Preliminary results of the evaluation of sanitary status of domestic and wild fauna that share pathologies and potential habitat with the Iberian Lynx (*Lynx pardinus*) - LIFE-Nature Project "Enhancing Habitat for the Iberian Lynx and Black Vulture in the Southeast of Portugal"

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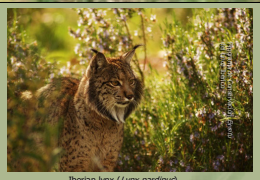
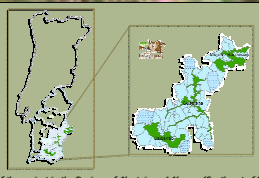
INTRODUCTION

Wildlife diseases can represent a serious conservation threat for free-living populations of endangered species, infectious diseases are one of the main five causes of global species extinctions [1]. Small, geographically isolated and genetically depleted populations, suffer from a progressive loss of diversity that potentially increases their susceptibility and decreases their response to infectious agents. The risk is higher in areas where the Iberian lynx (*Lynx pardinus*) shares its habitat with other wild and domestic species that also carry these infectious diseases.

Conservation Medicine approach to evaluate the sanitary status of animal populations reveal to be very important to the success of both *in situ* and *ex situ* conservation program of endangered species with declining populations [2], such as the Iberian lynx, considered the most endangered felid species on the planet [3].



Iberian lynx (*Lynx pardinus*)



Areas of the project in the Regions of Alentejo and Algarve (Southeast of Portugal)

For this purpose, an epidemiological survey was drawn, consisting on a sample collection and laboratorial testing of biological material from sympatric domestic and wild animals to detect and quantify the presence of pathogenic agents that might affect the Iberian Lynx at the areas of the LIFE-Nature Project 'Enhancing habitat for the Iberian Lynx and Black Vulture in the Southeast of Portugal' (LIFE08NATP000227), co-funded in 75% by the EU. This project aims to contribute to the improvement of the survival, feeding and breeding conditions of the Iberian lynx and the Black Vulture (*Aegypius monachus*) in southeast Portugal. The actions of this project are being implemented in the Natura 2000 Network areas of Moura, Mourão and Barrancos, Vale do Guadiana and Serra do Caldeirão.

Based on the scientific data available [4,5,6], the protocol has set up priority species and pathologies and based on existing population data a representative sampling of the domestic and wild populations was determined. According with the available scientific data, the expected prevalence for each pathology and group species was determined. Based on that, the sample size was calculated for a chosen confidence level.

The information gathered will allow the elaboration of a monitoring/control plan for the major pathologies found in the Iberian Lynx and will be integrated in the conservation strategy for this species.

OBJECTIVES

Evaluate the sanitary status of domestic and wild animals that can share pathologies and habitat with the Iberian lynx and determine the existence of any potentially dangerous disease reservoirs for the Iberian lynx.

METHODOLOGY

Priority diseases

Diseases that may affect the Iberian lynx as well as their degree of priority were defined by the veterinary technical group (GTV), within the framework of the Executive Committee (EC) of the action plan for the conservation of the Iberian lynx in Portugal (PACLIP). The degree of priority was based on the susceptibility of the Iberian lynx and on the impact of the disease. The current action will test all the high priority diseases and part of the medium priority diseases.

Priority	Disease agents
1 – High	FIV, FeLV, Feline Parvovirus, Morbillivirus, Coronavirus, Calicivirus, Herpesvirus, *Mycobacterium bovis* and *Cytauxzoon felis*
2 – Medium	Canine Parvovirus, *Leptospira interrogans*, *Brucella*, *Pasteurella haemolytica*, *Chlamidophila felis*, *Francisella tularensis*,, *Toxocara cati*, *Leishmania*, *Anaplasma*, *Ehrlichia canis*, *Mycobacterium paratuberculosis*, *Toxascaris leonina*
3 – Low	*Borrelia burgdorferi*, *Babesia*, *Theileria*, *Salmonella*, *Mycoplasma*, *Campylobacter*, *Shigella*, *Cryptosporidium*, *Sarcocystis*, *Ancylostoma caninum*, *Trichinella spiralis*, *Dirofilaria immitis*

Target species

The determination of target species and their priority was also based on the work done by the GTV, according with their abundance, likelihood of contact with the Iberian lynx and expected prevalence of the priority diseases. The species were grouped (1-6) according to the susceptibility to diseases and their descending degree of priority (1-3).

