# Factors that influence *Mycobacterium bovis* infection in red deer and wild boar in an epidemiological risk area for tuberculosis of game species in Portugal

Short title: *M. bovis* infection in red deer and wild boar in Portugal.

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**Abstract**

Bovine tuberculosis (bTB) is a worldwide zoonotic disease of domestic and wild animals. Eradication has proved elusive in those countries with intensive national programs but with ongoing transmission between wildlife and cattle. In Portugal, a high risk area for bTB was defined and specific measures implemented to assess and minimize the risk from wildlife. Data from the 2011 to 2014 hunting seasons for red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) were analyzed with bovine demographic and bTB information to assess factors that determined the occurrence and distribution of bTB in both species.

The likelihood of bTB-like lesions in wild boar was positively associated with density of red deer, wild boar and cattle, while for red deer only their density and age were significant factors. The likelihood of *M. bovis* isolation in wild boar was associated with density of cattle and red deer and also with the anatomical location of lesions, while for red deer none of the variables tested were statistically significant.

Our results suggest that, in the study area, the roles of red deer and wild boar may be different from those previously suggested for the Iberian Peninsula, as red deer may be the driving force behind *M. bovis* transmission to wild boar. These findings will assist government services and game managing bodies to better manage hunting zones and thereby enhance the success of the bTB eradication program.

**Keywords**

*Mycobacterium bovis*; Bovine tuberculosis; wild boar; red deer; epidemiology; Portugal.

**Introduction**

*Mycobacterium bovis* is the main cause of tuberculosis in animals, infecting wild and domestic species as well as humans (De Lisle et al., 2002). Although some countries have achieved eradication through test and slaughter programs and reinforced abattoir surveillance (Santos et al., 2009), the disease persists or is even reemerging in others.


**Figure 1.** Common pasturage for red deer and cattle in Idanha-a-Nova county (Source: Engenheiro Tiago Honrado).

In Portugal, eradication of bovine tuberculosis (bTB) has not yet been achieved despite an eradication program in operation since 1989. Two regions, Alentejo and Center have the highest prevalence (2013 animal prevalence of 0.06% and 0.07% respectively; Figures 1 and 2), although the trend has been downward trend for the last four years

(DGAV, 2015). Direct contact between wildlife and livestock or through sharing of feed and water resources has been reported as the major transmission mechanisms (Vieira-Pinto et al., 2011). The high risk area in Portugal is contiguous to Spain with no physical separation (Cunha et al., 2012) thus the disease can be considered as a transboundary disease risk. It may also present a threat for the conservation of endangered or protected wildlife species and a potential public health threat for hunters, veterinarians, game meat handlers and consumers (Vieira-Pinto et al., 2011).


**Figure 2.** Common pasturage for red deer, wild boar and cattle in Idanha-a-Nova county (infrared photography) (Source: Engenheiro Tiago Honrado).

In 2011, the Portuguese National Veterinary Authority issued an edict, a proclamation with legal force, defining a high risk area for bTB transmission between cattle and wild animals (Fig. 3) and establishing measures to monitor and control the situation. These

included the mandatory presence of a veterinarian during hunting sessions, to inspect carcasses for lesions compatible with bTB (LCTB) and collect samples for laboratory confirmation by histopathology and bacteriological culture.

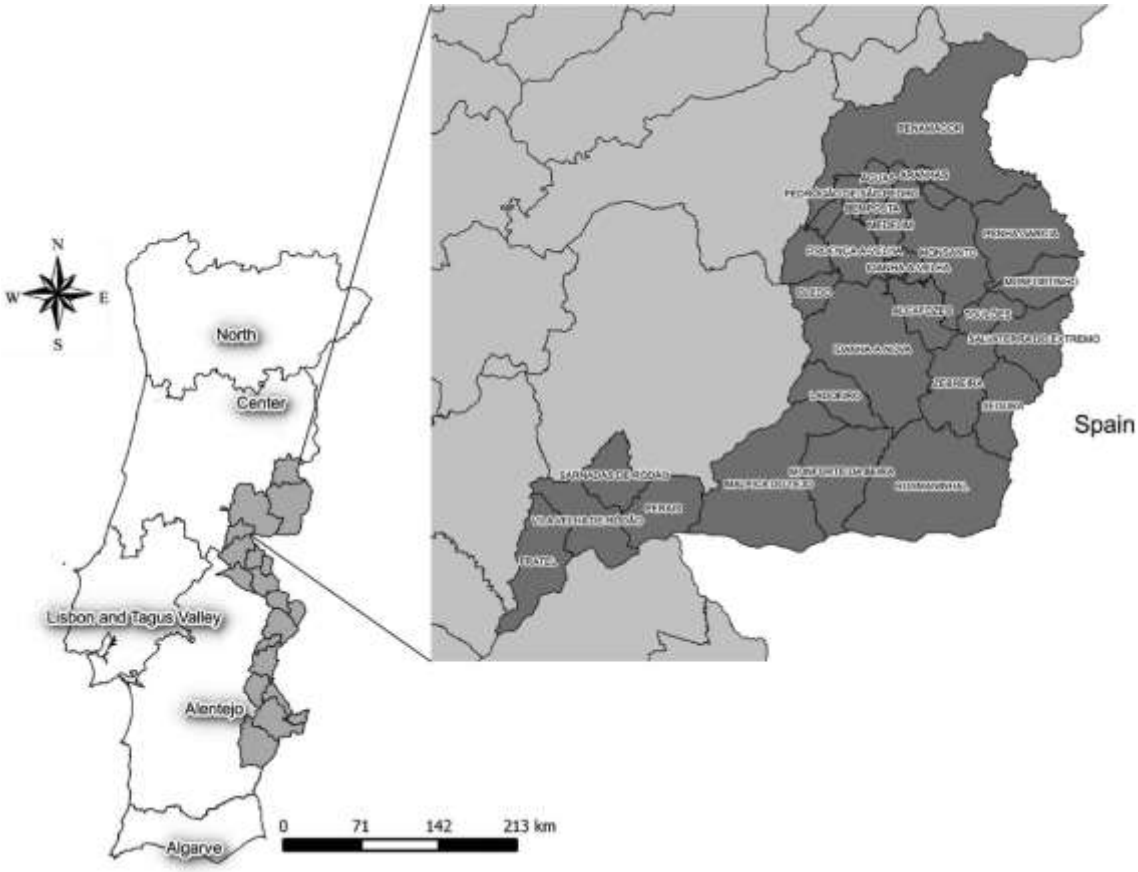


**Figure 3.** Regions of mainland Portugal and high-risk area for wildlife tuberculosis, as issued by Edict 1, with parishes under study zoomed in.

*M. bovis* and other mycobacteria belonging to *M. tuberculosis* complex have been isolated from wild boar and red deer, which share several *M. bovis* spoligotypes with cattle (Duarte et al., 2008; García-Jiménez et al., 2013; Rodríguez et al., 2011). Such links present a risk for livestock due to the possibility of spillover of infection, which might exacerbate the maintenance and spread of bTB in the high-risk area. However, the role of wild boar and red deer in the epidemiology of bTB in the different epidemiological contexts is not yet clear with some authors regarding these species as

reservoirs, while others consider them as maintenance hosts or dead end hosts (Aranaz et al., 2004; Cunha et al., 2011; Duarte et al., 2008; Gortázar et al., 2007; Santos et al., 2012).

