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Management practices and their association with *Mycobacterium tuberculosis* complex prevalence in red deer populations in Southwestern Spain

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ABSTRACT

Intensification of game management may increase the prevalence of tuberculosis (TB) in wildlife despite eradication programs implemented in cattle herds in the same areas. In this cross-sectional study, we investigated the association between wild game management practices and the presence of tuberculosis in red deer populations in Southwestern Spain. Five hundred and fifty-one animals were examined by necropsy to detect tuberculosis-like lesions in the main lymph nodes. Prevalence, as determined by TB-like lesions, was estimated to be 5.1% of animals, with 77% of TB-like lesions confirmed by PCR. Our results suggest that population density, in addition to factors which promote the local aggregation of animals, is factors associated with increased prevalence of TB in red deer populations. We suggest that management practices including supplementary feeding, fencing, water ponds and interaction with domestic livestock should be revised in order to prevent TB in wild deer both.

1. Introduction

Bovine tuberculosis (TB) is an ongoing concern in many regions because of affects on economics, health, animal welfare and conservation. In Spain, eradication programs based on tuberculin test-and-slaughter began in dairy cattle in the 1960s and have encompassed the national herd since 1986, resulting in a reduction in the percentage of test-positive cattle from 11.8% in 1987 to 1.6% in 2007, at an estimated cost of €8m (unpublished report of the Spanish Ministry of Environment, Marma, 2009). However, it is acknowledged that the reduction in TB prevalence has been less effective in areas where farmed cattle are free to range over large areas, sharing habitat with wild species which may act as reservoirs of infection (unpublished report of the Spanish Ministry of Environment). Several studies have shown associations between prevalence of tuberculosis in cattle and wildlife species, predominantly red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) (Aranaz et al., 2004; Hermoso de Mendoza et al., 2006; Parra et al., 2006; Vicente et al., 2006). Diagnosis of TB in wild boar based on *post-mortem* inspection and palpation has shown an increased proportion of carcass condemnation in the region of Extremadura (Southwestern Spain) from 0.8% in 1997–998 (Parra et al., 2006) to 2.9% in 2003/2004 (Hermoso de Mendoza et al., 2006). More recently, in vitro techniques for diagnosis have been developed including γ-interferon ELISA and PCR-based methods for direct detection of the organism, which can detect early stages of infection.

Alteration of natural environments and population structures as a consequence of management, may lead to

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Table 1
Tuberculosis-like lesion prevalence and PCR confirmation for different areas and the most relevant management practices at the estate level (n = 16) in a study of Mycobacterium tuberculosis complex prevalence in red deer populations in Southwestern Spain (level water ponds: L1 = from 0.17 to 0.21 water ponds/100 ha, N = 145; L2 = from 0.60 to 0.76, N = 145; and L3 = from 0.93 to 1.31, N = 116).

	TB-like lesions prevalence	P (Fisher exact test)	% of positive culture in lesions	TB prevalence confirmation	P (Fisher exact test)
Total red deer	5.1 (28/551)		77	3.1	
Sierra San Pedro	5.8 (22/381)	0.75	68.1	3. 7	0.68
National Park of Monfragüe	3.5 (6/170)		75	1.8	
Fenced	6.4 (21/328)	0.45	52.17	3.6	0.68
Unfenced	3.1 (7/223)		71.42	2.2	
With livestock	10.9 (22/202)	0.02	71.43	6.1	0.12
Without livestock	2.2 (5/231)		68.19	1.0	
With sowing	8 (28/320)	0.01	70	4.5	0.07
Without sowing	0 (0/105)		0	0	
Supplementary feeding	7 (26/348)	0.17	67.86	4.0	0.68
Non supplementary feeding	2.5 (2/77)		100	2.5	
Level water ponds/100 ha					
Level 1	3.1 (4/130)	0.06*	50	1.5	0.02*
Level 2	9.0 (12/133)		100	9.0	
Level 3	11.5 (12/104)		83.3	9.4	

* Chi-square test.

the introduction or exacerbation of diseases in wild animal populations (Corner, 2006). Reported examples are the spread of TB among several wildlife species that share the same habitats with extensive cattle in East Africa (Cleaveland et al., 2005). Other examples are TB transmission between cattle and wildlife in South Africa, and the remarkable case reported in Michigan, where winter supplementary feeding and increased density of white tailed deer have facilitated the aerosol transmission of TB that subsequently re-emerged in cattle after a successful eradication program (O'Brien et al., 2002).

