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Histopathological and immunohistochemical findings in lymphoid tissues of the endangered Iberian lynx (*Lynx pardinus*)

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Abstract

The Iberian lynx (*Lynx pardinus*) is the most threatened wild feline in the world. Little is known about the diseases and pathology that affect this animal. The aim of this study was to evaluate the histopathological status of the peripheral lymphoid tissues and thymus of Iberian lynxes necropsied between 1998 and 2003. Seventeen animals including females ($n=8$) and males ($n=9$), age range of 10 months to 16 years, with different causes of death were histopathologically and immunohistochemically (anti-CD3, CD79, MAC387, CD68) studied. Feline immunosuppressive virus laboratorial tests were negative. Five individuals presented neoplasia and/or tuberculosis. All animals presented some degree of both B and T cells depletion in peripheral lymphoid tissues and follicular hyalinosis in the center of depleted follicles. A viral origin of the lymphoid depletion is postulated although other causes (inbreeding, stress, toxic) are not ruled out. The loss of the effectiveness of the immune system increases the vulnerability of the critically endangered Iberian lynx to pathogens.

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Keywords: Iberian lynx; Histopathology; Immunohistochemistry; Immune depletion; CD3; CD79; MAC387; CD68; Tuberculosis

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Résumé

Le lynx d'Espagne (*Lynx pardinus*) est l'espèce de félin sauvage la plus menacée au monde. Les maladies affectant cette espèce sont mal connues. L'objectif de cette étude est d'examiner l'histopathologie des tissus et organes lymphoïdes secondaires et du thymus chez plusieurs lynx d'Espagne soumis à examen nécropsique entre 1998 et 2003. Les tissus et organes de dix sept lynx, dont 8 femelles et 9 males, d'âge compris entre 10 mois et 16 ans, et morts de différentes causes, ont été évalués par histopathologie et immunohistochimie (anti-CD3, CD79, MAC387, CD68). Les examens de laboratoire évaluant le statut d'infection vis à vis du virus de l'immunodéficience feline (FIV) se sont révélés négatifs. Cinq lynx présentaient une pathologie tumorale et/ou étaient atteints de tuberculose. Tous les animaux montraient un certain degré de déplétion lymphoïde, à la fois des territoires B- et T-dépendants des tissus lymphoïdes secondaires ainsi que des lésions de hyalinose folliculaire au centre des follicules lymphoïdes déplétés. Une origine virale à cette déplétion lymphoïde est proposée bien que d'autres causes (dont une consanguinité excessive, le stress ou une origine toxique) ne peuvent être exclues. L'immunodépression potentiellement associée est susceptible d'augmenter la susceptibilité aux agents pathogènes des lynx d'Espagne, dont la population est déjà en danger critique. © 2006 Elsevier Ltd. All rights reserved.

Mots clés: lynx d'Espagne; histopathologie; immunohistochimie; déplétion lymphoïde; CD3; CD79; MAC387; CD68; tuberculose

1. Introduction

The Iberian lynx (*Lynx pardinus*) is the most threatened wild feline in the world [1]. It is considered 'critically endangered' [2] and it is located only in two small isolated metapopulations in Southern Spain, the Doñana area and Sierra Morena [3]. The progressive disappearance of these animals in the past decades has been attributed to decreases in the populations of their main prey, the rabbit (*Oryctolagus cuniculus*), due to myxomatosis, traps and hunting [4–6]. Nowadays, the Iberian lynx is threatened by the fragmentation and loss of its habitat [7], estimating a total population under 200 individuals [3]. The conservation of this European large wild cat is of great concern to the European Union authorities who are given large amounts of funds for its conservation. The causes of 'non-natural' mortality (prompted by human intervention) of the Iberian lynx have been thoroughly studied during the past decades [8], but there are no references of mortality by diseases or necropsy findings. Little is known about diseases and pathology that affect the Iberian lynx; there are only a few publications about tuberculosis [9–11] and parasites [12–14] in the Iberian lynx, and there are no previous reports concerning histopathological findings. The aim of this study was to evaluate the histopathological status of the peripheral lymphoid tissues and thymus of Iberian lynxes necropsied between 1998 and 2003 in relation to other pathologies found in the animals.

