

P104- RENAL LESIONS IN DEER (*Cervus elaphus*) *Mycobacterium avium* subsp. *paratuberculosis* INVOLVEMENT

¹Matos AC, ¹Figueira L, ¹Martins MH, ²Matos M, ³Pires MA, ¹Álvares S, ¹Mendes A, ¹Sousa N, ³Coelho A, ³Pinto ML

¹Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal

²Department of Genetics and Biotechnology, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

³Department of Veterinary Sciences and Centre for Studies on Agricultural and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

INTRODUCTION

Paratuberculosis (Johne's disease) is a chronic infectious disease affecting wild and domestic ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). The disease is prevalent worldwide and has a significant financial impact on those affected¹. In Europe, paratuberculosis infection has been reported in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*)², fallow deer (*Dama dama*)³, and other exotic species. An epidemiological study was initiated in response to concerns about the prevalence of paratuberculosis in free ranging red deer in order to discern its economic impact.

MATERIALS AND METHODS

This study comprised 877 free-ranging red deer legally hunted in the Idanha-a-Nova (39° 55' 11" North, 7° 14' 12" West) and Penamacor (40° 10' 8" North, 7° 10' 14" West) cities (in Castelo Branco; Centre-western Portugal) during the period 2009-2011. Animals that presented any visible gross lesion or animals that showed loss of weight or a rough coat, were subjected to multiple organ and tissue collection. Following this procedure, kidneys from thirty-seven red deer were examined for the presence of Map by culture, IS900 polymerase chain reaction (PCR) and histopathology. Samples also included intestine and associated lymph nodes in which the same analytical procedures were performed. Culture methodology was performed as described by Juste et al. (1991). DNA from kidneys and from bacteria isolated on kidney culture was extracted by using commercial kits (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany and UltraClean® Microbial DNA Isolation, MO BIO Laboratories, Inc., 92010 Carlsbad, California, respectively), according to the manufacturer's instructions. DNA from kidney samples and from bacteria were tested in duplicate for Map using the primers RJ1 (GTT CGG GGC CGT CGCTTA GG) and PT91 (CCC ACG TGA CCT CGC CTC CA) flanking a region of 389 bp were used for amplification of the IS900 sequence of Map and PCR conditions as reported before by Garrido et al. (2000). Tissue samples were fixed in a 10% neutral buffered formol-saline-solution by immersion and processed for histopathology using routine techniques for paraffin embedding. Sections were stained with haematoxylin and eosin and the Ziehl-Neelsen technique.

DISCUSSION AND CONCLUSION

Map in kidneys was previously reported in cows with advanced paratuberculosis⁶, however it was not referred in wild deer. Map has been previously isolated from hepatic lymph nodes and spleen in deer⁷, but our results show that renal lesions may also be associated to Map infection. As far of our knowledge, this is the first time it was detected from deer kidneys and it's the first report of the disease in a free-ranging population in Portugal. Furthermore, our study demonstrates that Map circulates widely among populations of wild cervids, which represents a potential risk of infection to other susceptible species, such as wild mammals in this country.

RESULTS

Twenty-eight (93.3%) of the red deer had gross lesions in the intestine and associated lymph nodes compatible with paratuberculosis. Seven deer (18.9%) had also gross lesions in the kidneys. Microscopic lesions were observed in eight (21,6%) kidneys. Lesions were of solitary nature or multifocal, with the exception of one case of interstitial chronic nephritis (Figs. 1A and 1B). Lesions consisted of granulomas (Figs. 1C to 1F), which varied in size from microscopic to up to 1cm in diameter in the cases with a solitary lesion. In most of the cases, the core of the granuloma was composed by necrotic debris of caseous nature (10.8%), (Fig. 1D), surrounded by inflammatory cells, namely lymphocytes (16.2%), plasma cells, macrophages, and occasional epithelioid macrophages (Fig. 1E), but also neutrophils (13.5%) (Fig. 1F). Langhans multinucleated giant cells (13.5%) (Figs. 1E and 1F) were present in the granulomatous lesions but calcification was absent in all the observed cases. Only a small percentage (5.4%) presented liquefactive necrosis at the centre of the granulomatous lesion. No mycobacteria were visualized in the organ samples submitted to histopathological examination and bacilloscopy. Map was cultured from five (13.5%) kidney samples and Map PCR identification allowed us to detect thirty (81.1%) infected red deer.

