Histopathology lesions in red deer (*Cervus elaphus*) from Idanha-a-Nova and Lousã (Portugal): preliminary results

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Abstract

The red deer (*Cervus elaphus*) is a wild ungulate and game species with a broad distribution in Portugal. In Idanha-a-Nova (Castelo Branco) there is a particular interest in studying these populations due to the importance of the hunting industry to the regional economy, and the recognised role of ungulates as reservoirs of infectious agents to livestock and humans. This histopathology survey aimed to compare deer lesions from Idanha-a-Nova (*n*=16) and Lousã (*n*=4). Samples from the lung, liver and kidney of animals were collected during the hunting season. The results reveal a high prevalence of parasitic bronchitis (13/20; 65%), especially in Idanha-a-Nova (11/16; 68.8%). Moreover, many animals presented non-purulent nephritis (8/20; 40%). No statistically significant differences were found between regions or genders for any lesion. This survey provides an overview of the health status of the red deer populations in these regions. Further studies are crucial to better know the sanitary situation of these populations and under the One Health perspective, to apply protective measures for animal, hunter and consumer health.

Key words: *red deer; lungworm; congestion; non-purulent nephritis; histopathology; wildlife*

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Introduction

Red deer (*Cervus elaphus*) is a wild ungulate species with a broad distribution in Europe, including the Iberian Peninsula (Mattioli et al., 2022). *C. elaphus* is a potential carrier of several zoonotic pathogens, including *Mycobacterium bovis* (Vieira-Pinto et al., 2011), *Brucella abortus* and *B. melitensis* (Serrano et al., 2011), Shiga toxin-producing *Escherichia coli* (Lauzi et al. 2022), *Coxiella burnetti* (González-Barrio et al., 2015), and *Leptospira* spp. (Flint et al., 1986), to mention a few.

As a game species, populations of red deer (as well as wild boar, roe deer, and fallow deer) are in direct contact with hunters, and wild species meat consumers. Moreover, they share pasture, land and water resources with domestic animals, serving as potential reservoirs of important pathogens, including zoonotic infectious agents. In fact, the consumption of different types of wild meat and its role in zoonotic spillover are now gaining new attention worldwide, due to the recent emergence and re-emergence of zoonotic pathogens (Milbank and Vira, 2022). Notwithstanding, hunting and game meat consumption are a significant part of the natural resources and economy of most European countries (Fantechi et al., 2022), and therefore, wild populations should be carefully monitored for different health hazards and be a part of disease control programmes to avoid potential health consequences.

Idanha-a-Nova (Castelo Branco district, central-East Portugal) is considered a geographical area of concern for wild ungulate management, especially regarding certain infectious diseases such as tuberculosis. The hunting industry is an integrated part of the economy of this region, as well as livestock breeding. However, bovine tuberculosis has been on the rise in recent years, with clear evidence supporting the role of wild ungulates as reservoirs, compromising effective disease control and eradication, as has been the case in other European countries (Santos et al., 2015; Vieira-Pinto et al., 2011). In contrast, Serra da Lousã (Coimbra district, central Portugal) is mostly a naturally protected area with an excessive density of red deer, which is compromising the natural vegetation of the forest and agriculture in some areas (Monzón et al., 2012).

Wildlife histopathology is an integrated part of wildlife population health monitoring. Histopathology surveys have given meaningful contributions to our knowledge about wildlife diseases, and provided a first perspective of the state of health of wild populations (World Organisation for Animal Health (WOAH) 2015; McNamara 2016; Larenas-Muñoz et al. 2022).

This study aimed to present lesions observed on the lung, liver, and kidney of hunted red deer from two ecologically and epidemiologically distinct areas (Idanha-a-Nova and Lousã).

Materials and methods

Sampling

A total of 20 adult deer (3-6 years old) culled during August and September 2021 in Portugal were included in this study: 16 were from Idanha-a-Nova (Castelo Branco) and 4 were from Lousã (Coimbra). Eighteen were males and two were females. No criteria were applied to animal selection. Approximately 20 g of liver, kidney, and lung were kindly provided by hunters. All samples were collected right after the shot from fresh carcasses to avoid autolytic post-mortem

alteration, and were fixed in 10% neutral buffered formalin. No animals were killed specifically for the purposes of this study, and therefore no ethics approval was required for this study. All animals were killed according to Portuguese hunting legislation.