Management practices may further increase contact between livestock and wildlife species, such as red deer and wild boar, and may increase TB transmission and prevalence (Caley and Hone, 2002). In Spain, during the last 40 years landowners have fenced many hunting estates and intensified the management of red deer populations, maintaining high stocking densities and attempting to improve the quality of trophy animals (Carranza et al., 1996; Carranza, 1999; Martínez et al., 2002). Management in hunting estates may influence not only the population dynamics but also the behavior of the animals, promoting their aggregation at particular locations and thus facilitating contact between individuals and favoring the transmission of infectious diseases (Sánchez-Prieto et al., 2004; Gortázar et al., 2006; Hermoso de Mendoza et al., 2006; Morellet et al., 2007; Vicente et al., 2007; Castillo et al., 2010.).

Here we describe 16 hunting estates that manage populations of red deer in Southwestern Spain, some of them with domestic livestock sharing the same areas with red deer. Our objective in this work is to investigate which factors in the management of hunting estates may be associated with the prevalence of TB in red deer.

2. Materials and methods

2.1. Study area

Samples were collected from 16 different estates in two areas in Southwestern Iberian Peninsula: Sierra de San Pedro (14 estates with an approximate area of 18,500 ha) and Monfragüe National Park and surroundings (two estates with a total area of 5216 ha). Vegetation in these areas is predominantly composed of holm oak (*Quercus ilex*), cork oak (*Quercus suber*), scrublands (*Cystus* spp., *Phyllirea* spp., *Pistacia* spp., *Rosmarinus* spp., and *Erica* spp.), with some scattered pasturelands and small areas of farmed crops. The climate in this region is typically Mediterranean with mild winters and hot and dry summers; average rainfall is between 400 and 600 mm. Rainfall is concentrated in autumn and spring and almost absent in summer. Mean temperatures range from 3.5 °C in winter to 35 °C in summer.

2.2. Featured estates

Estates were selected so as to include some whose main purpose was recreational hunting, along with others encompassing both livestock rearing and hunting activity. Characteristics of the study estates were obtained from owners, guards and managers in the years of study, although management practices commonly did not change from one year to the next and the responders were asked to confirm that their answers equally applied to several (at least 2) previous years. Responders provided answers to questions about the management of the estate: fencing (yes/no), points of water supply (number/100 ha), supplementary feeding (yes/no), type of feeding (trail, crops or both), number of wild boars hunted and livestock sharing areas with deer (yes/no) (Table 1). For the interview, we considered as plantings closed areas with an electric fence maintained until the forage crop had grown up. Water ponds were available for all animals and did not have any chemical disinfection. The population density of 8 estates was obtained from Castillo et al. (2010) with values ranging between 15 and 40 deer/100 ha. Wild boar density was obtained from Fernández-Llario and Mateos-Quesada (2003) with values of 2.7wild boar/100 ha in Sierra de San Pedro or 3–4 wild boar in National Park of Monfragüe (Fernández-Llario et al., 2004). These areas have a low density of wild boar because its management is directed at deer, wild boar being a secondary use.

2.3. Animal sampling

Samples were collected from October to February in the hunting periods of 2007–2008 and 2008–2009. The hunting period commences after the rut, so the animals had suffered considerable loss of condition after the reproductive effort (mostly for males) and the hot and dry summer (for both sexes). Red deer examined for this study were shot in commercial hunt called montería. Normally, male deer of 2 years or more, or females of any age, can be shot in commercial montería, focused on stags, or in management hunts, aimed at reducing the density of hinds, respectively. Under these conditions, there is little opportunity for hunting bias towards particular individuals, and montería have been shown to be a less-biased procedure to obtain data from hunted red deer (Martínez et al., 2005). This kind of study does not encourage hunters to shoot additional deer (Carranza et al., 2004).