2. Materials and methods

2.1. Animals and samples

Tissue samples fixed in neutral 10% formalin of multiple organs from Iberian lynxes necropsied, found dead or that died in captivity between 1998 and 2003, were sent for

Table 1
Severity of lymphoid depletion/animal; relevant histological findings

Case no.	Iberian lynx name	Sex ^a	Age ^b	Origin ^c	Free	Cause of death	Relevant histological findings ^d	FIV, FeLV, FcoV, FPV, others ^e	Lymphoid depletion (H&E)
1	Elsa	F	Young (11 m)	D	Yes	Car accident	Unknown (autolysis)		Autolysis
2	Guiness	M	Young adult (2 y)	D	Yes	Disease	Unknown (autolysis)	FIP (PCR +)	Autolysis
3	Clara	F	Young adult (2–3 y)	D	Yes	Car accident	Traumatic lesions	Chlamydia (PCR +)	No
4	Romana	F	Young adult (2–3 y)	D	Yes	Car accident	Traumatic lesions		Mild
5	Pitu	F	Young adult (13 m)	D	Yes	Car accident	No		Mild
6	Ángeles	F	Adult (6–7 y)	D	No	Disease	Chronic pneumonia Septicemia In situ squamouscell carcinoma (skin)		Mild
7	Rada	M	Young adult (16 m)	D	Yes	Car accident	No		Mild
8	Zoe	M	Young adult (17 m)	D	Yes	Car accident/ disease	Traumatic lesions Chronic pneumonia Chronic cystitis		Mild
9	Villar	M	Young adult (20 m)	SM	Yes	Trap	Pulmonary thrombosis		Mild
10	Moralejo	M	Young (11 m)	D	Yes	Car accident	Fatty liver Traumatic lesions		Mild
11	Isabel	F	Aged (13 y)	D	No	Disease	Pulmonary tuberculosis Squamous cell carcinoma (skin) with pulmonary metastases	<i>M. bovis</i> (PCR +, IHC +)	Moderate Moderate
12	Celia	F	Aged (12 y)	D	No	Disease	Squamous cell carcinoma (skin)		Moderate

(continued on next page)

Table 1 (continued)

Case no.	Iberian lynx name	Sex ^a	Age ^b	Origin ^c	Free	Cause of death	Relevant histological findings ^d	FIV, FeLV, FCoV, FPV, others ^e	Lymphoid depletion (H&E)
							In situ transitional cell carcinoma (urinary bladder)		
13	Hinojo	M	Adult (3–4 y)	D	Yes	Car accident	Traumatic lesions		Moderate
14	Niña	F	Young adult (1–2 y)	D	Yes	Car accident	Traumatic lesions		Moderate
15	Justo	M	Young (11 m)	D	Yes	Disease	Interstitial bronchopneumonia (calicivirus)	FIP (PCR+)	Moderate
							No FIP lesions	Calicivirus (IHC+, IF-, PCR-) Distemper (IHC-) Toxopl (PCR+)	
16	Pablo	M	Aged (16–17 y)	D	Yes	Quarrel/disease	Generalized tuberculosis Interstitial bronchopneumonia (Chlamydia)	<i>M. bovis</i> (PCR+, IHC+) Chlamydia (PCR+)	Severe
								Toxopl (PCR+)	
17	Fermín	M	Young adult (2 y)	SM	No	Disease	Generalized tuberculosis	<i>M. bovis</i> (PCR+, IHC+) Chlamydia (PCR-, IF+)	Severe

^a Sex: F, female; M, male.

^b Age: m, months; y, years.

^c Origin: D, Doñana; SM, Sierra Morena.

^d Parasitic lesions are not included.