Target Species Group	Priority
1 – Domestic, feral and wild felids	1
2 – Domestic and feral dogs	1
3 – Other wild carnivores	2
4 – Domestic (free range) and wild ungulates	2
5 – Wild lagomorphs and rodents	3
6 – Vectors (*Ixodidae* and *Argasidae*)	3

Group 1	• Capture • Run overs	**Group 4**	• Blood sampling • Hunting
Group 2	• Blood sampling	**Group 5**	• Capture • Hunting
Group 3	• Capture • Run overs • Hunting	**Group 6**	• Captured animals • Vegetation

Sampling

The sample size for each group and for each of the areas of the project was calculated considering the estimated existing populations for an expected prevalence based on bibliography available for a confidence level of 95% and a 10% error (Bold). Samples collection are depending on group type (the right table also shows the percentage of collected samples till October 2013).

Group\Project Area	Caldeirão	Guadiana	Moura/Barrancos	Total samples
Group 1	**10/96** (10,4%)	**18/96** (18,8%)	**22/91** (18,8%)	**50/283** (18,8%)
Group 2	**99/93** (106,4%)	**103/93** (110,7%)	**108/93** (116,1%)	**310/279** (111,1%)
Group 3	**31/79** (39,2%)	**22/86** (25,5%)	**92/70** (131,4%)	**145/235** (61,7%)
Group 4	**10/62** (15,1%)	**5/62** (8,0%)	**17/62** (27,4%)	**32/186** (17,2%)
Group 5	**13/62** (20,9%)	**68/62** (109,6%)	**61/62** (98,3%)	**142/186** (76,3%)
Group 6	**0/97** (0%)	**91/97** (93,8%)	**15/97** (15,4%)	**106/291** (36,4%)
Total samples	**150/489** (30,6%)	**304/496** (61,3%)	**311/475** (65,5%)	**785/1.460** (53,7%)

Laboratory tests to be used

Virology: ELISA, PCR;

Bacteriology: ELISA, complement fixation, PCR, culture, microscopic agglutination, biochemical tests;

Parasitology: Optical microscopy, immunofluorescence, direct agglutination.

Software

WinEpiscope, Survey Toolbox, ArcGIS 9.3, SPSS and R.

PRELIMINARY RESULTS

Sample collection (figures bellow) data per Group, from August 2011 till October 2013 are shown in the upper table and its distribution at the right map.

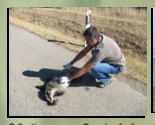
Cage trap used for capture of wild carnivores

European Wildcat (*Felis silvestris*) captured in the Guadiana area

Common genet (*Genetta genetta*) captured in the Guadiana area

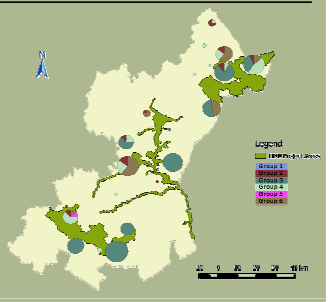
Blood sampling on a Common genet (*Genetta genetta*)

Eurasian badger (*Meles meles*) victim of road traffic collision



Collecting run over Eurasian badger (*Meles meles*)

Hunting samples collecting - Eurasian wild boar (*Sus scrofa*)



Samples collected per group from August 2011 till October 2013

Laboratory results (agent disease prevalence (%)=positive/sampled; confidence intervals) available are displayed on the next tables (method "Sterne", confidence level 95%). Group 6 (vectors) laboratory results are not display since till the present time they have not been processed, also absent are the disease agents not tested.