Histopathology analysis

Deer samples (lung, liver and kidney) were analysed in the Histopathology Laboratory at the University of Trás-os-Montes and Alto Douro (UTAD, Portugal), and processed for light microscopy using routine histological technique. Slides were stained with haematoxylin and eosin, and specific stains (Giemsa, Gram, Ziehl Neelsen stains) where needed. Histopathology slides were observed under a blind test using an optical microscope (Nikon E600®, Nikon Instruments Inc., Melville, NY, USA).

Statistical Analysis

SPSS® Statistics version 27.0 was used for descriptive statistics and statistical inference analysis. Fisher's Exact test was used for qualitative data analysis. A confidence interval of 95% was considered (p-critical value = 0.05).

Results

The summary of the diagnosed lesions is presented in Table 1.

Lung

More than half of the analysed deer showed verminous bronchitis (13/20; 65%), characterised by catarrhal inflam-

Table 1. Frequency of positive cases for each histopathologic lesion from each location (Idanha-a-Nova and Lousã) and the total.

Lesions	Idanha-a-nova (*n*=16) *n* (%)	Lousã (*n*=4) *n* (%)	Total (*n*=20) *n* (%)
Lung			
Verminous bronchitis	11 (68.8%)	2 (50%)	13 (65%)
Haemorrhage	3 (18.8%)	2 (50%)	5 (20%)
Congestion	1 (6.3%)	0	1 (5%)
Atelectasis	1 (6.3%)	0	1 (5%)
Liver			
Congestion	7 (43.8%)	2 (50%)	9 (45%)
Vacuolar change	4 (25%)	1 (25%)	5 (20%)
Biliary hyperplasia	1 (6.3%)	2 (50%)	3 (15%)
Portal eosinophils infiltration	1 (6.3%)	0	1 (5%)
Kidney			
Non-purulent nephritis (subacute; chronic)	6 (37.5%)	2 (50%)	8 (40%)
Congestion	3 (18.8%)	0	3 (15%)

Note: Some samples presented more than one lesion, while others did not show any lesions.

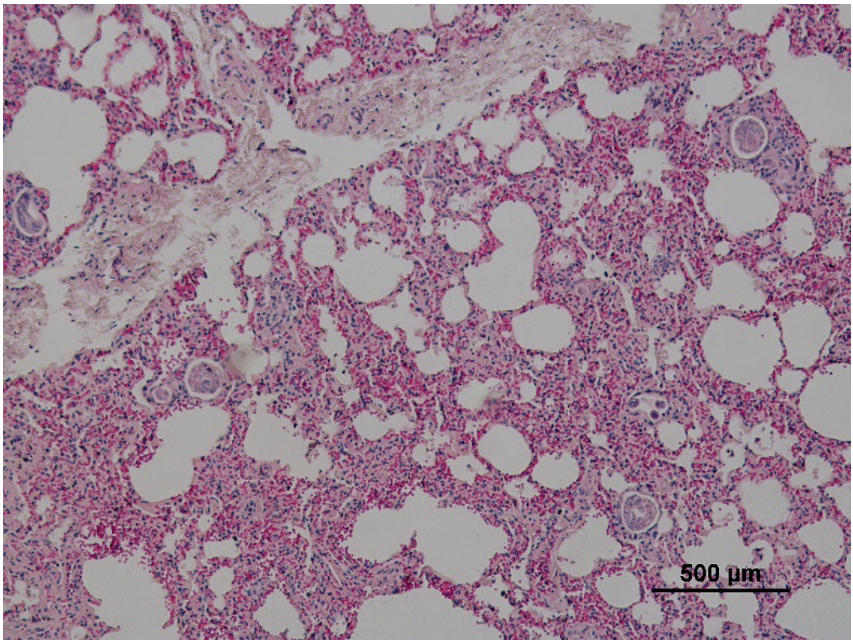

Figure 1. Verminous pneumonia in a deer (10x; 4x)