^e FIV, feline immunodeficient virus; FeLV, feline leukaemia virus; FCoV, feline coronavirus; FPV, feline panleukopenia virus; PCR, polymerase chain reaction; IHC, immunohistochemistry; IF, indirect immunofluorescence. Only positive results are shown.

histopathological diagnosis to the Service of Veterinary Pathology (Veterinary School of Madrid), together with the macroscopic necropsy findings. This made a total of 17 very different animals including females ($n=8$) and males ($n=9$), age range from 10 months to 16 years, wild ($n=14$) and captive ($n=3$), from different populations (15 from the Doñana population and two from the Sierra Morena population), and different causes of death (mainly trauma, $n=11$, caused by road accidents, traps...) (Table 1). The presence of

infectious agents in the dead animals was investigated from tissue samples or carcass fluids, whenever possible, by PCR or serology depending on the availability of the samples. The positive results and the corresponding method of detection are displayed on Table 1.

2.2. Histopathology and immunohistochemistry

Formalin tissue samples were paraffin embedded, cut in 4 µm sections, deparaffined and H&E stained. A complete histopathological study was performed in each case. Ziehl–Neelsen technique for acid-fast bacilli and streptavidin–biotin–peroxidase complex anti-*Mycobacterium tuberculosis* (mouse monoclonal primary antibody, reacts with other members of *M. tuberculosis* complex, i.e. *Mycobacterium bovis*, Novocastra NCL-MT, 1:40, incubation overnight 4 °C; secondary antibody) were performed in the tuberculosis granulomas to locate the bacilli ‘in situ’. The lymphoid tissues histologically evaluated included spleen, lymph nodes (at least three per animal; lymph nodes without granulomas were studied to establish cellular depletion in the cases with tuberculosis), MALT (respiratory and intestinal mucosa-associated lymphoid tissue) and thymus (three samples of animals aged 16, 17 months and 2 years old). Thymus involution was evaluated according to histological criteria published for the domestic cat [15].

The severity of lymphoid depletion/animal (LD) was categorized in none, mild, moderate and severe considering two variables: (a) number and type of lymphoid tissues affected and (b) grade of lymphoid depletion/tissue: normal, mild, moderate and severe (Table 2). Immunohistochemistry (streptavidin–biotin complex peroxidase method) was carried out on deparaffined sections after a high temperature antigen unmasking protocol (in a pressure cooker, sections were immersed in citrate buffer pH 6.0 and boiled for 2 min). The primary antibodies used were: rabbit polyclonal antibody anti-CD3, pan-T cell marker (DAKO A452, dilution 1/100, incubation overnight 4 °C); mouse monoclonal antibody anti-CD79, mature B cells marker (clone HM57, DAKO M7051, dilution 1/10, incubation overnight 4 °C); mouse monoclonal antibody clone MAC 387 myeloid/histiocyte antigen (DAKO M0747, dilution 1/200, incubation overnight 4 °C); and mouse monoclonal antibody anti-CD68 macrophage

Table 2
Scoring system of lymphoid depletion (H&E)

Severity of lymphoid depletion/animal	Spleen ^a	LN _a , LN _b , LN _c ^b	MALT(R), MALT(I) ^c
None	0	0,1	0,1
Mild	1	0,1,2	0,1,2
Moderate	2	1,2,3	1,2,3
Severe	3	2,3	2,3

Tissue depletion was categorized in: 0=none, 1=mild, 2=moderate and 3=severe.

^a Grade of lymphoid depletion/animal was based mainly on the splenic depletion.

^b LN, lymph nodes; the median of the tissue depletion scores of the three lymph nodes studied (a, b, c) was considered.

^c MALT(R), respiratory mucosa-associated lymphoid tissues; MALT(I), intestinal mucosa-associated lymphoid tissues; when depletion was different in both tissues, the less altered one was chosen for the total scoring. Lymph nodes and MALT tissues were considered only if regional pathologies were ruled out.

