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INTRODUCTION

The Eurasian wild boar (*Sus scrofa*) is the most widespread free-ranging ungulate in large areas of Europe¹. Their rooting habits, omnivorous diet, ability to cross barriers and contact with a broad range of wild and domestic animals², as well as their susceptibility to mycobacterial infections, makes them useful as disease sentinels³, but the presence of *Mycobacterium avium* subsp. *paratuberculosis* (Map) was never assessed in this species in Portugal.

MATERIAL AND METHODS

During the time period of 2009-2011, 589 free-ranging wild boars 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) were examined. A preliminary study that included the mesenteric lymph nodes of 97 animals randomly selected, with and without gross lesions, was performed. Lymph nodes were tested for the presence of Map by culture, IS900 polymerase chain reaction (PCR) and histopathology. Culture methodology was performed as described by Juste et al. (1991). DNA from lymph nodes as well as from bacteria isolated by culture was extracted 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 lymph nodes 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. Tissue sections were stained with haematoxylin and eosin and the Ziehl-Neelsen technique.

RESULTS

Gross and microscopic lesions were observed in 28 (28,8%) mesenteric lymph nodes from wild boars. These lesions were always multifocal and ranged from occasional proliferative lesions, with less than 1 cm (71.4%) to large areas of granulomatous lesions, more than 1 cm (28.6%) in diameter, of either necrotic or necrotic calcified granulomas (Fig. 1A and 1B, respectively). The presence of lymphocytes (96.4%) (Fig. 1 C) and caseous necrosis (78.6%) were the most common features. Macrophages were also abundant (63,3%) and multinucleated giant cells (Langhans type) were observed in 46,67% of the granulomatous lesions (Figs. 1C and 1D). No mycobacteria were visualized in the organ samples submitted to histopathological examination and bacilloscopy. Of the 28 mesenteric lymph nodes with granulomatous lymphadenitis, 46.4% were either PCR positive or 21.4% culture positive.

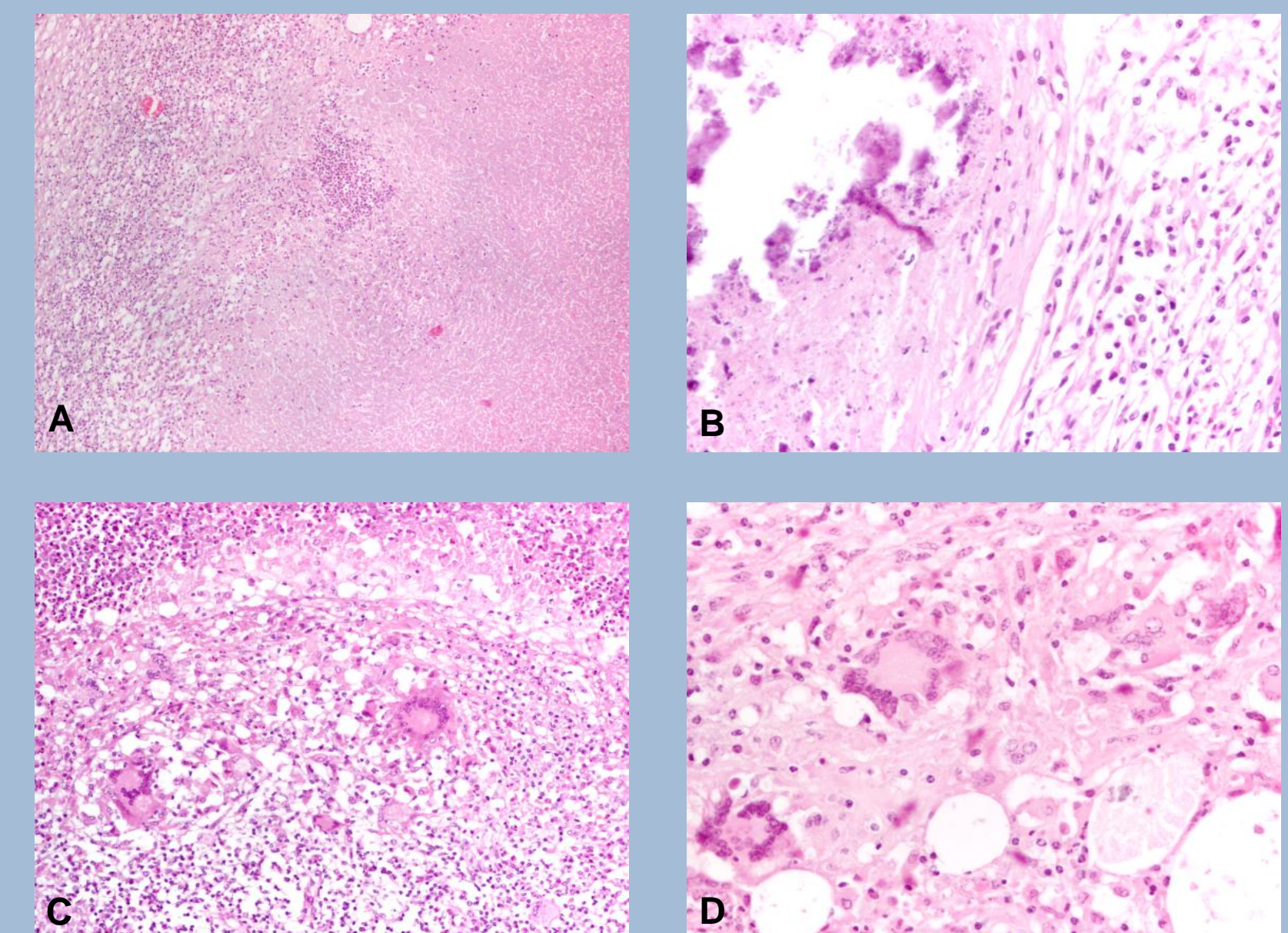


Fig.1: Morphological aspects of the granulomatous lesions in the mesenteric lymph nodes of Eurasian wild boars (*Sus scrofa*): caseous material in the necrotic centre (A), which may be calcified (B), surrounded by lymphocytes, macrophages (C) and giant multinucleated cells (Langhans type) (C and D). Hematoxylin and Eosin. Original magnifications: A, 10x; B and D, 40x; C 20x.

DISCUSSION AND CONCLUSION

Reports in which Map was isolated from wild boars reveal that the infection can occur with or without lesions, being the last the most frequent cases. Our preliminary results are in accordance to what was previously described³ and demonstrate the presence of Map in the mesenteric lymph nodes of wild boars with granulomatous lesions similar to those observed in tuberculosis. Furthermore, we confirm that wild boar is both susceptible and may be a vector of Map infections, which poses a risk of disease transmission to other wild and livestock animals. It is also noteworthy that according to these preliminary results, 37.9% of the infected animals were approved for human consumption.

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