The relationship between animal and habitat variables requires further analysis to help explain the epidemiological role of wild species in bTB maintenance and dissemination. The purpose of this study was to better understand factors affecting the occurrence and distribution of macroscopic lesions compatible with bTB and determinants of *M. bovis* isolations in red deer and wild boar in the bTB high-risk area in Portugal.

## Materials and Methods

### *Study area*

The study area comprised 31 parishes of the high risk area and included all 17 parishes of Idanha-a-Nova, all four parishes of Vila Velha de Ródão counties, eight parishes from Penamacor and two parishes from Castelo Branco counties (DGAV, 2011; Fig. 3).

### *Sample collection and analysis*

Samples from red deer and wild boar hunted in these area during three hunting seasons (June 1st to May 31st 2011/2012, 2012/2013 and 2013/2014) were collected in the following way: each carcass was systematically examined for macroscopic LCTB by a private veterinarian (the "designated veterinarian") accredited by the official services and who followed procedures as defined by Edict number 1 and Regulation 853/ 2004 (DGAV, 2011; EC, 2004). When LCTB were detected, samples of affected organs and lymph nodes were collected and sent to the National Reference Laboratory for Bovine Tuberculosis (*Instituto Nacional de Investigação Agrária e Veterinária* -

INIAV) for histopathological and bacteriological examination, while the carcass was then destroyed. Laboratory techniques are detailed in Vieira-Pinto et al., 2011; a laboratory result was classified as positive when *M. bovis* was cultured whilst a negative result was recorded when no organisms were isolated or other Mycobacterium species (*M. avium* and other non-tuberculous Mycobacteria (NTM)) were cultured. Where multiple lesions were detected, samples of LCTB belonging to the same animal were pooled. Lesio0ns were grouped by anatomical location where the "head" group included the submaxillary, submandibular, mandibular, parotid or retropharyngeal lymph nodes (Lymph nodes.), the "thorax" group included the mediastinal and bronchial Lymph nodes. and the lung, and the "abdomen" group comprised lesions in the mesenteric Lymph nodes. or the liver, kidney, spleen or intestine. Lesions located elsewhere were included in an "other" group.

***Data collection and analysis***

Data were collected on hunting events (number, species, age and sex of animals killed in the study area) during the three hunting seasons and the result of the veterinary initial examination and laboratory results; all were managed in an MS Access® 2007 database. Calculations were performed at parish level, the smallest administrative division which formed the spatial unit used in this study. Data on cattle densities in each parish were obtained from the databases of the official veterinary services. As there are no reports on wild boar and red deer densities, the number of hunted animals within a parish in relation to its size was used as a proxy for density, as has been done elsewhere (Vicente et al., 2013). Apparent bTB prevalence calculated as the number of animals with *M. bovis* isolation divided by the number of hunted animals

and the 95% confidence intervals were calculated using the package "prevalence" in R, function propCI, choosing an exact method. Fisher's exact test was used to test the statistical significance of differences between species with respect to likelihood of LCTB and isolation of *M. bovis*. Pearson's correlation test was applied to assess, at parish level, the association between animal densities (red deer, wild boar and cattle), the respective prevalence of LCTB in each of the wild species and of the likelihood of *M. bovis* isolation. Cluster analyses to detect areas of extremes in risk of *M. bovis* infection were undertaken in SatScan® for red deer and wild boar separately, using parish as spatial unit. For each of the two species, multivariate logistic regression was used to assess factors influencing the likelihood of presence of LCTB and of *M. bovis* isolation, using stepwise forward variable selection. The variables included in the analysis are listed in table 1. For both red deer and wild boar, animals aged one or more years were classified as 'adult' while the remainder were classified as 'young'. Odds ratios and the respective 95% confidence intervals were calculated as measures of the magnitude of effects. Apart from the cluster analysis, all statistical analyses were performed in R (version 3.2.0) (R Development Core Team, 2008). In all analyses, a probability lower than 5% was considered as statistically significant.

**Table 1.** Explanatory variables used for logistic regression models. For continuous variables minimum, mean and maximum values are displayed.

| Variable | Values/range |
|---|---|
| Age | Adult; young |
| Gender | Male; female |
| Location of lesions on the body | Head; thorax; abdomen; other |
| Density of red deer | mean= 0.93 animals/Km$^2$, min= 0 animals/Km$^2$, max= 6.95 animals/Km$^2$, median= 0.29 animals/Km$^2$ |
| Density of wild boar | mean= 1.04 animals/Km$^2$, min= 0.06 animals/Km$^2$, max= 3.91 animals/Km$^2$, median= 0.87 animals/Km$^2$ |
| Density of cattle | mean= 31.76 animals/Km$^2$, min= 0 animals/Km$^2$, max= 142.1 animals/Km$^2$, median= 16.19 animals/Km$^2$ |

## Results

During the three hunting seasons (study period), a total of 5924 animals were hunted, comprising 3733 red deer (63%) and 2191 wild boar (37%) (Table 2).

**Table 2.** Number of red deer and wild boar hunted during study period, with lesions suspected of tuberculosis (LCTB) and *M. bovis* isolation, by gender and age group, in the study area.

| Species | Class | Number of hunted animals | Number of animals with LSTB (% of the hunted animals, by species) | Number of animals with *M. bovis* isolation | Apparent prevalence of *M. bovis* and CI at 95%, by species age and gender |
|---|---|---|---|---|---|
| **Red deer** | Female | 2101 | 148 (7.0) | 127 | 6.0 [5.1; 7.2] |
| | Male | 1632 | 114 (7.0) | 95 | 5.8 [4.7; 7.1] |
| | Adult | 3044 | 235 (7.7) | 201 | 6.6 [5.8; 7.5] |
| | Young | 689 | 27 (3.9) | 21 | 3.1 [1.9; 4.6] |
| | **Total** | **3733** | **262 (7.0)** | **222** | **6.0** [5.2; 6.8] |
| **Wild boar** | Female | 1284 | 189 (14.7) | 116 | 9.0 [7.5; 10.7] |
| | Male | 907 | 132 (14.5) | 75 | 8.3 [6.6; 10.3] |
| | Adult | 1593 | 238 (14.9) | 145 | 9.1 [7.7; 10.6] |
| | Young | 598 | 83 (13.9) | 46 | 7.7 [5.7; 10.1] |
| | **Total** | **2191** | **321 (14.6)** | **191** | **8.7** [7.6; 10.0] |
| **Total wild boar and red deer** | | **5924** | **583 (9.8)** | **413** | **7.0** [6.3; 7.7] |

### *Prevalence and anatomical distribution of LCTB*

The proportion of hunted animals presenting LCTB was significantly higher in wild boar (14.6%) than in red deer (7%) (OR = 2.3, 95% CI = 1.9 – 2.7; p < 0.001]. Most animals

with LCTB had only one carcass site affected (97.2% - wild boar; 94.3% - red deer). The distribution of these single lesions differed significantly between the two species (Table 3); in wild boar, single lesions were located mostly in the head (80.4%) while in red deer, most were located in the abdomen (45.3%) and thorax (33.2%) (p-value <0.001) (Table 3).