(clone PG-M1) (DAKO M0876, dilution 1/20, incubation overnight 4 °C). *M. tuberculosis*, CD79, MAC387 and CD68 sections were subsequently incubated with anti-mouse biotinylated secondary antibody (Dako E04233, dilution 1:200, 30 min at room temperature). CD3 sections were subsequently incubated with anti-rabbit biotinylated secondary antibody (Vector Laboratories BA1000, 1:400, 30 min at room temperature). Next, all the slides were incubated with streptavidin conjugated with peroxidase (Zymed P50242, 1:400, 30 min, at room temperature). All washes and dilutions were made with Tris-buffered-saline (TBS) (pH 7.4). The slides were developed with a chromogen solution containing 3-3' diaminobenzidine tetrachloride (Sigma Chemical Co. D5059) and H₂O₂ in distilled water and counterstained in hematoxylin (Sigma GH5-2-16). Negative control slides were used by substituting the primary antibody by TBS. Evaluation of the leucocyte populations labeled with each antibody was made by two observers simultaneously and scored as increased, normal, mild decreased, moderately decreased and severely decreased. For immunohistochemical validation of the antibodies to the Iberian lynx tissues, healthy domestic cat tissues, with known reactivity for the antibodies employed, were used as positive controls.

3. Results

The grade of lymphoid depletion/tissue (normal, mild, moderate and severe) and the severity of lymphoid depletion/animal (LD) (none, mild, moderate and severe) on H&E slides are depicted in Tables 1 and 3. The spleen was the lymphoid organ in which the cellular depletion (number of follicles and cellular density) was more evident and frequent (Table 3). It was not possible to carry out the histological evaluation due to autolysis in two cases. One case did not show immune depletion with H&E and the rest of the animals studied presented some degree of lymphoid depletion (7 mild, 5 moderate and 2 severe). The grade of LD per animal did not depend on the origin of the animal, whether it was free or captive, or the cause of death, and it increased with age: mild LD was found mainly in young or young-adult animals while moderate and severe LD was more frequent in aged animals. LD was also severe in two Iberian lynxes suffering tuberculosis (Cases 16 and 17). Hyaline substance deposition was commonly observed in lymphoid follicles with evident lymphoid depletion (Fig. 1). Besides traumatic lesions, the most interesting histopathological findings were pneumonia, epithelial neoplasms and tuberculosis (Table 1). Five individuals (Cases 6, 11, 12, 16 and 17) presented neoplasia (squamous cell carcinoma of the skin and urinary bladder 'in situ' carcinoma) and/or tuberculosis (*M. bovis*, PCR). In one of the

Table 3
Grade of lymphoid depletion/tissue (H&E)

	Spleen	Lymph nodes	MALT ^a
Normal (%)	17.1	27.3	37.5
Mild (%)	42.7	27.3	37.5
Moderate (%)	35.7	27.3	25.0
Severe (%)	14.3	18.2	0

^a MALT, mucosa-associated lymphoid tissue.

