virus (FeLV)-infected and FeLV/feline immunodeficiency virus (FIV)-coinfected symptomatic cats. *J. Vet. Intern. Med.* **4**, 477–482.

Miyazawa, T. (2002). Infections of feline leukemia virus and feline immunodeficiency virus. *Front. Biosci.* **7**, 504–518.

Pacitti, A.M. (1987). Latent feline leukemia virus infection: a review. *J. Small Anim. Pract.* **28**, 1153–1159.

Palomares, F. & Caro, T.M. (1999). Interspecific killing among mammalian carnivores. *Am. Nat.* **153**, 492–508.

Palomares, F., Delibes, M., Ferreras, P., Fedriani, J.M., Calzada, J. & Revilla, E. (2000). Iberian lynx in a

fragmented landscape: predispersal, dispersal, and postdispersal habitats. *Conserv. Biol.* **14**, 809–818.

Palomares, F., Ferreras, P., Fedriani, J.M. & Delibes, M. (1996). Spatial relationships between Iberian lynx and other carnivores in an area of south-western Spain. *J. Appl. Ecol.* **33**, 5–13.

Pertoldi, C., Garcia-Perea, R., Godoy, J.A., Delibes, M. & Loeschcke, V. (2006). Morphological consequences of range fragmentation and population decline on the endangered Iberian lynx (*Lynx pardinus*). *J. Zool. (Lond.)* **268**, 73–86.

Pontier, D., Fromont, E., Courchamp, F., Artois, M. & Yoccoz, N.G. (1998). Retroviruses and sexual size dimorphism in domestic cats (*Felis catus L.*). *Proc. Biol. Sci.* **265**, 167–173.

Rezanka, L.J., Rojko, J.L. & Neil, J.C. (1992). Feline leukemia virus: pathogenesis of neoplastic disease. *Cancer Invest.* **10**, 371–389.

Rodríguez, A. & Delibes, M. (1992). Current range and status of the Iberian Lynx (*Felis pardina* Temminck 1824) in Spain. *Biol. Conserv.* **61**, 189–196.

Roelke, M.E., Johnson, W.E., Millán, J., Palomares, F., Revilla, E., Rodríguez, A., Calzada, J., Ferreras, P., León-Vizcaíno, L. & Delibes, M. (2008). Exposure to disease agents in the endangered Iberian lynx (*Lynx pardinus*). *Eur. J. Wildl. Res.* **54**, 171–178.

Ryser, A., Scholl, M., Zwahlen, M., Oetliker, M., Ryser-Degiorgis, M.P. & Breitenmoser, U. (2005). A remote-controlled teleinjection system for the low-stress capture of large mammals. *Wildl. Soc. Bull.* **33**, 721–730.

Sarma, P.S. & Log, T. (1973). Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests. *Virology* **54**, 160–169.

Sleeman, J.M., Keane, J.M., Johnson, J.S., Brown, R.J. & Woude, S.V. (2001). Feline leukemia virus in a captive bobcat. *J. Wildl. Dis.* **37**, 194–200.

Tandon, R., Cattori, V., Gomes-Keller, M.A., Meli, M.L., Golder, M.C., Lutz, H. & Hoffmann-Lehmann, R. (2005). Quantitation of feline leukemia virus viral and proviral loads by TaqMan real-time polymerase chain reaction. *J. Virol. Methods* **130**, 124–132.

Tandon, R., Cattori, V., Willi, B., Lutz, H. & Hofmann-Lehmann, R. (2008). Quantification of endogenous and exogenous feline leukemia virus sequences by real-time PCR assays. *Vet. Immunol. Immunopathol.* **123**, 129–133.

Torres, A.N., O'Halloran, K.P., Larson, L.J., Schultz, R.D. & Hoover, E.A. (2008). Development and application of a quantitative real-time PCR assay to detect feline leukemia virus RNA. *Vet. Immunol. Immunopathol.* **123**, 81–89.

Weijer, K., UytdeHaag, F. & Osterhaus, A.D. (1987). Feline leukemia virus (FeLV) and FeLV-associated diseases in cats: a review. *Tijdschr Diergeneeskd* **112**, 726–737.