**Table 3**. Anatomical location of single LCTB in wild boar and red deer.

| | **Wild boar (%)** | **Red deer (%)** |
|---|---|---|
| Head | 251 (80.4) | 25 (10.1) |
| Thorax | 10 (3.2) | 82 (33.2) |
| Abdomen | 23 (7.4) | 112 (45.3) |
| Other | 28 (9.0) | 28 (11.3) |
| **Total** | **312 (100)** | **247 (100)** |

Multiple lesions were detected in nine wild boar (2.8%) and 15 red deer (5.7%), and of these 19 (79.2%) were positive on culture. Most multiple lesions In wild boar were located in the head and thorax or in the head and abdomen, while for red deer the most common multiple location comprised the thorax and abdomen (Table 4).

**Table 4**. Distribution of multiple lesions in wild boar and red deer.

| | **Wild boar** | **Red deer** |
|---|---|---|
| Head and thorax | 4 | 0 |
| Head and abdomen | 3 | 0 |
| Head and other | 1 | 0 |
| Thorax and abdomen | 1 | 10 |
| Thorax and other | 0 | 4 |
| Abdomen and other | 0 | 1 |
| **Total** | **9** | **15** |

***Apparent prevalence of infection with M. bovis and other Mycobacteria***

The apparent prevalence of *M. bovis* in wild boar was significantly higher than in red deer [191/2191 (8.72%; 95% CI = 7.57 – 9.98) and 222/3733 (5.95%; 95% CI = 5.21 – 6.75) respectively (OR = 1.5; 95% CI = 1.2-1.8; p <0.001)]. However, *M. bovis* was isolated from a significantly higher percentage of red deer with LCTB than from wild

boar with LCTB [222 (84.7%) and 191 (59.5%) respectively; OR = 3.8; 95% CI = 2.5 – 5.8; p <0.001].

*M. bovis* was the most frequent species of mycobacteria isolated (Table 5). *M. avium* was isolated from significantly more lesions in wild boar than red deer (8.1% vs. 0.8%; OR=0.09, 95% CI = 0.01 - 0.36; p < 0.001) and two wild boar were positive to *M. tuberculosis* compared to no isolation in red deer.

**Table 5.** Results of bacterial culture of samples from wild boar and red deer with LCTB.

| | **Wild boar** | | **Red deer** | |
|---|---|---|---|---|
| | **Number (%) of positive isolation** | **Apparent prevalence (%), CI 95%** | **Number (%) of positive isolation** | **Apparent prevalence (%), CI 95%** |
| ***M. bovis*** | 191 (59.5) | 8.72 [7.57; 9.98] | 222 (84.7) | 5.95 [5.21; 6.75] |
| ***M. avium*** | 26 (8.1) | 1.19 [0.78; 1.73] | 2 (0.8) | 0.05 [0.01; 0.19] |
| ***M. tuberculosis*** | 2 (0.6) | 0.09 [0.01; 0.33] | 0 (0.0) | 0 |
| **Non-tuberculous mycobacterium** | 10 (3.1) | 0.40 [0.22; 0.84] | 3 (1.1) | 0.08 [0.02; 0.23] |
| **Negative** | 84 (26.2) | - | 33 (12.6) | - |
| **Na** | 8 (2.5) | - | 2 (0.8) | - |
| **Total** | **321 (100)** | **-** | **262 (100)** | - |

Na- inconclusive results

In red deer, the probability of isolating *M. bovis* from LCTB was very similar across all body locations (p=0.84) while in wild boar there was considerable variation, although the differences were not statistically significant (p=0.06).

**Table 6**. Anatomic distribution of *M. bovis* isolations in animals with single LCTB.

| | **Wild boar** | | **Red deer** | |
|---|---|---|---|---|
| | **Number (%) of animals with *M. bovis* isolation** | **Rate of *M. bovis* isolation in LCTB in each body location** | **Number (%) of animals with *M. bovis* isolation** | **Rate of *M. bovis* isolation in LCTB in each body location** |
| Head | 151 (81.2) | 60.2 | 20 (9.6) | 80.0 |
| Thorax | 3 (1.6) | 30.0 | 70 (33.6) | 85.4 |
| Abdomen | 7 (3.8) | 30.4 | 98 (47.1) | 87.5 |
| Other | 25 (13.4) | 89.3 | 20 (9.6) | 71.4 |
| **Total** | **186 (100)** | **59.6** | **208 (100)** | **84.2** |

***Spatial patterns of LCTB and M. bovis isolation***

Data on cattle, wild boar and red deer densities, LCTB apparent prevalence and BTB prevalence are presented, by parish, in Table 1 Annex. Parishes with higher apparent prevalence of *M. bovis* in wild boar were also those with the highest red deer densities.

The results of the correlation analysis at parish level are presented in table 7. High correlations occurred between red deer density and prevalence of LCTB / *M. bovis* in red deer (r= 0.876 and r= 0.882 respectively) as well with prevalence of LCTB / *M. bovis* in wild boar (r=0.670 and r=0.783 respectively). By contrast, prevalence of LCTB / *M. bovis* in wild boar only show very weak correlation with wild boar density (r=0.034 and r=-0.057 respectively).

**Table 7.** Correlation matrix of variables cattle density, red deer density, wild boar density, prevalence of LCTB in red deer, prevalence of *M. bovis* in red deer, prevalence of LCTB in wild boar, prevalence of *M. bovis* in wild boar, at parish level.

| | **Density Cattle** | **Density RD** | **Density WB** | **Prev LCTB RD** | **Prev LCTB WB** | **Prev *M. bovis* RD** | **Prev *M. bovis* WB** |
|---|---|---|---|---|---|---|---|
| **Density Cattle** | 1 | -0.212 | 0.065 | -0.161 | 0.049 | -0.221 | -0.067 |
| **Density RD** | - | 1 | 0.177 | 0.876 | 0.670 | 0.882 | 0.783 |
| **Density WB** | - | - | 1 | 0.298 | 0.034 | 0.227 | -0.057 |
| **Prev LCTB RD** | - | - | - | 1 | 0.683 | 0.957 | 0.750 |
| **Prev LCTB WB** | - | - | - | - | 1 | 0.614 | 0.956 |
| **Prev *M. bovis* RD** | - | - | - | - | - | 1 | 0.676 |

Prev - prevalence; LCTB - Lesions compatible with tuberculosis; WB - wild boar; RD - red deer.

Cluster analysis identified Rosmaninhal as a parish with a significantly higher risk for *M. bovis* infection for red deer and cattle than the other parishes in the high-risk area (Red deer high-risk cluster: Relative risk (RR) =3.4; p-value=$2.2e^{-16}$; Cattle high-risk cluster: RR = 70.2; p-value< $1.0e^{-17}$). For wild boar, the identified high prevalence spatial cluster is bigger including not only Rosmaninhal but also 2 contiguous counties:

Monforte da Beira and Zebreira (Wild boar high-risk cluster: RR=6.8; p-value< 1.0e-$^{17}$) (Fig. 4).