Five hundred and fifty-one animals were examined by necropsy to detect TB-like lesions in the retropharyngeal, mediastinal and mesenteric lymph nodes; the ileocecal valve, lungs, kidneys and liver were also examined. The evaluation protocol included detailed inspection and palpation followed by incision of potential lesions. Inspections were carried out by qualified staff.

TB-like lesions (enlarged, caseated or calcified) were sampled, collected in a sterile container and refrigerated to 4 °C until processing, typically within 48 h. Samples were processed for microscopic examination, decontamination and culture in Lowenstein Jensen Medium with pyruvate and without glycerol. Positive cultures were confirmed as *Mycobacterium bovis* by PCR amplification of IS*6110* (Parra et al., 2008).

2.4. Statistical analysis

A cross-sectional study to identify the relationship between some management factors and the prevalence of TB in red deer was carried out. Firstly, we explored the association between TB prevalence and the different management conditions using non-parametric tests (Fisher exact tests; Statistical package version 8).

Secondly, we conducted a Generalized Linear Model (SPSS software *vs.* 13, SPSS, Inc., Chicago, IL, USA) analysis to investigate the combined effect of several management conditions on the presence of red deer with TB lesions confirmed by positive culture and PCR. The dependent variable followed a binomial distribution, so we used a binomial model (logit link) to estimate the effect of management conditions on the odds of TB in red deer. As fixed effects we included all the management practices for which we had sufficient and suitable data, as shown in the exploratory analyses.

In this case, a forward stepwise method was used to test for the significant changes in −2log-likelihood when including or removing a variable, which is generally more reliable than the Wald statistic for testing the contribution of each factor to the explanatory model.

After having analysed the effect of the management conditions on TB prevalence, we were interested in knowing whether the presence of another species also reported as a reservoir, the wild boar, had an effect on red deer TB. For this purpose we refit the Generalized Linear Model with exactly the same variables and procedures but adding the number of wild boars hunted in the estate as a new fixed factor. The number of boars in each estate was previously categorized into two levels (level 1 with density ≤2.5 wild boar/ha and level 2 with density >2.5 wild boar/ha). The study has been reported in accordance with the STROBE statement (Von Elm et al., 2007).

3. Results

A total of 551 free ranging red deer were examined for TB-like lesions of which 5.1% (28/551) were positive. Samples from the lesions of the 28 individuals were cultured and 77% of them were a PCR positive for *Mycobacterium tuberculosis* complex (3.1% of the total red deer examined). Management factors studied and their associated prevalences are shown in Table 1.

A forward stepwise was used including presence of livestock, supplementary feeding and number of water ponds/100 ha. This last variable was firstly included as a covariate, but the model showed the best-fitting values of the information criteria (logit likelihood and AIC: Akaike's Information Criterion) when it was included as a categorical variable with three levels (L1 = from 0.17 to 0.21 water ponds/100 ha, N = 145; L2 = from 0.60 to 0.76, N = 145; and L3 = from 0.93 to 1.31, N = 116). Due to the presence of empty cells, only the main effects could be considered in the model. This model allows for an estimate of the differences between levels within each fixed factor. Nevertheless, we decided to conduct another model (a Logistic Regression Analysis) on the same variables in order to obtain new information (odds ratios, Hosmer–Lemeshow test, percentage of correct classification and Nagelkerke R^2 statistic) to confirm the previous model fit.

Results from the Generalized Linear Model showed significant effects of supplementary feeding, number of water ponds/100 ha and presence of livestock on the prevalence of TB. Both supplementary feeding and presence of livestock in the estate increased TB prevalence (Table 2). These two factors were correlated, as all estates without livestock provided supplementary feeding to red deer, whereas only some of the estates with livestock did so (exact Fisher test: P < 0.001; Fig. 1a). Our results also showed a significant effect of the number of water ponds/100 ha. Although adding this factor to a Logistic Regression model resulted in poorer model fit (Hosmer–Lemeshow fit-goodness test P < 0.05), its inclusion produced a significant change in the −2log-likelihood (change = 6.430, df = 2, P = 0.04). Notably, TB prevalence was 3-fold higher in estates with intermediate level of water ponds compared with estates with the highest level of water ponds/100 ha. However, estates with a low level of water ponds did not show higher TB prevalences compared to those with the highest level of water ponds/100 ha (see Table 2). The management practice of building water ponds is mainly oriented towards livestock