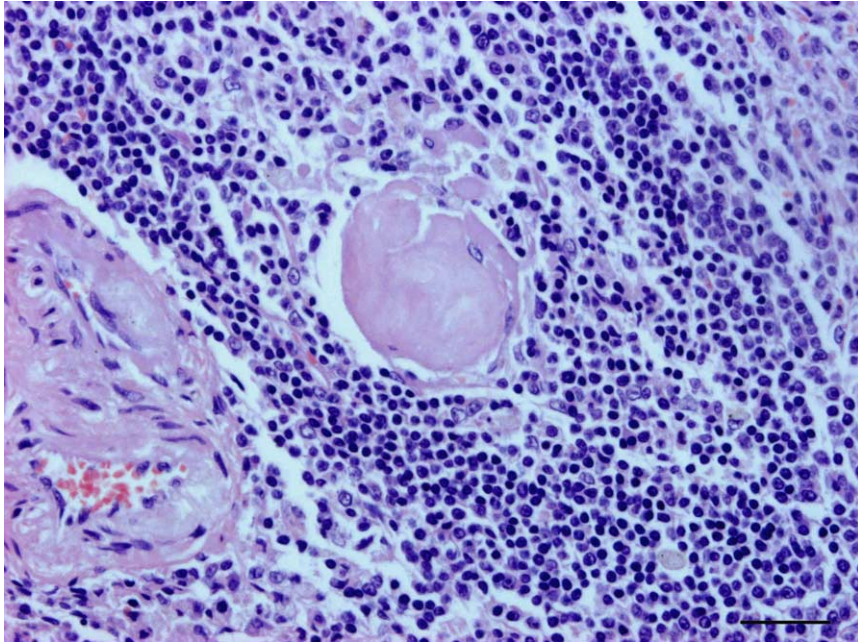


Fig. 1. Iberian lynx 'Pitu'; spleen H&E. Hyaline substance deposition in lymphoid follicle. Bar=65 μ m.

cases with tuberculosis (Case 11), the granulomas were only found in the lung (tuberculous primary complex); in another case with disseminated tuberculosis (Case 16) the main tuberculosis lesions were found in liver and lymph nodes, although small granulomas were also observed in the lung; another animal with a history of blindness (since 3 months of age) with generalized tuberculosis (Case 17), granulomas of tuberculosis were found in lung, liver, lymph nodes, both adrenal glands, and in both ocular globes (bilateral tuberculous granulomatous uveitis). In all granulomas acid-fast bacilli were histologically identified by the Ziehl–Neelsen technique and immunohistochemistry. Multinucleated cells (2–5 nuclei) were observed in the granulomas.

No evidence of feline immunosuppressive virus infection (feline immunodeficiency virus, feline leukaemia virus, feline coronavirus and feline panleukopenia virus) was found in any of the individuals.

CD3, CD79, MAC387 and CD68 immunohistochemistry is represented in Fig. 2. CD3 immunostaining was seen labeling cells in T areas (periarteriolar lymphoid sheets and marginal zones in splenic white pulp and parafollicular areas in lymph nodes and MALT) (Fig. 3); CD79 positive cells were found in B areas (lymphoid follicles) (Fig. 4a and b). MAC387 stained large macrophages mainly located in splenic red pulp, medular lymph node and MALT (Fig. 4c); CD68 labeled scarce macrophages, dendritic cells and follicular dendritic cells (Fig. 4d). The spleen was the organ with less immunostaining of the cellular subpopulations analyzed. Comparing the results among the leukocyte subpopulations studied (Fig. 2), T cells (CD3-positive cells) were seriously decreased in