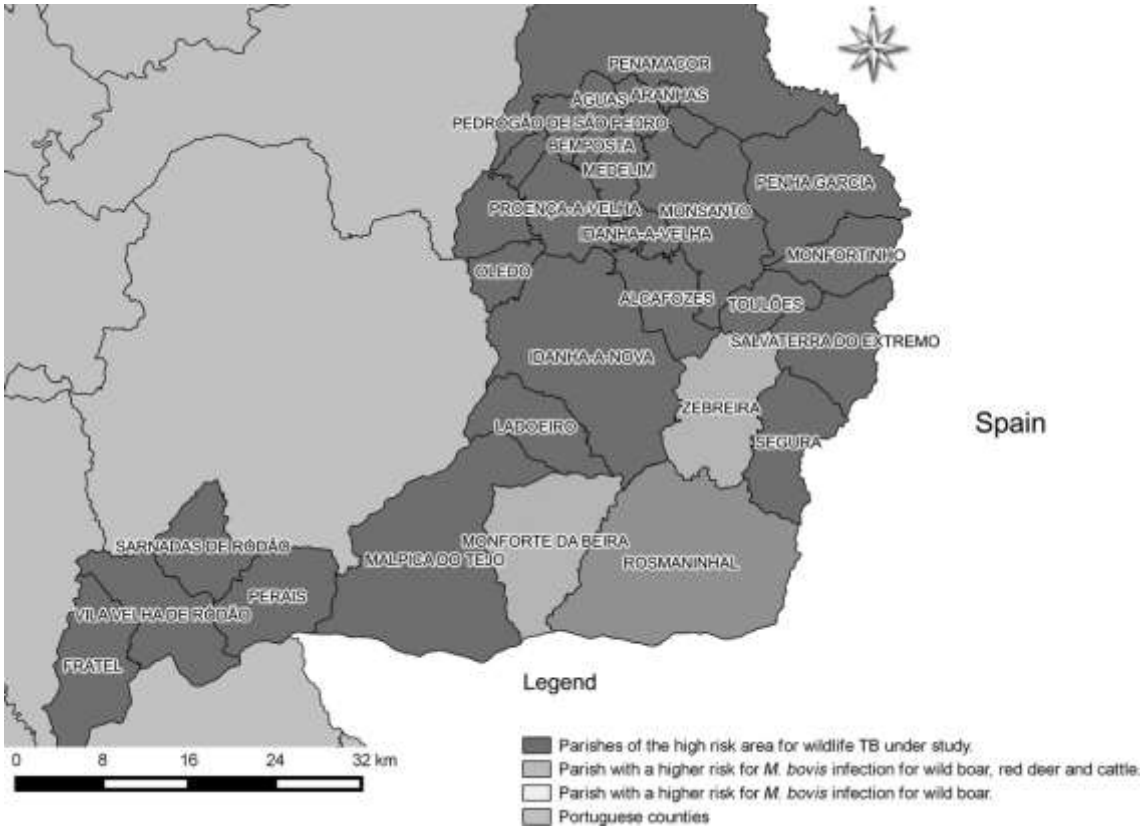


**Figure 4.** Parishes with higher risk for *Mycobacterium bovis* infection in red deer, wild boar and cattle, identified by the cluster analysis, within the officially defined high-risk area for wildlife tuberculosis.

### *Determinants of the likelihood of LCTB and M. bovis in wild boar and red deer*

The results of the final logistic regression models are presented in Table 8. Factors significantly associated with the likelihood of LCTB in wild boar were density of wild boar, density of red deer and density of cattle. For red deer only density of red deer and age group were significant, with adult animals being almost 2.5 times more likely to have LCTB than young animals.

**Table 8**. Final logistic regression models for determinants of the likelihood of LCTB in wild boar and red deer.

| Species | Variables | P-value | Coefficients | OR and 95% CI |
|---|---|---|---|---|
| **Wild boar** | Intercept<br>Density WB<br>Density RD<br>Density Cattle | $<2e^{-16}$ ***<br>0.0001 ***<br>$< 2e^{-16}$ ***<br>0.0275 * | -2.1469 0.3698<br>0.2878 0.0053 | 0.12 [0.08;0.17] 0.69 [0.57;0.83] 1.33 [1.26;1.41] 1.01 [1.00;1.01] |
| **Red deer** | Intercept<br>Density RD<br>Age group ('adult'=1) | $< 2e^{-16}$ ***<br>$< 2e^{-16}$ ***<br>$1.72e^{-05}$ *** | -4.9611<br>0.3029<br>0.9007 | 0.007 [0.00; 0.01]<br>1.35 [1.27;1.45]<br>2.46 [1.66; 3.79] |

* Significant ; *** Highly significant; WB – Wild boar; RD – Red deer; OR – Odds ratio; CI – Confidence interval

Factors affecting the likelihood of *M. bovis* isolation (given LCTB) were also analyzed (Table 9), and for wild boar those were the density of red deer, density of cattle and the location of the lesions in the body, with lesions located in the abdomen and the thorax having a lower chance of *M. bovis* isolation compared to the head, used as reference (WB density, although a significant predictor for the likelihood of LCTB, was not a statistically significant determinant of *M. bovis* isolation – p-value= 0.6). For red deer none of the variables tested were statistically significant predictors for the likelihood of isolating *M. bovis* from LCTB.

**Table 9**. Final logistic regression model for determinants of the likelihood of *M. bovis* isolation in wild boar (for red deer none of the variables tested were statistically significant predictors of the likelihood of *M. bovis* isolation).

| Species | Variables | P-value | Coefficients | OR and 95% CI |
|---|---|---|---|---|
| **Wild boar** | Intercept<br>Density RD<br>Density Cattle<br>Location on the body (head as reference) | 0.1267<br>0.0036 **<br>0.0022 **<br>other: 0.0735<br>abdomen: 0.0185 *<br>thorax: 0.034* | 0.56<br>0.18<br>-0.02<br>1.14<br>-1.18<br>-1.56 | 1.75 [0.86; 3.64]<br>1.20 [1.06; 1.36]<br>0.98 [0.97; 0.99]<br>3.14 [1.03; 13.70]<br>0.31 [0.11; 0.80]<br>0.21 [0.04; 0.83] |

* Significant ; ** Very significant; RD – Red deer; OR – Odds ratio; CI – Confidence interval

## Discussion

A species can act as maintenance host (where infection can persist without external source) of *M. bovis* or spillover host (infection disappears when disease eliminated

from external source) depending on factors such as animal density, environmental factors and management practices (Corner, 2006; Gortázar et al., 2006; Vicente et al., 2007., Palmer et al., 2012). Examples of maintenance hosts are the Eurasian badger (*Meles meles*) in the United Kingdom, white tailed deer (*Odocoileus virgianus*) in the United States and the brushtailed possum (*Trichosurus vulpecula*) in New Zealand (Corner, 2006). In cases of overabundance and when effective intra-species transmission occurs, a spillover host can become a maintenance host as occurred with the ferret (*Mustela furo*) in New Zealand (Ryan et al., 2006).

In the Iberian Peninsula, wild boar and red deer are considered to be maintenance hosts as some populations maintain a high bTB prevalence rates despite long-term lack of contact with cattle (Gortázar et al., 2008; Vicente et al., 2006). Others suggest that *M. bovis* is a multihost pathogen within a multi-species ecosystem (Gortázar et al., 2012; Renwick et al., 2007) in which pathogen persistence and spread is dependent on the density of each maintenance host species and also on the effective interspecies contact rate. In this multihost system, and where host densities vary widely between areas, the distinction between maintenance and spillover host is likely to be blurred (Nugent, 2011). Understanding the factors that influence *M. bovis* infection can assist veterinary authorities in controlling the disease as the most efficient disease control efforts are aimed at maintenance hosts (Palmer et al., 2012).

