Short Communication

No evidence that wild red deer (*Cervus elaphus*) on the Iberian Peninsula are a reservoir of *Mycobacterium avium* subspecies *paratuberculosis* infectionT. Carta^a, M.P. Martin-Hernando^a, M. Boadella^a, I.G. Fernández-de-Mera^{a,b}, A. Balseiro^c, I.A. Sevilla^d, J. Vicente^a, E. Maio^{a,e}, M. Vieira-Pinto^e, J. Alvarez^{a,b}, J.M. Pérez-de-la-Lastra^a, J. Garrido^d, C. Gortazar^{a,*}^a IREC (CSIC – UCLM – JCCM), Ronda de Toledo s.n., Ciudad Real, Spain^b VISAVET and Departamento de Sanidad Animal, Universidad Complutense de Madrid, 28040 Madrid, Spain^c SERIDA, Laboratorio de Sanidad Animal, 33299 Jove, Gijón, Spain^d NEIKER-TECNALIA, Department of Animal Health, Bizkaia 48160, Spain^e Universidad de Trás-os-Montes e Alto Douro, Vila Real, Portugal

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ABSTRACT

The potential role of red deer (*Cervus elaphus*) as a reservoir of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection is largely unknown. A total of 332 wild red deer were investigated using post-mortem examination, bacteriology and serology. Only three animals (1.12%) were found to have lesions on histopathological examination and no MAP bacteria were recovered on culture. The results suggest it is unlikely that wild red deer make a significant contribution to the maintenance of MAP infection in the region. The cross-reactivity of the ELISAs used indicates this diagnostic modality is ineffective in the detection of MAP infection in this species. The implications of these results for the control of this important pathogen in both livestock and wildlife are discussed.

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Paratuberculosis (Johne's disease) is a chronic enteritis of ruminants with a worldwide distribution. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Wildlife can contribute to the maintenance of MAP infection in livestock (Kopečna et al., 2008). While a high prevalence of MAP infection (33%) has been reported in wild red deer (*Cervus elaphus*) in the Italian Alps (Robino et al., 2008), other studies have found much lower prevalence of infection (0–0.5%) (Álvarez et al., 2005; Kopečna et al., 2008).

Antibodies to paratuberculosis protoplasmic antigen (PPA)-3 were found in only 4% of Norwegian red deer (Tryland et al., 2004), in contrast to the serological evidence of widespread exposure to MAP or cross-reacting mycobacteria in Spain (30%) (Reyes-Garcia et al., 2008). In southern Spain, MAP was detected in red deer by PCR (Reyes-Garcia et al., 2008), although infection was not confirmed by culture (Álvarez et al., 2005). Throughout the Iberian Peninsula the red deer population is expanding, reaching densities of up to 70 per km². We hypothesised that MAP infection could be present in a significant proportion of these animals.

Samples from 332 hunter-killed wild red deer were obtained between 2005 and 2009 (Fig. 1 and Table 1 and Supplementary material). The presence of macroscopically visible lesions suspicious of tuberculosis (Martín-Hernando et al., 2010) was recorded, and samples of the jejunal and ileal mesenteric lymph nodes, and of the

ileocaecal valve (ICV) were fixed in formalin and processed for histopathological examination (Balseiro et al., 2008). Samples of pooled mesenteric lymphoid tissue were inoculated onto mycobactin-supplemented Herrold's egg yolk, Löwestein-Jensen media and Middlebrook 7H11 media (Sevilla et al., 2007). An adsorbed ELISA was performed to detect serum antibodies to MAP and *Mycobacterium bovis* (Reyes-Garcia et al., 2008) (see Supplementary material).

No macroscopically visible lesions suspicious of paratuberculosis were observed and there was no evidence of diarrhoea in any of the sampled animals. On histopathological examination, three animals (1.12%; 95% CI 0.3–3.3%) had small granulomas in the inter-follicular areas of their intestinal lymphoid tissue consistent with paratuberculosis. No acid-fast bacilli were present on Ziehl-Neelsen staining of the lesions, and MAP was not cultured (95% CI 0–1.2%). Two of these deer were from population 'A' (2/83, 2.4%) and one was from population 'I' (1/20, 5%) (Fig. 1), neither of which population had any contact with deer farms. Lesions consistent with tuberculosis were found in 25 animals (8.4%). Serology detected antibodies to PPA-3 and bovine purified protein derivative (bPPD), and these results were correlated ($r_s = 0.37$; $P < 0.05$) (see Supplementary material).

The results of this survey suggest it is unlikely that wild red deer make a significant contribution to the maintenance of MAP infection in the region. The cross-reactivity of the ELISAs we used indicates this diagnostic modality is ineffective in the detection of MAP infection in this species. The 'negative' results do however

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Fig. 1. Map of Iberian Peninsula illustrating the 13 sampling sites (indicated by letters A–M). The numbers within the circles indicate the number of red deer examined using histopathological examination, bacteriological culture and ELISA, respectively. Populations sampled in south-central Spain included sites with high to very high animal densities (mean 20; maximum 69 per km²). Sampling sites in northern Spain included areas of high animal density where there was close contact with domestic livestock (mainly cattle). No farmed deer were sampled.

Table 1

Details of numbers of samples processed for histopathological examination, bacteriological examination and for ELISA.