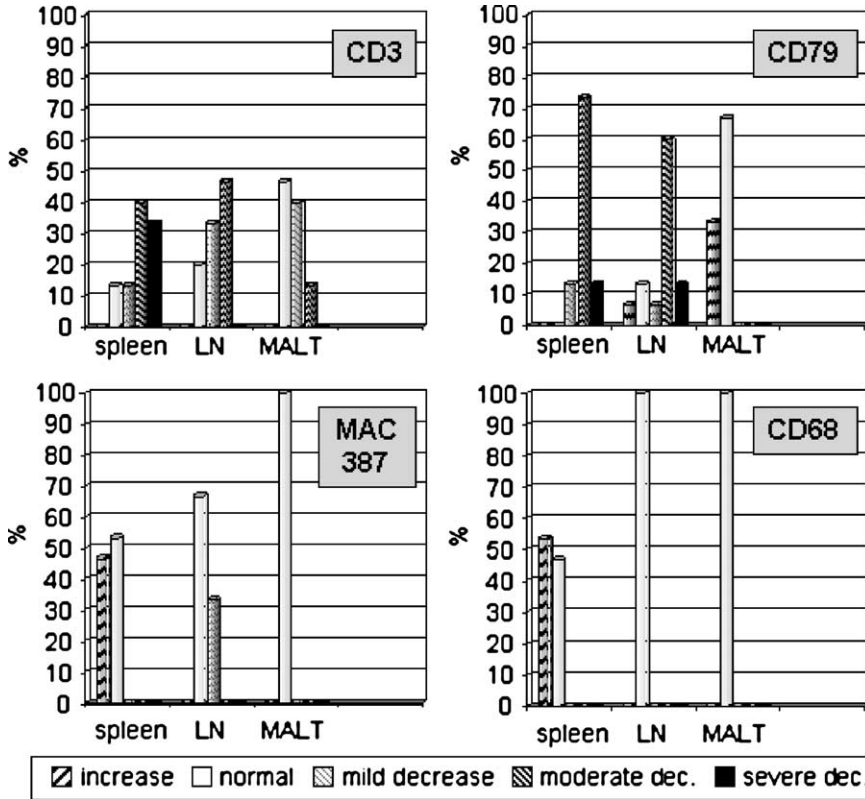


Fig. 2. Immunohistochemistry of CD3, CD79, MAC387 and CD68. Columns indicate the percentage of lymphoid tissues with immunostained subpopulations. LN, lymph node; MALT, mucosa associated lymphoid tissue.

most of the tissues analyzed, being the spleen the tissue with the most striking reduction; B cells were decreased in many cases in the spleen and lymph nodes but normal or increased in MALT; In many animals, coexisting reductions in both B (CD79) and T (CD3) cell subpopulations was observed in the spleen (13/15, 86.6%) and lymph nodes (12/15, 80.0%). Levels of macrophages and dendritic cells (positive cells to MAC387 and CD68, respectively) were normal in MALT samples; MAC387-positive cells were mildly reduced in some lymph nodes (5/15, 33.3% of the cases); splenic MAC387 and CD68 positive subpopulations were normal (MAC387 8/15, 53.3%; CD68 6/15, 46.6%) or increased (MAC387 7/15, 46.6%; CD68 8/15, 53.3%).

Histologically, the 2-year-old Iberian lynx thymus sample was involuted with abundant interlobular adipose tissue and low cellularity of lymphoid tissue, therefore, it was not included in the immunohistochemical study. The other two samples of thymus (from Iberian lynxes aged 16 and 17 months) were mildly involuted with defined thymic lobules and medium cellularity. In these two cases, there were mild and severe reductions of CD3 positive subpopulations.

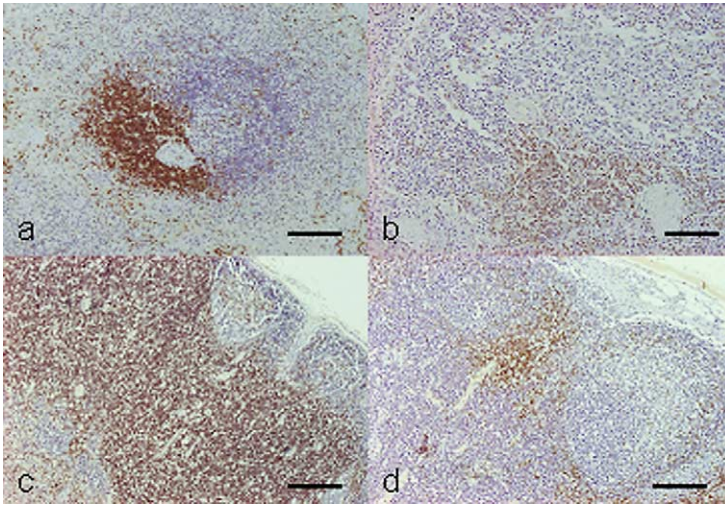


Fig. 3. Streptavidin–biotin–peroxidase complex anti-CD3. (a) Control spleen; bar=700 μ m. (b) Iberian lynx 'Hinojo' spleen; severe decrease of CD3 cells; bar=280 μ m. (c) Control lymph node; bar=700 μ m. (d) Iberian lynx 'Pitu' lymph node; moderate decrease; bar=280 μ m.