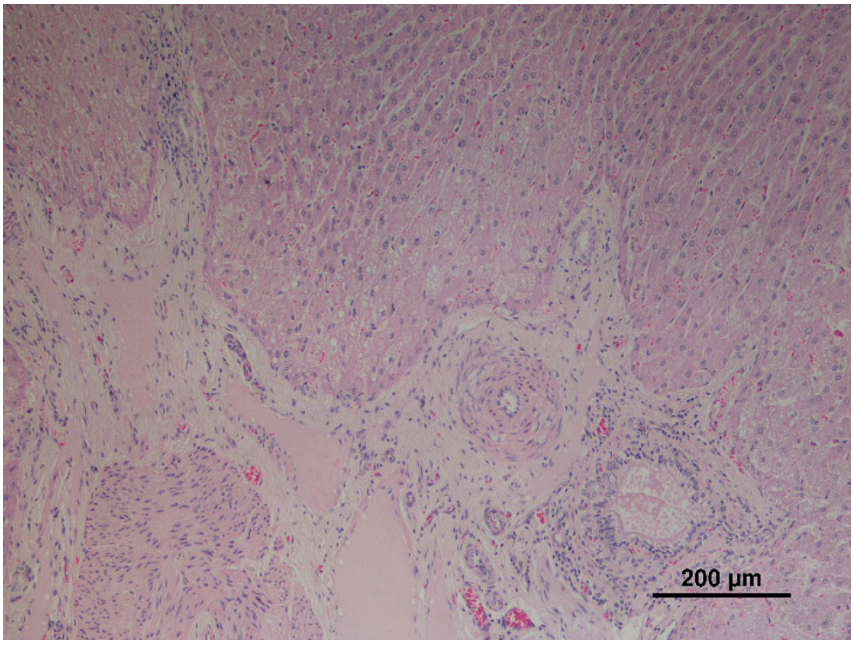

Figure 2. Liver cells presenting vacuolar change and biliary hyperplasia (10 x; 20 x)

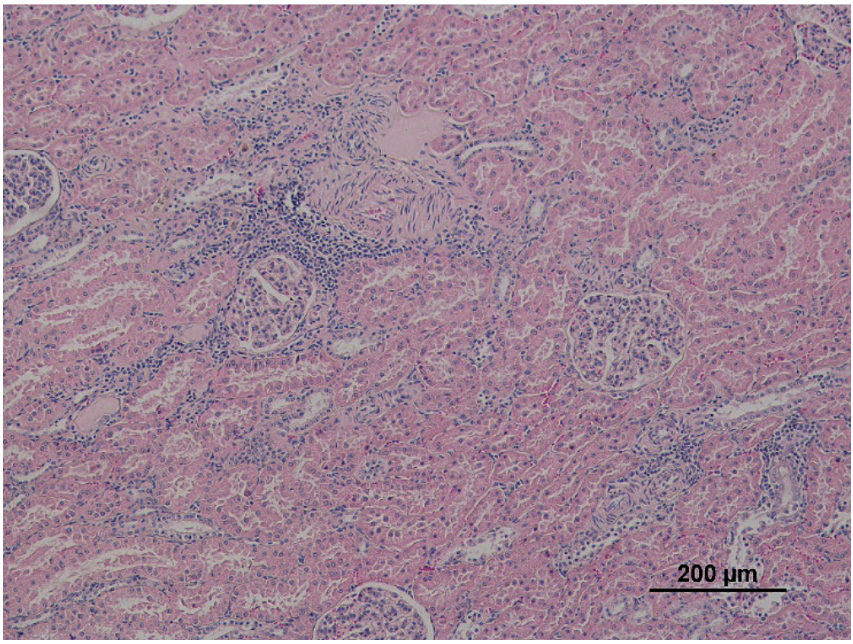

Figure 3. Non-purulent nephritis in a deer (10 x; 10 x)

mation, with the presence of parasite specimens in bronchial and bronchiolar lumens, with varying degrees of lymphoid hyperplasia (Fig. 1). The percentage of infected animals was higher from Idanha-a-Nova than Lousã, although this difference was not statistically significant (P=0.587). Similarly, no significant differences were found between the sexes (P=0.521).