The study area is known as the last stronghold for red deer in Portugal as large populations of red deer are known to occur (Cunha et al., 2012), reflected in the high number of red deer killed during the three seasons under study (63% of all the hunted animals.

In the study population, the apparent prevalence of *M. bovis* in the sampled carcasses (8.7% in wild boar [95% CI = 7.57 - 9.98], 5.9% in red deer [95% CI = 5.21 – 6.75] were lower, but not significantly different than a previous survey in the same area by Vieira-Pinto et al., (2011) who identified a prevalence of 15.9% (±15.6) for wild boar and 10.3% (± 10.08) for red deer. A significantly higher number of wild boar were positive for *M. avium* and *M. tuberculosis*, as well as other non-tuberculous mycobacterium, than red deer. *M. tuberculosis* in the two wild boar was likely due to their scavenging nature, omnivorous feeding habits and proximity to human populations (Cahill et al., 2012).

In both species, animals were found with multiple lesions but the proportion was higher in red deer. Such lesions may arise from reduced genetic resistance whereby the immune system may not be capable of containing the infection, with subsequent generalization of disease (Acevedo-Whitehouse et al., 2005; Clifton-Hadley and Wilesmith, 1991; Mackintosh et al., 2004; Naranjo et al., 2008; Zanella et al., 2008). The distribution of *M. bovis* lesions in the carcass may be linked to the primary route of infection (Biet et al., 2005) and can affect the extent to which an animal species may disseminate the agent in an ecosystem and therefore the epidemiology of bTB in wildlife (Zanella et al., 2008). In the sampled carcasses, the majority of LCTB and *M. bovis* isolations in red deer occurred in the abdomen and the thorax, whereas most lesions in wild boar occurred in the head. Head or mesenteric lymph nodes lesions are more suggestive of the oral/digestive route while lesions in the lungs and associated lymph nodes are indicative of aerogenous infection (Aranaz et al., 2004; Cunha et al., 2012). Wild boars usually eat carrion, which might explain the high number of lesions associated with the digestive route of infection (Gortázar et al., 2003) while for red

deer, there appeared to be a combination of digestive and respiratory routes. In some hunting regions in Spain, lesions have been recorded mainly in the thoracic lymph nodes and lungs in wild boar (Gortázar et al., 2003; Parra et al., 2006) and in the head lymph nodes in others (Martín-Hernando et al., 2007). Such geographical differences likely reflect differences in environmental factors such as animal density, gatherings of animals or availability of food (Parra et al., 2006). Our findings agree with Martín-Hernando et al., (2010) who reported lesions more commonly in the abdominal region than in the thoracic region in red deer. In this species lesions have been also described in lungs as well as mesenteric, tracheobronchial, and medial retropharyngeal lymph nodes. (Cunha et al., 2012; Martín-Hernando et al., 2010; Zanella et al., 2008). Lesions located in the lungs, as is the case for red deer, can lead to excretion of mycobacteria through respiratory secretions and contamination of feed (Johnson et al., 2008; Palmer et al., 2001). This may have epidemiological significance where close contact occurs or in cases of environmental, feed or water contamination.

For red deer the likelihood of LCTB was significantly influenced by red deer density and age, while the density of wild boar did not seem to have a measurable effect. The positive association with age is expected and explained by the endemic and chronic nature of bTB in red deer in this area.

In wild boar, the presence of LCTB was influenced by the density of red deer, density of wild boar and density of cattle. The likelihood of *M. bovis* isolation from wild boar presenting LCTB was significantly affected by densities of red deer and cattle in the parish and *M. bovis* was more likely to be isolated from LCTB in the head than the thorax or abdomen. The density of red deer was the strongest determinant for both the likelihood of LCTB and *M. bovis* isolation in wild boar (r = 0.67 and 0.88,

coefficients: 0.28 and 0.18 respectively) while cattle density, although statistically significant, had a weak positive (r = 0.049, coefficient: 0.005) and a weak negative (r = -0.067, coefficient: -0.02) effect on both outcomes. Paradoxically, wild boar density had a negative coefficient in the LCTB likelihood model, most likely due to the low density of wild boar in those counties with higher prevalence of *M. bovis* infection in wild boar but which have high red deer density and associated high bTB prevalence. Such findings are consistent with a hypothesis of red deer being the driving force behind *M. bovis* transmission to wild boar. Highest red deer densities are recorded in the parish of Rosmaninhal, which has the highest prevalence of bTB for the three species studied. The parish is bound by two rivers, Tejo in the south and Erges in the east and is very close to the Spanish border, which facilitates the movement of animals from one country to another. The region is a remote and wild untouched area with little human presence, so the animals tend to aggregate and become established there.

Gortázar et al., (2007) state that when wild boars coexist with deer, 84% of the populations of wild boar were TB-positive whereas only 75% were TB-positive when wild boar were present in the absence of deer. Nebbia et al., (2000) and Vicente et al., (2006), have also referred to the red deer overabundance as a risk factor for mycobacterial diseases in the species. This often reflects management practices such as feed supplementation, which affects both the population dynamics and the behavior of the animals, promoting aggregation and increased contact and thereby increased probability of transmission between individuals (Castillo et al., 2011; Martínez-López et al., 2014). In all the models, red deer density was consistently the most relevant and significant factor influencing the presence of LCTB and *M. bovis* infection in both wild species. This, together with the together with the apparent

higher susceptibility of red deer, expressed by the higher proportion of multiple lesions, and the possible higher potential for excretion of the disease agent, denoted by the location of lesions, are all consistent with the prominent role for red deer in the epidemiology of bTB. Their role is exacerbated by the environmental contamination with mycobacteria, particularly at watering sites, and other indirect routes, which could also play a role in disease transmission (Santos et al., 2012).

The results of this study suggest that the roles of wild boar and red deer in the study area are different from those described in other countries and in the Iberian Peninsula region, whereby red deer may be the actual infectious force, acting more as a maintenance host with spillover to wild boar, at least in the red deer habitat. Control strategies should therefore focus on limiting contact between wild species, and between them and cattle, and removing or reducing artificial feeding to avoid animal concentrations. The zoonotic potential of *M. bovis* , particularly for abattoir workers, farmers, veterinarians, hunters and forest guards (Aranaz et al., 1999; Cunha et al., 2012) necessitates ongoing surveillance and control and analyses such as descrived here, to improve understanding of the epidemiology of the disease.

**Acknowledgements**

The authors would like to thank Eng. Tiago Honrado for the field photos (Fig. 1 and Fig. 2), Professor Carla Nunes for her statistical support, Eng. Luís Caiola for advice on geographical information systems and database, and CIISA (UID/CVT/00276/2013) for financing this publication.

Thanks are also due to the game managers and veterinarians in the field responsible for the initial examination, for their role in the collection of samples.

We acknowledge also all the laboratory staff responsible for the laboratory analysis and results.

**Conflict of interest statement**

The authors declare that no conflicting financial or personal interests exist.

## References

Acevedo-Whitehouse, K., Vicente, J., Gortazar, C., Höfle, U., Fernandez-de-Mera, I.G., et al, 2005. Genetic resistance to bovine tuberculosis in the Iberian wild boar. Mol. Ecol. 14, 3209–3217.