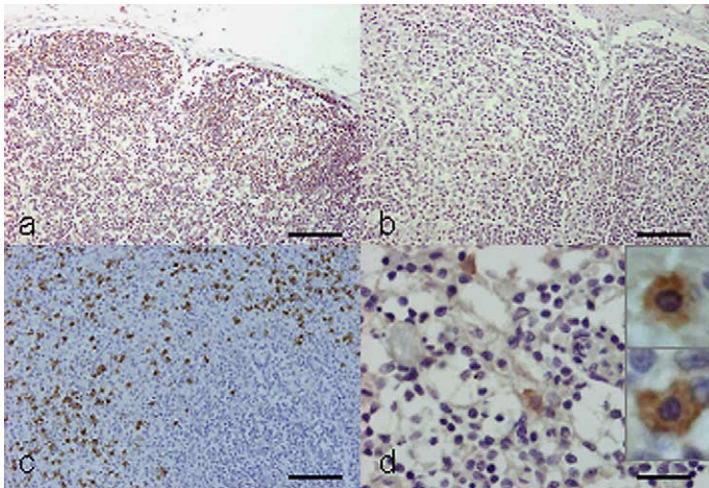


Fig. 4. (a) Immunohistochemistry anti-CD79; control lymph node; bar=280 μ m. (b) Immunohistochemistry anti-CD79; Iberian lynx 'Pitu'; lymph node; bar=280 μ m. (c) Immunohistochemistry MAC387; Iberian lynx 'Rada'; spleen; bar=280 μ m. (d) Immunohistochemistry anti-CD68, control; positive dendritic cells; bar=70 μ m. Upper right: Iberian lynx 'Pitu'; splenic macrophage CD-68 positive. Lower right: Iberian lynx 'Rada'; thymic macrophage CD-68 positive.

4. Discussion

This is a descriptive study in which reductions of immune cells were found by histopathology (H&E) and immunohistochemistry in peripheral lymphoid tissues of 15 Iberian lynxes. This is a very representative population considering that the total estimated population of this species left is of 200 animals. The leukocyte subpopulations detected by immunohistochemical methods was restricted to T lymphocytes, B lymphocytes and macrophages, since all the samples available for the study were formalin-fixed and paraffin embedded. Antibodies anti-human CD3, human MAC387, human CD79 and human CD68 (clone PG-M1, clone EBM11) have been tested in formalin-fixed tissues of domestic species, including the domestic cat [15–18]. According to our results, the antibodies used also show cross-reaction with lynx tissues. To our knowledge, this is the first report of immune detection of leukocyte subpopulations in wild felids.

In most animals, the lymphoid depletion observed with H&E had parallel decreases in CD3 and CD79 positive cells which indicate a partial combined immunodeficiency affecting T and B cells subpopulations. Spleen was the lymphoid organ with more evident hypocellularity and decreases of CD3 and CD79 populations (T and B lymphocytes). B cells were decreased in many cases in the spleen and lymph nodes but normal or increased in MALT. Minor reduction in T and B subpopulations, together with increases of macrophages (MAC387 and CD68 positive-cells) in lymph nodes and MALT samples could have been influenced by local pathologies (i.e. intestinal parasitic infestations in mesenteric lymph nodes and intestinal MALT). The increased number of macrophages and dendritic cells (MAC387 and CD68 positive cells) found in the splenic red pulp and lymph node medulla of tissues with T and B cell reductions indicates a proliferation of these cell types that remains unexplained although it could represent a compensating situation. T and B cell depletion and increase macrophage proliferation has been indicated in lymphoid tissues of domestic cats with experimental feline infectious peritonitis (Feline coronavirus) [15]. Follicular hyalinosis in the center of depleted follicles is a common histopathological finding in domestic cats with panleukopenia (feline parvovirus, FPV) [19].

In our opinion, the histopathology and immunohistochemistry of the three samples of thymus analyzed in this study are not representative. To evaluate properly the cellularity of the thymus, it is necessary to know the normal thymic involution in the lynx, and there are no studies on this species. In our cases, times and histological characteristics of thymic involution had to be compared with data published in the domestic cat [15]. Nevertheless, the two animals with similar ages (16 and 17 months) were both categorized as mild involution but one showed mild and the other severe T-cell reduction indicating that, at least in the latter case, it was a true depletion of T-lymphocytes and not a normal thymic involution.