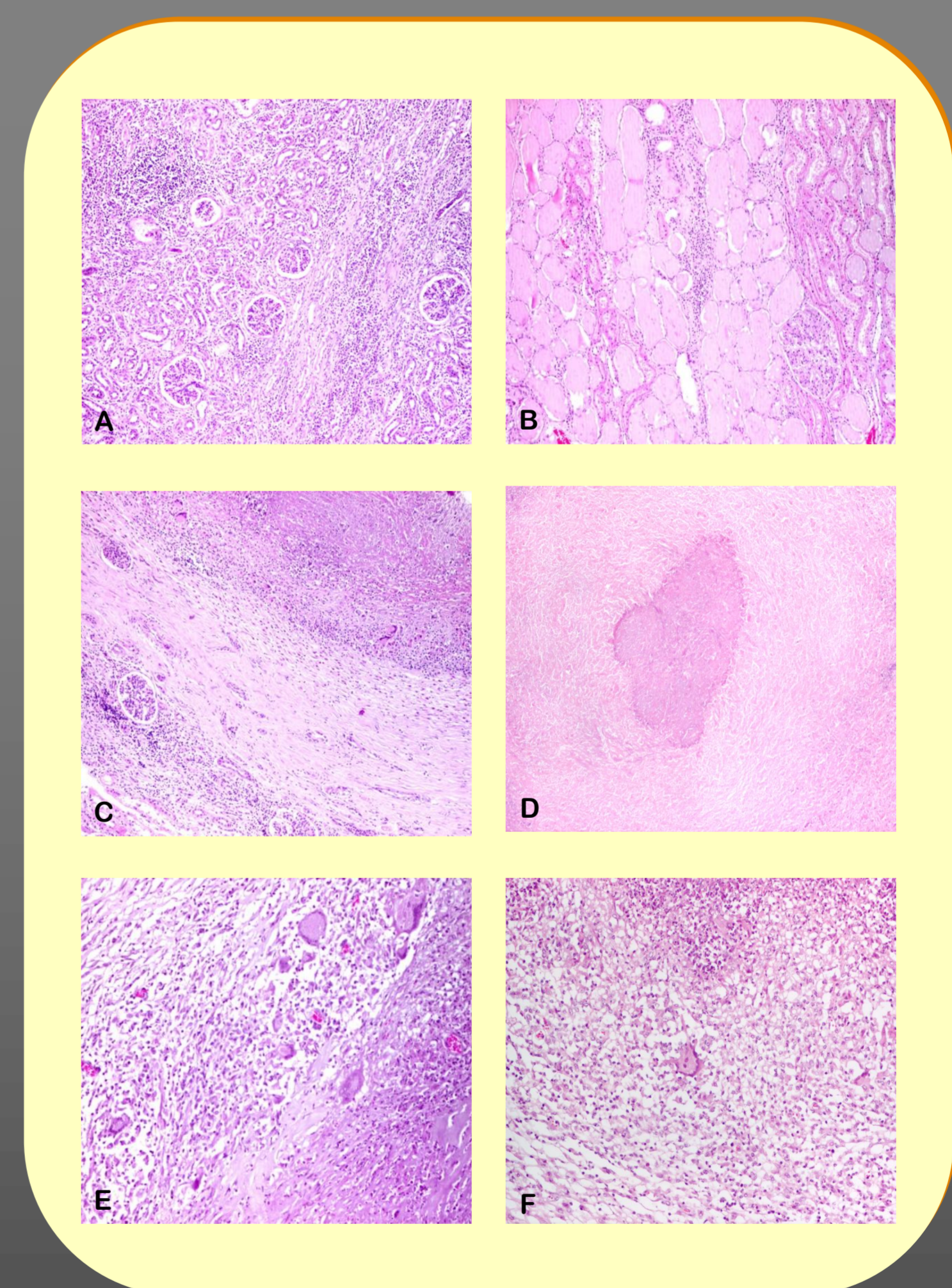


Figure 1 Morphological aspects of the interstitial chronic nephritis and granulomatous lesions in the kidneys of deer (*Cervus elaphus*): lymphocytes and plasma cells in the renal interstitium (A), and dilated renal tubules filled with hyaline material (B). Granulomatous lesion in the renal parenchyma, surrounded a fibrous capsule (C). Centre of the granuloma composed by caseous debris (D), which are surrounded by lymphocytes, macrophages and giant multinucleated cells (Langhans type) (E and F), and to a lesser extent, neutrophils (F). Haematoxylin and Eosin. Original magnifications: A, B and C 10x; E and F, 20x.

REFERENCES

- 1-Losinger, 2006. Journal of Dairy Research 73, 33-43.
- 2-Robino et al. 2008. European Journal of Wildlife Research 54, 357-360.
- 3- Marco et al. 2002. Journal of Wildlife Diseases 38, 629-632.
- 4- Juste et al. 1991. Veterinary Microbiology 28, 385-390.
- 5- Garrido et al. 2000. Veterinary Microbiology 77, 379-386.
- 6- Mutharia et al. 2010. International Journal of Food Microbiology 136, 340-344.
- 7- Machackova-Kopecna et al. 2005. Veterinary Microbiology 105, 261-268.