Anonymous, 2013. Working document on bovine tuberculosis infection rate. Direção Geral de Alimentação e Veterinária (DGAV).

Aranaz, A., de Juan, L., Montero, N., Sanchez, C., Galka, M., Delso, C., Alvarez, J., Romero, B., Bezos, J., Vela, A.I., Briones, V., Mateos, A., Dominguez, L., 2004. Bovine Tuberculosis (Mycobacterium bovis) in Wildlife in Spain. J. Clin. Microbiol. 42, 2602–2608. doi:10.1128/JCM.42.6.2602-2608.2004

Aranaz, A., Liébana, E., Gómez-Mampaso, E., Galán, J.C., Cousins, D., Ortega, A., Blázquez, J., Baquero, F., Mateos, A., Súarez, G., Domínguez, L., 1999. Mycobacterium tuberculosis subsp. caprae subsp. nov.: a taxonomic study of a new member of the Mycobacterium tuberculosis complex isolated from goats in Spain. Int. J. Syst. Bacteriol. 49 Pt 3, 1263–1273.

Biet, F., Boschiroli, M.L., Thorel, M.F., Guilloteau, L.A., 2005. Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Vet. Res. 36, 411–436. doi:10.1051/vetres:2005001

Cahill, S., Llimona, F., Cabañeros, L., Calomardo, F., 2012. Characteristics of wild boar (Sus scrofa) habituation to urban areas in the Collserola Natural Park (Barcelona) and comparison with other locations. Anim. Biodivers. Conserv. 35, 221–233.

Castillo, L., Fernández-Llario, P., Mateos, C., Carranza, J., Benítez-Medina, J.M., García-Jiménez, W., Bermejo-Martín, F., Hermoso de Mendoza, J., 2011. Management practices and their association with Mycobacterium tuberculosis complex prevalence in red deer populations in Southwestern Spain. Prev. Vet. Med. 98, 58–63. doi:10.1016/j.prevetmed.2010.11.008

Clifton-Hadley, R.S., Wilesmith, J.W., 1991. Tuberculosis in deer: a review. Vet. Rec. 129, 5–12.

Coelho, A.C., Pinto, M. de L., Matos, A., Matos, M., Pires, M. dos A., 2013. Mycobacterium avium Complex in Domestic and Wild Animals, in: Payan Carreira, R. (Ed.), Insights from Veterinary Medicine. InTech.

Corner, L.A.L., 2006. The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: How to assess the risk. Vet. Microbiol. 112, 303–312. doi:10.1016/j.vetmic.2005.11.015

Cunha, M.V., Matos, F., Canto, A., Albuquerque, T., Alberto, J.R., Aranha, J.M., Vieira-Pinto, M., Botelho, A., 2012. Implications and challenges of tuberculosis in wildlife ungulates in

Portugal: A molecular epidemiology perspective. Res. Vet. Sci. 92, 225–235. doi:10.1016/j.rvsc.2011.03.009
Cunha, M.V., Monteiro, M., Carvalho, P., Mendonça, P., Albuquerque, T., Botelho, A., 2011. Multihost Tuberculosis: Insights from the Portuguese Control Program. Vet. Med. Int. 2011, 1–10. doi:10.4061/2011/795165
De Lisle, G.W., Bengis, R.G., Schmitt, S.M., O'Brien, D.J., 2002. Tuberculosis in free-ranging wildlife: detection, diagnosis and management. Rev Sci Tech Int Epiz 21, 317–334.
DGAV, 2011. Edital N$^{o}$ 1 - Tuberculose em Caça Maior.
DGAV, D.G. de A. e V., 2015. Programa Nacional de Controlo e Erradicação da Tuberculose Bovina para 2015.
Duarte, E.L., Domingos, M., Amado, A., Botelho, A., 2008. Spoligotype diversity of Mycobacterium bovis and Mycobacterium caprae animal isolates. Vet. Microbiol. 130, 415–421. doi:10.1016/j.vetmic.2008.02.012
EC, 2012. Decisão de Execução n.$^{o}$ 2012/204/UE.
EC, E.C., 2008. Commission Decision 2008/425/EC of 25 April 2008, 2008/425/EC.
EC, E.C., 2004. Regulation 853/2004 of the European Parliament and of the Council of 29 April 2004.
García-Jiménez, W.L., Benítez-Medina, J.M., Fernández-Llario, P., Abecia, J.A., García-Sánchez, A., Martínez, R., Risco, D., Ortiz-Peláez, A., Salguero, F.J., Smith, N.H., Gómez, L., Hermoso de Mendoza, J., 2013. Comparative Pathology of the Natural infections by Mycobacterium bovis and by Mycobacterium caprae in Wild Boar (Sus scrofa): Natural Infection of Wild Boar by Mycobacterium bovis and Mycobacterium caprae. Transbound. Emerg. Dis. 60, 102–109. doi:10.1111/j.1865-1682.2012.01321.x
Gavier-Widén, D., Cooke, M.M., Gallagher, J., Chambers, M.A., Gortázar, C., 2009. A review of infection of wildlife hosts with Mycobacterium bovis and the diagnostic difficulties of the "no visible lesion" presentation. N. Z. Vet. J. 57, 122–131. doi:10.1080/00480169.2009.36891
Glawischnig, W., Allerberger, F., Messner, C., Schonbauer, M., Prodinger, W.M., 2003. Tuberculosis in free-living red deer (Cervus elaphus hippelaphus) in the northern Alps. Wien. Tierarztl. Monatsschr. 90, 38–44.
Gortázar, C., Acevedo, P., Ruiz-Fons, F., Vicente, J., 2006. Disease risks and overabundance of game species. Eur. J. Wildl. Res. 52, 81–87. doi:10.1007/s10344-005-0022-2
Gortázar, C., Delahay, R.J., Mcdonald, R.A., Boadella, M., Wilson, G.J., Gavier-Widen, D., Acevedo, P., 2012. The status of tuberculosis in European wild mammals: The status of TB in European wild mammals. Mammal Rev. 42, 193–206. doi:10.1111/j.1365-2907.2011.00191.x
Gortázar, C., Ferroglio, E., Höfle, U., Frölich, K., Vicente, J., 2007. Diseases shared between wildlife and livestock: a European perspective. Eur. J. Wildl. Res. 53, 241–256. doi:10.1007/s10344-007-0098-y
Gortázar, C., Torres, M.J., Vicente, J., Acevedo, P., Reglero, M., de la Fuente, J., Negro, J.J., Aznar-Martín, J., 2008. Bovine Tuberculosis in Doñana Biosphere Reserve: The Role of Wild Ungulates as Disease Reservoirs in the Last Iberian Lynx Strongholds. PLoS ONE 3, e2776. doi:10.1371/journal.pone.0002776
Gortázar, C., Vicente, J., Gavier-Widen, D., 2003. Pathology of bovine tuberculosis in the European wild boar(Sus scrofa). Vet. Rec. 152, 779–780.
Johnson, L.K., Liebana, E., Nunez, A., Spencer, Y., Clifton-Hadley, R.S., et al., 2008. Histological observations of bovine tuberculosis in lung and lymph node tissues from British deer. Vet. J. 175, 409–412.
Mackintosh, C.G., De Lisle, G.W., Collins, D.M., Griffin, J.F., 2004. Mycobacterial diseases of deer. N. Z. Vet. J. 52, 163–174.
Martínez-López, B., Barasona, J.A., Gortázar, C., Rodríguez-Prieto, V., Sánchez-Vizcaíno, J.M., Vicente, J., 2014. Farm-level risk factors for the occurrence, new infection or

persistence of tuberculosis in cattle herds from South-Central Spain. Prev. Vet. Med. 116, 268–278. doi:10.1016/j.prevetmed.2013.11.002