In 20% of animals, haemorrhage was detected. Congestion and atelectasis were also detected. All analysed animals showed some pathological changes in the pulmonary tissue, making the lung the most affected analysed organ. No statistically significant differences were found between sexes or geographical locations for these alterations.

Liver

Congestion was present in nine cases (40%), seven from Idanha-a-Nova (43.8%) and two from Lousã (50%). Cellular hepatic alterations (specifically vacuolar change) were also present in 20% of all deer (Fig. 2). More rarely, deer showed biliary hyperplasia (3/20; 15%) and eosinophils in the parenchyma (1/20; 5%). Eight deer did not present any liver change. None of the alterations presented statistically significant differences between the sexes or geographical locations.

Kidney

The most frequent change was non-purulent nephritis (Fig. 3), diagnosed in eight animals (49%), six from Idanha-a-Nova (37.5%), and two from Lousã (50%). A less common alteration was congestion (15%). None of these alterations presented statistically significant differences by sex or geographical location. Four deer did not show any lesions in the kidney.

Discussion

In this study, the lung, kidney, and liver of 20 red deer killed during August and September 2021 in Idanha-a-Nova (*n*=16) and Lousã (*n*=4) were analysed.

The lung was the most affected organ, with a high prevalence of verminous bronchitis (13/20; 65%), especially in Idanha-a-Nova (11/16; 68.8%). Several lungworms may affect red deer, such as *Dictyocaulus viviparous*, *Protostrongylus* spp., *Orthostrongylus macrotis* and *Varestongylus* spp. (Howerth et al., 2018). Parasitology morphology and molecular analysis would be needed to confirm which species are involved in these pathologic lesions. As with other pathogens, high densities of deer in some regions contribute to high amounts of parasites (including lungworms) and the presence of parasitic bronchitis (Pichon et al., 1999; Page-Karjian et al., 2020). Deer have been reported to be potential reservoirs of lung parasites for domestic species in some regions of contact between wildlife and domestic species (Gortázar et al., 2007). Therefore, further research must be conducted to verify this phenomenon in these locations, specially Idanha-a-Nova, considering the higher detected prevalence of parasitic lesions and the more intense livestock industry in the area, compared to Lousã.

Cellular changes (particularly vacuolar change) (5/20; 20%) and congestion (9/20; 45%) were observed in the liver. These alterations are nonspecific. Good nutrition (associated with glycogen storage) may explain the observed cellular changes (Pérez-García et al. 2021) and congestion likely suggests that the corpse was not properly bled. Stress, infectious agents and xenobiotics (such as metals and persistent organic pollutants) are among the possible causes of this type of lesion in multiple organs, including the liver (Howerth et al., 2018; Page-Karjian et al., 2020). Biliary hyperplasia (3/20; 15%) also has multiple possible causes including different toxic substances (Haschek et al., 2010; Maronpont 2022) or trematode infestation, such as by *Fasciola hepatica*, *Dicrocoelium dendriticum* or *Fascioloides magna* (Craig, 2009). Fluke and nematode infections are also related to eosinophilic infiltrates, also found in one of the analysed deer (1/20; 5%). Nevertheless, no parasitic forms were identified in the liver parenchyma during the histopathology analysis. Further research, including biochemical, microbiological, toxicological and coprological exams, is needed to clarify this issue.

Non-purulent nephritis was diagnosed in more than one-third of deer (8; 40%). This can have distinct origins, including an infectious agent or contact with substantial doses of toxic substances from the environment. Several cervid species (including red deer) are susceptible to the malignant catarrhal fever (MCF) virus, and this pathogen may be responsible for foci of acute nephritis. The disease was first reported in domestic herbivores in Portugal in 2001(Cortez et al., 2001). Other viruses that may induce kidney damage include different orbiviruses, such as bluetongue viruses (BTV) and epizootic haemorrhagic diseases viruses (EHDV) (Howerth et al., 2018; Navas-Suárez et al., 2018). Both diseases have been registered in Spain and the potential of red deer as sentinels and maintenance hosts of BTV has been studied (Rodríguez-Sánchez et al., 2010; Freath et al., 2022). Regarding bacteria, several zoonotic pathogens need to be considered as possible causes, including *Leptospira* spp., *Clostridium* spp. or, more rarely, *Brucella* spp. (Ayanegui-Alcerreca et al., 2011; Andreoli et al., 2014; Howerth et al., 2018; Navas-Suárez et al., 2018). The correlation between the presence of *Leptospira* spp. in wild populations (as reservoirs) and occasional disease outbreaks in humans