Disease Prevalence (etiological agents) per Group

	FIV	FeLV	* Feline Parvovirus	* Morbillivirus	* Calicivirus	* Herpesvirus	Coronavirus	*Chlamidophila felis* **	*Toxoplasma gondii*
Group 1	10,3% (2,9-27,2%)	17% (7-35,9%)	50% (29-71%)	0% (0-12,3%)	7,4% (1,9-23,7%)	0% (0-12,3%)	11,5% (3,2-30%)	0% (0-13,9%)	75% (54,2-88,5%)

	***Morbillivirus	Coronavirus	***Canine Parvovirus	***Leptospira interrogans	Brucella spp	Leishmania
Group 2	100% (98,7-100%)	87,9% (83,7-91,2%)	88,3% (84,5-91,7%)	20,1% (13,7-28,5%)	0% (0-1,6%)	34,1% (28,7-39,8%)

	* Morbillivirus	* Coronavirus	* Canine Parvovirus	* *Micobacterium bovis*	*Leptospira interrogans*	*Brucella* spp	*Pasteurella haemolytica***	*Leishmania*
Group 3	33% (24,4-42,9%)	1,5% (0,03-6,9%)	47% (37,2-56,8%)	3% (0,15-15,1%)	33% (24,4-42,9%)	0% (0-8,5%)	0% (0-8,5%)	0% (0-8,5%)

	Herpesvirus (ADV)	*M. Para_ tuberculosis*	*Leptospira interrogans*	*Brucella* spp
Group 4	41,6% (18,1-70,6%)	0% (0-15,1%)	0% (0-19,6%)	15,2% (5,4-34,3%)

	*Coccidea***	*Toxoplasma gondii*
Group 5	24% (11,7-42,3%)	11,1% (3,8-26%)

(Etiological agent disease prevalence (%) = positive/sampled (confidence intervals) determined by serological test except PCR *, Culture **; Serology of serum from animals vaccinated ***; Method "Sterne"; Confidence level 95%; 100% tests specificity and sensibility were assumed).

CONCLUSIONS AND FUTURE WORK

The evaluation and assessment of the laboratory final results will provide baseline data about the diseases that affect the resident populations in each area, as well as its potential impact on the Iberian Lynx.

Also, it may allow the detection of reservoirs of disease that are potentially dangerous for the Iberian lynx.

Additionally, at the end of the project, according to the results, reports will be made proposing some measures to reduce the contamination risk, to be carried out by the responsible authorities, in areas where this risk might be a problem for the conservation of the species.

The information gathered will allow the elaboration of a monitoring/control plan for the major diseases found in the study area.

REFERENCES:

1. Smith K.F., Sax D.F., Lafferty K.D. (2006) Evidence for the Role of Infectious Disease in Species Extinction and Endangerment *Conservation Biology* 20 (5), 1349 1357.
2. Aguirre A.A., Ostfeld R.S., Tabor G.M., House C., Pearl M.C. (2002) Conservation Medicine: Ecological Health in Practice. New York:Oxford University Press.
3. IUCN (2011) IUCN Red List of Threatened Species. http://www.iucnredlist.org/details/12520/0.
4. Roelke M.E., Johnson WE, Millán J., Palomares F., Revilla E., Rodriguez A., Calzada J., Ferreras P., León-Vizcaino L., Delibes M., O`Brien S.J. (2008) Exposure to disease agents in the endangered Iberian lynx (*Lynx pardinus*). *Eur J Wildl* es;Epub aehad of print: DOI 10.1007/s10344-10007-10122-10342.
5. Millán, J., Candela, M. G., Palomares, F., Cubero, M. J., Rodriguez, A., Barral, M., de la Fuente, J., Almeria S., and Leon-Vizcaino, L. (2009). Disease threats to the endangered Iberian lynx (*Lynx pardinus*). *Veterinary Journal* 182, 114–124. doi:10.1016/j.tvjl.2008.04.005.
6. Meli, M.L., Cattori, V., Martínez, F., López, G., Vargas, A., Simón, M.A., Zorrilla, I., Muñoz, A., Palomares, F., López-Bao, J.V., Pastor, J., Tandon, R., Willi, B., Hofmann-Lehmann, R., Lutz, H. (2009). Feline leukemia virus and other pathogens as important treats to the survival of the critically endangered Iberian lynx (*Lynx pardinus*). *PLoS One*4, e4744.

AKNOWLEGEMENTS:

The Veterinary Technical Group of the Action Plan for the Conservation of the Iberian Lynx in Portugal (PACLIP). Staff of the INIAV-LNIV. All the collaborators of the LIFE-Nature Project 'Enhancing habitat for the Iberian Lynx and Black Vulture in the Southeast of Portugal'. Team members engaged at the samples collection.