Martín-Hernando, M.P., Höfle, U., Vicente, J., Ruiz-Fons, F., Vidal, D., Barral, M., Garrido, J.M., de la Fuente, J., Gortazar, C., 2007. Lesions associated with Mycobacterium tuberculosis complex infection in the European wild boar. Tuberculosis 87, 360–367. doi:10.1016/j.tube.2007.02.003

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. J. Comp. Pathol. 142, 43–50. doi:10.1016/j.jcpa.2009.07.003

Miller, R., Kaneene, J.B., Fitzgerald, S.D., Schmitt, S.M., 2003. Evaluation of the influence of supplemental feeding of white-tailed deer (Odocoileus virginianus) on the prevalence of bovine tuberculosis in the Michigan wild deer population. J. Wildl. Dis. 39, 84–95. doi:10.7589/0090-3558-39.1.84

Naranjo, V., Gortazar, C., Vicente, J., de la Fuente, J., 2008. Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. Vet. Microbiol. 127, 1–9. doi:10.1016/j.vetmic.2007.10.002

Nebbia, P., Robino, P., Ferroglio, E., Rossi, L., Meneguz, G., Rosati, S., 2000. Paratuberculosis in red deer (Cervus elaphus hippelaphus) in the western Alps. Vet. Res. Commun. 24, 435–443.

Nugent, G., 2011. Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: A New Zealand case study. Vet. Microbiol. 151, 34–42. doi:10.1016/j.vetmic.2011.02.023

Palmer, M.V., Thacker, T.C., Waters, W.R., Gortázar, C., Corner, L.A.L., 2012. Mycobacterium bovis: A Model Pathogen at the Interface of Livestock, Wildlife, and Humans. Vet. Med. Int. 2012, 1–17. doi:10.1155/2012/236205

Palmer, M.V., Whipple, D.L., Waters, W.R., 2001. Experimental deer-to-deer transmission of Mycobacterium bovis. Am. J. Vet. Res. 62, 692–696.

Parra, A., García, A., Inglis, N.F., Tato, A., Alonso, J.M., Hermoso de Mendoza, M., Hermoso de Mendoza, J., Larrasa, J., 2006. An epidemiological evaluation of Mycobacterium bovis infections in wild game animals of the Spanish Mediterranean ecosystem. Res. Vet. Sci. 80, 140–146. doi:10.1016/j.rvsc.2005.05.010

R Development Core Team, 2008. R.

Renwick, A.R., White, P.C.L., Bengis, R.G., 2007. Bovine tuberculosis in southern African wildlife: a multi-species host-pathogen system. Epidemiol. Infect. 135, 529–540. doi:10.1017/S0950268806007205

Rodríguez, S., Bezos, J., Romero, B., de Juan, L., Álvarez, J., Castellanos, E., Moya, N., Lozano, F., Javed, M.T., Sáez-Llorente, J.L., Liébana, E., Mateos, A., Domínguez, L., Aranaz, A., Spanish Network on Surveillance and Monitoring of Animal Tuberculosis, 2011. Mycobacterium caprae infection in livestock and wildlife, Spain. Emerg. Infect. Dis. 17, 532–535. doi:10.3201/eid1703.100618

Ryan, T.J., Livingstone, P.G., Ramsey, D.S.L., de Lisle, G.W., Nugent, G., Collins, D.M., Buddle, B.M., 2006. Advances in understanding disease epidemiology and implications for control and eradication of tuberculosis in livestock: the experience from New Zealand. Vet. Microbiol. 112, 211–219. doi:10.1016/j.vetmic.2005.11.025

Santos, N., Correia-Neves, M., Almeida, V., Gortázar, C., 2012. Wildlife tuberculosis: a systematic review of the epidemiology in Iberian Peninsula. Epidemiol. Insights.

Santos, N., Correia-Neves, M., Ghebremichael, S., Källenius, G., Svenson, S.B., Almeida, V., 2009. Epidemiology of Mycobacterium bovis infection in wild boar (Sus scrofa) from Portugal. J. Wildl. Dis. 45, 1048–1061.

Santos, N., Geraldes, M., Afonso, A., Almeida, V., Correia-Neves, M., 2010. Diagnosis of tuberculosis in the wild boar (Sus scrofa): a comparison of methods applicable to hunter-harvested animals. PloS One 5. doi:10.1371/journal.pone.0012663

Vicente, J., Barasona, J.A., Acevedo, P., Ruiz-Fons, J.F., Boadella, M., Diez-Delgado, I., Beltran-Beck, B., González-Barrio, D., Queirós, J., Montoro, V., de la Fuente, J., Gortazar, C., 2013. Temporal trend of tuberculosis in wild ungulates from Mediterranean Spain. Transbound. Emerg. Dis. 60 Suppl 1, 92–103. doi:10.1111/tbed.12167

Vicente, J., Höfle, U., Garrido, J.M., Fernández-de-mera, I.G., Acevedo, P., Juste, R., Barral, M., Gortazar, C., 2007. Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in south central Spain. Vet. Res. 38, 451–464. doi:10.1051/vetres:2007002

Vicente, J., Höfle, U., Garrido, J.M., Fernández-De-Mera, I.G., Juste, R., Barral, M., Gortazar, C., 2006. Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Vet. Res. 37, 107–119. doi:10.1051/vetres:2005044

Vieira-Pinto, M., Alberto, J., Aranha, J., Serejo, J., Canto, A., Cunha, M.V., Botelho, A., 2011. Combined evaluation of bovine tuberculosis in wild boar (Sus scrofa) and red deer (Cervus elaphus) from Central-East Portugal. Eur. J. Wildl. Res. 57, 1189–1201. doi:10.1007/s10344-011-0532-z

Vingada, J., Fonseca, C., Cancela, J., Ferreira, J., Eira, C., 2010. Ungulates and their management in Portugal., in: European Ungulates and Their Management in the 21st Century. Cambridge University Press, pp. 392–418.

Wilson, K., Bjørnstad, O.N., Dobson, A.P., Merler, S., Poglayen, G., Randolph, S.E., Read, A.F., Skorping, A., 2002. Heterogeneities in macroparasite infections: patterns and processes. Ecol. Wildl. Dis. 6–44.

Zanella, G., Duvauchelle, A., Hars, J., Mouton, F., Boschiroli, M.L., Durand, B., 2008. Patterns of lesions of bovine tuberculosis in wild red deer and wild boar. Vet. Rec. 163, 43–47.