has previously been proven, especially in disaster climatic events (Michna and Campbell, 1970; Storck et al., 2008). Thus, and also considering its pathogenicity, testing these animals for *Leptospira* spp. (at least) would be relevant. Infections by the filarial worm *Elaeophora elaphi* can also occasionally originate in interstitial nephritis (Carrasco et al., 1995). This parasite was previously reported in red deer from Spain (Santín-Durán et al., 2000).

Further research and other laboratory analysis (including microbiological cultures, molecular analysis, or toxicological methods) are needed to find the real causes of the observed lesions. A more complete assessment allows a more detailed understanding of the health threats of red deer in Portugal, as well as its role as a reservoir of zoonotic pathogens for other wildlife species, livestock, and even humans. A complete wildlife pathology and histopathology survey provides knowledge on a population's health status and allows a comparison between animal populations (and locations) with distinct characteristics (McNamara, 2016). However, it undeniably presents a limited capacity to provide definitive diagnoses and draw definitive conclusions, especially when just some tissues are available for analysis. Possibly, by increasing the number of sampled animals (especially in Lousã), more accurate conclusions could be taken to examine whether there are significant differences between the sexes or regions (even though animals may change habitats and migrate between the two locations). Notwithstanding, this study may provide a first orientation to design future monitoring assessments of deer populations.

Conclusions

Overall, verminous bronchitis was the most common lesion, present in more than half of samples, followed by hepatic congestion and non-purulent nephritis. No significant differences were found between the sexes or geographical locations.

Some of these lesions may have zoonotic agents with public health relevance (namely *Leptospira* spp., *Brucella* spp. or fluke worms). Therefore, hunters, veterinarians, or consumers should adopt protective measures when contacting or consuming red deer. Collaboration between veterinarians and hunters to perform surveys and surveillance programmes should be encouraged to improve the general knowledge on this subject.

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Patohistološke lezije u običnog jelena (*Cervus elaphus*) iz mjesta Idanha-a-Nova i Lousã (Portugal): preliminarni rezultati

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Obični je jelen (*Cervus elaphus*) divlji kopitar i vrsta divljači koja je vrlo raširena u Portugalu. U mjestu Idanha-a-Nova (Castelo Branco) postoji poseban interes za istraživanje ovih populacija uslijed važnosti lovne industrije za regionalnu ekonomiju, uz prepoznatu ulogu kopitara kao rezervoara uzročnika infekcija stoke i ljudi. Ovaj smo patohistološki pregled pisali s ciljem usporedbe lezije jelena iz mjesta Idanha-a-Nova (*n*=16) i Lousã (*n*=4). Tijekom sezone lova prikupljeni su uzorci iz pluća, jetre i bubrega svih životinja. Rezultati su otkrili veliku prevalenciju bronhitisa prouzročenog parazitima (13/20; 65 %), posebno u mjestu Idanha-a-Nova (11/16; 68,8 %), a brojne životinje su imale i negnojni nefritis (8/20; 40 %). Nisu otkrivene nikakve statistički značajne razlike između regija i spolova za bilo koju leziju. Ova studija daje pregled zdravstvenog statusa populacije običnog jelena u tim regijama. Dodatne studije su ključne za bolje poznavanje zdravstvenih prilika tih populacija, iz perspektive Jednog zdravlja, u svrhu poduzimanja mjera zaštite zdravlja potrošča, lovaca i životinja.

Ključne riječi: *obični jelen, plućni crv, kongestija, negnojni nefritis, patohistologija, divlje životinje*