Table 2
Generalized Linear Model of the association between management practices and conditions and the presence of tuberculosis-like lesions confirmed by culture and PCR in red deer in a study of *Mycobacterium tuberculosis* complex prevalence in red deer populations in Southwestern Spain.

Parameter	*B*	Standard error	Wald's Chi-square	*df*	Sig.	Odds ratio
Intersection	−2.190	0.334	64.128	1	0.00	
Supplementary feeding						
Without *vs.* with	−2.183	0.803	7.392	1	0.01	0.113
Livestock						
Without *vs.* with	−2.495	0.822	9.223	1	0.00	0.082
Water ponds						
Level 1 *vs.* level 3	−.198	1.337	0.022	1	0.88	0.821
Level 2 *vs.* level 3	1.173	0.514	5.214	1	0.02	3.233

Nagelkerke R^2 = 0.196. Percentage of correct classification = 94.2%. Hosmer–Lemeshow test (second step: livestock and supplementary feeding): P = 0.98.

and this is why the estates with few water ponds were also free of livestock (Fig. 1).

To investigate the effects of the presence of wild boar in the same area, the previous model was refit including the presence of wild boar hunted during the hunting season in that year. The results showed no significant effect of the presence of wild boar hunting in the area (P = 0.998).

4. Discussion

Our results show that some management factors favoring the aggregation of animals, such as food supplementation, presence of water ponds or presence of livestock were associated with high prevalences of TB in red deer.

Recently, a number of articles have been published describing an increase in TB prevalence in the southwest of the Iberian Peninsula: 0.8% in 1997–1998 (Parra et al., 2006) to 2.9 in 2004 (Hermoso de Mendoza et al., 2006). Our results support the same trend in prevalence in this area. Despite this increase in TB prevalence in the southwest of the Iberian Peninsula, our estimates are lower than those presented by Vicente et al. (2006) (15.84%) in South-Central Spain.

Similarities were observed between the situation reported here and that of white tailed deer in Michigan, USA. In the latter case, a TB positive deer was detected after the cattle on the farm had gained a tuberculosis-free status (Schmitt et al., 1997). Later, the prevalence in deer increased to 0.54% and resulted in 19 infected cattle herds (O'Brien et al., 2002). By inference, we should pay special attention to this outcome because the TB control measures implemented for cattle in our area may not be as efficient as they could, because of the risk of continuous re-infections due to the high prevalences of TB occurring in wild ungulates.

Comparing our situation to that of white tailed deer of Michigan, the contagion in both cases appears associated with the aggregation of deer. In white tailed deer it has been reported that the main management practice responsible for disease transmission was supplementary feeding in winter (Miller et al., 2003). Similarly, in our experience the main routes for transmission are related to supplementary feeding, water ponds and livestock uses. In the same way, Vicente et al. (2007) investigated TB prevalences under different management practices and found a relationship with supplementary feeding and water ponds, although the presence of livestock was not considered in that study.

Supplementary feeding may be necessary for red deer survival during limiting periods, for maintaining immune response and body condition to acceptable levels (Cichon et al., 2002). Therefore, the impact of aggregation resulting from supplementary feeding may, in some part, be compensated for by an improvement in overall immune health and resilience to *Mycobacterium bovis* infection and