Method	Number of samples	Number of sites	Comments
Recording of grossly visible lesions suspicious of paratuberculosis (abdomen)	332	13	See Balseiro et al. (2008)
Recording of grossly visible lesions suspicious of tuberculosis (head, thorax, abdomen)	296	13	See Martín-Hernando et al. (2010)
Histopathology	267	12	Both haematoxylin and Eosin and Ziehl–Neelsen staining carried out
Bacteriological culture	324	13	Mesenteric lymph node or ileo-caecal valve
ELISA	238	13	Using paratuberculosis protoplasmatic antigen-3 (PPA-3) and bovine purified protein derivative (bPPD)

have interesting implications for wildlife disease surveillance. Firstly, given that clinical paratuberculosis occurs in farmed deer, care should be taken to avoid introducing the infection by releasing farmed deer into the wild. Secondly, since paratuberculosis is uncommon in wild deer, it should not interfere with the diagnosis of tuberculosis in this species (Martín-Hernando et al., 2010).

Why MAP infection is much more commonly found in farmed than in wild red deer remains unclear (Fernandez-De-Mera et al., 2009). One possible explanation is related to the fact that calves

in the wild are born away from the main herd, only joining the herd some weeks later (Putman, 1988), a behaviour that could decrease the likelihood of neonates becoming infected (Mackintosh et al., 2010). However, the findings of the current survey are difficult to reconcile with the high prevalence of infection reported in red deer in the Alps (Robino et al., 2008).

A further point of interest is the high MAP infection prevalence observed in fallow deer populations on the Iberian Peninsula vs. sympatric red deer (Population A). Balseiro et al. (2008) detected a high MAP infection prevalence among fallow deer, including cases of multibacillary paratuberculosis suggesting significant MAP excretion. However, none of the samples from animals from this location analysed in the context of the present study were found to be infected. The fact that fallow deer are more gregarious and graze, as opposed to browse, more than red deer (Putman, 1988), might account for their greater and/or earlier exposure risk. Our results in relation to red deer are similar to those obtained for other wild ruminants such as roe deer (*Capreolus capreolus*), mouflon (*Ovis aries*), and chamois (*Rupicapra pyrenaica*), suggesting that wildlife plays a minor role in the maintenance of MAP infection on the Iberian Peninsula.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tvjl.2011.08.010](https://doi.org/10.1016/j.tvjl.2011.08.010).

References

- Álvarez, J., De Juan, L., Briones, V., Romero, B., Aranaz, A., Fernández-Garayzábal, J.F., Mateos, A., 2005. *Mycobacterium avium* subspecies paratuberculosis in fallow deer and wild boar in Spain. *Veterinary Record* 156, 212–213.
- Balseiro, A., García Marín, J.F., Solano, P., Garrido, J.M., Prieto, J.M., 2008. Histopathological classification of lesions observed in natural cases of paratuberculosis in free-ranging fallow deer (*Dama dama*). *Journal of Comparative Pathology* 138, 180–188.
- Fernandez-De-Mera, I.G., Vicente, J., Hofle, U., Fons, F.R., Ortiz, J.A., Gortazar, C., 2009. Factors affecting red deer skin test responsiveness to bovine and avian tuberculin and to phytohaemagglutinin. *Preventive Veterinary Medicine* 90, 119–126.
- Kopečna, M., Trčka, I., Lamka, J., Moravkova, M., Koubek, P., Heroldova, M., Mrlik, V., Kralova, A., Pavlik, I., 2008. The wildlife hosts of *Mycobacterium avium* subsp. paratuberculosis in the Czech Republic during the years 2002–2007. *Veterinarni Medicina* 53, 420–426.
- Mackintosh, C.G., Clark, R.G., Thompson, B., Tolentino, B., Griffin, J.F.T., de Lisle, G.W., 2010. Age susceptibility of red deer (*Cervus elaphus*) to paratuberculosis. *Veterinary Microbiology* 143, 255–261.
- Martín-Hernando, M.P., Torres, M.J., Aznar, J., Negro, J.J., Gandía, A., Gortázar, C., 2010. Distribution of lesions in red and fallow deer naturally infected with *Mycobacterium bovis*. *Journal of Comparative Pathology* 142, 43–50.
- Putman, R.J., 1988. *The Natural History of Deer*. Comstock Publishing Associates. Cornell University Press, Ithaca, NY, USA.
- Reyes-Garcia, R., Perez-de-la-Lastra, J.M., Vicente, J., Ruiz-Fons, F., Garrido, J.M., Gortazar, C., 2008. Large-scale ELISA testing of Spanish red deer for paratuberculosis. *Veterinary Immunology and Immunopathology* 124, 75–81.
- Robino, P., Nebbia, P., Tramuta, C., Martinet, M., Ferroglio, E., De Meneghi, D., 2008. Identification of *Mycobacterium avium* subsp. paratuberculosis in wild cervids (*Cervus elaphus hippelaphus* and *Capreolus capreolus*) from Northwestern Italy. *European Journal of Wildlife Research* 54, 357–360.
- Sevilla, I., Garrido, J.M., Geijo, M., Juste, R.A., 2007. Pulsed-field gel electrophoresis profile homogeneity of *Mycobacterium avium* subsp. paratuberculosis isolates from cattle and heterogeneity of those from sheep and goats. *BMC Microbiology* 7, 18.
- Tryland, M., Olsen, I., Vikoren, T., Handeland, K., Arnemo, J.M., Tharaldsen, J., Djonne, B., Josefsen, T.D., Reitan, L.J., 2004. Serologic survey for antibodies against *Mycobacterium avium* subsp. paratuberculosis in free-ranging cervids from Norway. *Journal of Wildlife Diseases* 40, 32–41.