## Annexes:

**Table 1 Annex** - Density, number of hunted animals, number of animals presenting LSTB and *M. bovis* positive animals, proportion of animals with LCTB and *M. bovis* apparent prevalence with confidence intervals at 95%, by parish and for the 3 species under study.

| | WILD BOAR | | | | | | RED DEER | | | | | | CATTLE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parishes** | **Density (animals /Km$^2$)** | **Hunted** | **LSTB** | ***M. bovis*** | **%LSTB/ hunted** | **% Apparent prevalence and CI 95%** | **Density (animals/Km$^2$)** | **Hunted** | **LSTB** | ***M. bovis*** | **%LSTB/ hunted** | **% Apparent prevalence and CI 95%** | **Density (animals/Km$^2$)** | **Tested** | ***M. bovis*** | **Apparent prevalence and CI 95%** |
| **Águas** | 0.07 | 1 | 1 | 0 | 100.0 | 0.0 [0; 98] | 0.0 | 0 | 0 | 0 | - | - | 27.52 | 417 | 0 | 0.0 [0;0.9] |
| **Alcafozes** | 0.7 | 40 | 3 | 1 | 7.5 | 2.5 [0.06; 13.2] | 0.14 | 8 | 0 | 0 | 0.0 | 0.0 [0; 36.9] | 72.7 | 4131 | 0 | 0.0 [0;0.09] |
| **Aldeia de Santa Margarida** | 0.44 | 6 | 0 | 0 | 0.0 | 0.0 [0; 46] | 0.0 | 0 | 0 | 0 | - | - | 7.33 | 100 | 0 | 0.0 [0;3.6] |
| **Aranhas** | 0.91 | 5 | 0 | 0 | 0.0 | 0.0 [0; 52] | 0.55 | 3 | 0 | 0 | 0.0 | 0.0 [0; 70.8] | 0 | 0 | 0 | - |
| **Fratel** | 0.75 | 73 | 5 | 3 | 6.8 | 4.1 [0.9; 11.5] | 0.54 | 53 | 1 | 1 | 1.9 | 1.9 [0.05;10] | 0.17 | 17 | 0 | 0.0 [0;19.5] |
| **Idanha-a-Nova** | 1.05 | 240 | 31 | 4 | 1.,9 | 1.7 [0.46; 4.2] | 0.01 | 2 | 0 | 0 | 0.0 | 0.0 [0; 84.2] | 88.73 | 20228 | 10 | 0.05 [0.02; 0.09] |
| **Idanha-a-Velha** | 0.96 | 20 | 0 | 0 | 0.0 | 0.0 [0; 16.8] | 0.29 | 6 | 0 | 0 | 0.0 | 0.0 [0; 45.9] | 40.47 | 841 | 0 | 0.0 [0;0.4] |
| **Ladoeiro** | 0.35 | 22 | 3 | 1 | 13.6 | 4.5 [0.1; 22.8] | 0.0 | 0 | 0 | 0 | - | - | 142.1 | 8990 | 3 | 0.03 [0.01; 0.1] |
| **Lardosa** | 0.31 | 14 | 0 | 0 | 0.0 | 0.0 [0; 23.2] | 0.0 | 0 | 0 | 0 | - | - | 1.64 | 73 | 0 | 0.0 [0;4.9] |
| **Malpica do Tejo** | 0.87 | 215 | 52 | 24 | 24.2 | 11.2 [7.3; 16.2] | 1.95 | 480 | 16 | 12 | 3.3 | 2.5 [1.3; 4.3] | 13.82 | 3401 | 3 | 0.09 [0.02; 0.3] |
| **Monforte da Beira** | 0.85 | 102 | 66 | 34 | 64.7 | 33.3 [24.3; 43.6] | 3.64 | 438 | 22 | 19 | 5.0 | 4.3 [2.6; 6.7] | 10.47 | 1260 | 2 | 0.2 [0.02;0.6] |
| **Monfortinho** | 2.47 | 133 | 21 | 10 | 15.8 | 7.5 [3.7; 13.4] | 0.56 | 30 | 1 | 0 | 3.3 | 0.0 [0; 11.6] | 34.48 | 1855 | 0 | 0.0 [0;0.2] |
| **Monsanto** | 0.17 | 23 | 0 | 0 | 0.0 | 0.0 [0; 14.8] | 0.01 | 2 | 0 | 0 | 0.0 | 0.0 [0; 84.2] | 8.99 | 1187 | 0 | 0.0 [0;0.3] |
| **Oledo** | 3.91 | 108 | 2 | 0 | 1.9 | 0.0 [0; 3.4] | 0.0 | 0 | 0 | 0 | - | - | 98.96 | 2735 | 0 | 0.0 [0;0.1] |
| **Penamacor** | 0.06 | 21 | 0 | 0 | 0.0 | 0.0 [0; 16.1] | 0.03 | 13 | 0 | 0 | 0.0 | 0.0 [0; 24.7] | 8.13 | 3044 | 0 | 0.0 [0;0.1] |
| **Penha Garcia** | 1.81 | 233 | 14 | 9 | 6.0 | 3.9 [1.8; 7.2] | 2.83 | 363 | 2 | 2 | 0.6 | 0.6 [0.07; 2.0] | 16.21 | 2082 | 0 | 0.0 [0;0.2] |
| **Perais** | 1.28 | 105 | 15 | 8 | 14.3 | 7.6 [3.4; 14.5] | 0.93 | 76 | 1 | 1 | 1.3 | 1.3 [0.03; 7.1] | 7.22 | 592 | 0 | 0.0 [0;0.6] |
| **Rosmaninhal** | 0.96 | 256 | 95 | 74 | 37.1 | 28.9 [23; 35] | 6.95 | 1856 | 202 | 172 | 10.9 | 9.3 [8.0; 10.7] | 16.19 | 4322 | 182 | 4.2 [3.6; 4.9] |
| **Salgueiro do Campo** | 0.16 | 5 | 0 | 0 | 0.0 | 0.0 [0; 52.2] | 0.0 | 0 | 0 | 0 | - | - | 0 | 0 | 0 | - |
| **Salvaterra do** | 1.55 | 127 | 9 | 1 | 7.1 | 0.8 [0.02; 4.3] | 0.62 | 51 | 0 | 0 | 0.0 | 0.0 [0; 7.0] | 51.84 | 4258 | 1 | 0.02 [0.0006; 0.1] |

| **Extremo** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **São Miguel de Acha** | 0.46 | 19 | 4 | 0 | 21.1 | 0.0 [0; 17.6] | 0.0 | 0 | 0 | 0 | - | - | 52.55 | 2169 | 11 | 0.5 [0.3; 0.9] |
| **Segura** | 2.44 | 180 | 43 | 14 | 23.9 | 7.8 [4.3; 12.7] | 2.60 | 192 | 14 | 13 | 7.3 | 6.8 [3.7; 11.3] | 34.6 | 2555 | 1 | 0.04[0.001; 0.2] |
| **Touloes** | 1.27 | 46 | 1 | 0 | 2.2 | 0.0 [0; 7.7] | 0.08 | 3 | 0 | 0 | 0.0 | 0.0 [0; 70.8] | 4.26 | 154 | 0 | 0.0 [0;2.4] |
| **Vila Velha de Rodao** | 1.65 | 149 | 3 | 0 | 2.0 | 0.0 [0; 2.5] | 0.9 | 81 | 2 | 2 | 2.5 | 2.5 [0.3; 8.6] | 0 | 0 | 0 | - |
| **Zebreira** | 0.44 | 46 | 14 | 8 | 30.4 | 17.4 [9.1; 30.7] | 0.58 | 60 | 1 | 0 | 1.7 | 0.0 [0; 6.0] | 55.55 | 5738 | 11 | 0.2 [0.1; 0.3] |