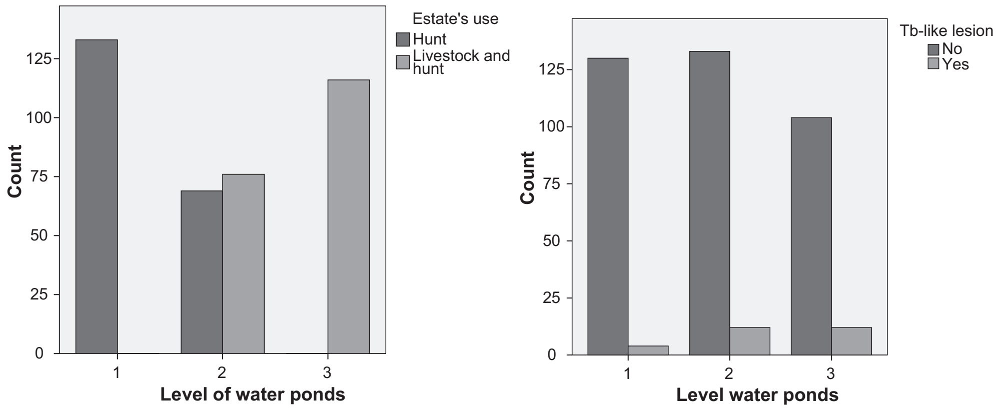

Fig. 1. Number of estates with different densities of water ponds (three levels) separated as a function of (left) their economic use (livestock and hunting or only hunting) and (right) the presence or absence of cases with TB-like lesions in a study of *Mycobacterium tuberculosis* complex prevalence in red deer populations in Southwestern Spain.

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other potential pathogens. However, our results suggest that provision of supplementary feeding along a trail on the ground increases rather than decreases the prevalence of TB-like lesions; this suggests that it may be advisable to explore alternative methods for providing supplementary feeding that minimize excessive aggregation and direct contact among animals, should supplementary feeding be necessary to maintain the condition of animals through the limiting season.

In contrast to Michigan, where supplementation is implemented in winter when snow impedes access to food sources (Miller et al., 2003), in our study area supplementary food is provided in summer. The Extremadura summer is characterized by high temperatures with mean maximum temperatures close to 40 °C, accompanied by low humidity which does not promote survival of the mycobacterial bacillus in the environment (Duffield and Young, 1985). Despite these conditions, supplementary feeding seems to have higher effect than water ponds on the prevalence of TB. However, transmission of the pathogen may be facilitated in places where humidity and temperature are more favorable for the survival of TB bacilli, as may occur around pond areas. Thus, areas at greater risk of promoting disease transmission such as water ponds during the summer could benefit from management practices directed towards preventing the access of animals (such as fencing some water ponds), provided that they can have access to alternative water sources.

Livestock management in the same areas used by red deer populations appeared to be associated with the highest prevalences of TB. In this sense, the worst situation for red deer TB occurs where there is shared habitat with livestock coupled with restricted access to water sites. This situation not only seems to increase TB prevalences in red deer, but also it may have an impact on disease control and TB management in livestock. As Hermoso de Mendoza et al. (2006) reported, a variety of strain types are shared between wildlife and livestock. Studies have confirmed that cattle, red deer and wild boar are all TB reservoirs (Vicente et al., 2006); the main difference being the natural TB resistance of wild boar and the ability of this species to move freely, even through fences, thus becoming a very efficient spreader of TB in affected areas. Despite the potential role of wild boar in the maintenance of the disease, our results did not show a relationship between hunting of this species in the area and TB prevalence in red deer. One possible explanation is that the data on the presence of wild boar hunted in the area was too crude to capture the actual risk of TB transmission by wild boars to red deer or cattle. Further studies are needed focusing on the interactions between wild boar, red deer and cattle and their role in the spread and transmission of TB.

Despite management practices, high stocking density is a common risk factor impacting the prevalence of infectious diseases, for example see Lugton et al. (1998), Garner (2001), and Griffin et al. (2005). A decrease in deer density, as encouraged by the Michigan Government (Miller et al., 2003), should result in the reduction of TB prevalence because the risk of contact is minimized and the body condition and overall health and wellbeing of animals improve.

In summary, high population density and the presence of sites that promote the aggregation of animals may play a central role in the prevalence of TB in red deer populations in Southwestern Spain. Supplementary feeding, water ponds and livestock appear the most important factors when considering management practices for TB control in red deer. Reducing the risks of contact between wildlife and cattle might also improve the success of bovine TB eradication programs.

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