Letters

WILDLIFE

Granulomatous lymphadenitis caused by *Nocardia* species in hunted wild boar (*Sus scrofa*) in Portugal

Nocardia species infections in mammals cause pyogranulomatous lesions in a variety of organs and are described in a variety of mammals (Pier and Fichtner 1981), including marine mammals (Leger and others 2009) and domestic pigs (Koehne and Giles 1981).

In humans, *Nocardia* species have the potential to cause localised or disseminated infection (Cooper and others 2014). This genus has previously been detected in wild ungulates (Vemireddi and others 2007, Domenis and others 2009) but not in wild boar (*Sus scrofa*). Although hundreds of ungulates are hunted in the Iberian Peninsula annually, no case of nocardiosis from wild boar has been reported.

Seven samples of lymph nodes with granulomatous lymphadenitis, with gross visible tuberculosis-like lesions but negative to mycobacteria in culture and PCR, from seven wild boar were analysed. Gram-stained smears revealed Gram-

positive short filaments, coccoid forms, and branching rods in all samples. In the Ziehl-Neelsen stain, the samples were partially acid fast.

Diagnostic cultures were performed in all animals according to microbiological protocols (Saubolle and Sussland 2003). Material was inoculated onto 5 per cent sheep blood agar plates, chocolate agar and potato dextrose agar for up to two to three weeks.

Tissues were processed for detection of *Nocardia* species DNA. DNA was extracted from tissues with a commercial kit (DNeasy Tissue Kit; Qiagen) according to the manufacturer's instructions. A sample of sterile water was extracted in parallel with the tissue samples (extraction negative control). DNA samples were tested by PCR as described previously by Laurent and others (1999) and Wada and others (2003). Nocardial 16S rDNA was amplified in two segments. The primers specific for the 16S rDNA of genus *Nocardia* were: forward (NG1): 5'-ACC GAC CAC AAG GGG G-3' and reverse (NG2): 5'-GGT TGT AAA CCT CTT TCG A-3', according to Laurent and others (1999).

Another set of primers designed by Wada and others (2003) were used: forward (NF): 5'-CGT GCT TAA CAC ATG CAA GT-3' and reverse (NR): 5'-TTC ACC GCT ACA CCA GGA AT-3'.

Gross and microscopic lesions were observed in all lymph nodes. The lesions were multifocal in nature, capsulated, and centred by caseous and/or liquefied material. Two cases also showed calcification at the centre of the lesion. The surrounding inflammatory infiltrate was composed of abundant neutrophils, macrophages, lymphocytes and few plasmocytes. In both cytological and histological preparations, the inflammatory pattern was suppurative with necrosis or a mixed suppurative-granulomatous reaction. Organisms, when present, were always seen in areas of suppurative inflammation.

Two male wild boar (28.6 per cent) had positive results for *Nocardia* species by PCR. *Nocardia* species was not confirmed in culture.

There is little information about nocardiosis in wild ungulates. *Nocardia* species are considered to be opportunistic pathogens that typically cause systemic disease in immunosuppressed individuals, including those stressed by environmental stressors or undergoing long-term corticosteroid therapy (Leger and others 2009).

In Portugal, the role of *Nocardia* species may be underestimated in causing tuberculosis-like lesions and could lead to misinterpretations of the diagnostic test results when no other differential diagnoses techniques are used.

To our knowledge, these are the first documented cases of nocardiosis in wild boar: this infection should be considered a differential diagnosis for granulomatous lymphadenitis in ungulates.

A. C. Matos, **A. P. Dias**, **M. Morais**, **M. Matos**, **M. L. Pinto**, **A. C. Coelho**, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal
L. Figueira, Escola Superior Agrária, Instituto Politécnico de Castelo Branco, Castelo Branco, Portugal

Correspondence to:
A. C. Matos, School of Agriculture, Polytechnic Institute of Castelo Branco, 6001-909 Castelo Branco, Portugal
e-mail: acmatos@ipcb.pt

References

COOPER, C. J., SAID, S., POPP, M., ALKHATEEB, H., RODRIGUEZ, C., AGUILAR, M. P. & ALOZIE, O. (2014) A complicated case of an immunocompetent patient with disseminated nocardiosis. *Infectious Disease Reports* **6**, 5327

DOMENIS, L., PECORARO, P., SPEDICATO, R., CORVONATO, M., PELETTO, S., ZUCCON, F. & ACUTIS, P. (2009) *Nocardia otitidiscaviarum* pneumonia in an Alpine chamois (*Rupicapra rupicapra rupicapra*). *Journal of Comparative Pathology* **141**, 70-73

KOEHNE, G. & GILES, R. J. (1981) *Nocardia asteroides* abortion in swine. *Journal of the American Veterinary Medical Association* **179**, 478-479

LAURENT, F. J., PROVOST, F. & BOIRON, P. (1999) Rapid identification of clinically relevant *Nocardia* Species to genus level by 16S rRNA gene PCR. *Journal of Clinical Microbiology* **37**, 99-102

LEGER, J. A. S., BEGEMAN, L., FLEETWOOD, M., FRASCA, S., GARNER, M. M., LAIR, S., TREMBLEY, S., LINN, M. J. & TERIO, K. A. (2009) Comparative pathology of nocardiosis in marine mammals. *Veterinary Pathology* **46**, 299-308

PIER, A. C. & FICHTNER, R. E. (1981) Distribution of serotypes of *Nocardia asteroides* from animal, human, and environmental sources. *Journal of Clinical Microbiology* **13**, 548-553

SAUBOLLE, M. A. & SUSSLAND, D. (2003) Nocardiosis: review of clinical and laboratory experience. *Journal of Clinical Microbiology* **41**, 4497-4501

VEMIREDDI, V., SHARMA, A., WU, C. C. & LIN, T. L. (2007) Systemic nocardiosis in a reindeer (*Rangifer tarandus tarandus*). *Journal of Veterinary Diagnostic Investigation* **19**, 326-329

WADA, R., ITABASHI, C., NAKAYAMA, Y., ONO, Y., MURAKAMI, C. & YAGIHASHI, S. (2003) Chronic granulomatous pleuritis caused by *Nocardia*: PCR based diagnosis by nocardial 16S rDNA in pathological specimens. *Journal of Clinical Pathology* **56**, 966-969

doi: 10.1136/vr.h3988