Iberian lynxes included in the study presented generalized immune depletion, which coexisted with high prevalence of neoplasms and tuberculosis (*M. bovis*). It is known that devastating diseases (late generalization of tuberculosis) and metastatic tumors can cause depression of the immune system [19]. In our opinion, the presentation of these neoplastic and infectious diseases were promoted by the defects in the immune system although the alternative hypothesis is equally valid. The coexistence in the same animal of pulmonary tuberculosis and squamous cells carcinoma of the skin and, in another case, the coexistence of two different epithelial carcinomas (skin and urinary transitional epithelium), could be

coincidental, but also could be the result of an immunodeficiency status. To our knowledge this is the first report of any kind of tumor in the Iberian lynx. There are several publications describing tuberculosis in the Iberian lynx [9–11]. In two of our three cases, the primary pulmonary lesions indicate that the contamination was probably by inhalation from contaminated food as it has been indicated in a previous case of tuberculosis in the Iberian lynx and in other wild felids [10,20]. In the remaining case, the distribution and size of the lesions do not confirm an inhalation route of contamination and suggest a digestive origin of the tuberculosis. Multinucleated cells (2–5 nuclei) observed in the granulomas did not display the typical aspect of Langhans' cells as in other species infected with *M. bovis* [19]. The presence of multinucleated cells in tuberculosis of Iberian lynx have not been previously indicated.

It is interesting to note that one animal with disseminated tuberculosis (Case 17) presented granulomas in both adrenal glands and in both ocular globes. In the previous report about the pathology of one Iberian lynx with tuberculosis [10], both adrenal glands were also affected. This fact could indicate a special predisposition of these animals to have adrenal tuberculosis, since adrenal glands are not a common site for tuberculosis lesions in other animal species [19]. Ocular tuberculosis is an uncommon but documented finding in the domestic cat [21] that has never been referred to in wild felids and could be also a consequence of an immune deficiency status. In our case, the early appearance of blindness (3-month-old) seems to indicate an ocular primoinfection rather than a dissemination, possibly due to contact with contaminated food. Nevertheless, the high prevalence of tuberculosis in our series of cases can be also influenced by the high prevalence of the disease in wild and domestic ungulates living in the same areas [11].

By far, the information available does not allow to establish the most likely cause of the immune depletion. All the animals were negative to feline immunosuppressive viruses (FeLV, FIV, FCoV, FPV) using conventional techniques for the domestic cat, which do not exclude the possibility of infection with lynx-specific viral strains or unknown viruses. In fact, the histopathological findings of the present study are coincident with those found in cats infected with immunosuppressive viruses, specially in non-acute diseases or carrier stages [19,22]. Stress should also be taken into account as a possible origin of the immune depletion but only in the captive animals ($n=4$). Finally, there are several reasons to consider a primary immune system defect due to the inbreeding of the Iberian lynx. Evidences for reduced genetic variability in the Iberian lynx, due to the low number of individuals separated in two small subpopulations, have been recently published [7].

The results of our study indicate that all animals presented some degree of immune depletion, affecting both B and T cells, which could only be explained in a few cases by old age and/or concomitant diseases (tuberculosis, tumors). Prospective investigations are necessary to assess the clinical immune system parameters (serum concentrations of IgA, IgG, IgM; in vitro lymphocyte blastogenesis with mitogens; flow cytometric quantification of blood subpopulations of leukocytes) in appropriate samples of living Iberian lynxes to know (a) if the histological cellular depletion found in this series of cases is present in the left population of Iberian lynxes; (b) if there are important clinical consequences and (c) the possible existence of a viral infection non-detected by conventional laboratorial techniques. The free status of the animals and therefore delicate management of this species makes it difficult to carry out such studies; in any case, sampling of living animals

requires capture and anesthesia. Our preliminary results of an ongoing prospective study indicate the presence of leukopenia and lymphopenia in some of the animals.

The reduction of the effectiveness of the immune system could increase the state of vulnerability in the critically endangered Iberian lynx to pathogens. According to our results, the Iberian lynx could be in serious risk of extinction not only due to known causes (habitat destruction, road accidents, traps...) but also to its immune vulnerability.

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