Abstract

Body condition (BC) is a measure to assess the health status of domestic and wild animals. When food resources are abundant, a decrease in BC may indicate an increase in the energetic expenditure due to the effects of growth, reproduction, or disease. BC impoverishment is one of the most common clinical effects of diseases progressing chronically, such as animal tuberculosis (TB) caused by the *Mycobacterium tuberculosis* complex. The Eurasian wild boar (*Sus scrofa*) is the main wild TB reservoir in the Mediterranean basin. In this work, we used the kidney fat index (KFI), to assess the impact of TB progression on the BC of 1,372 hunter-harvested free-ranging wild boar in seven populations in southern Spain. Surprisingly, TB had only slight effects on wild boar BC and individuals exhibiting severe TB showed greater BC than TB-free individuals. The age (adults had greater BC than juveniles) and sex (females had greater BC than males) were the main BC determinants in wild boar. Sampling population and season explained more BC variability than individual factors, suggesting that other external factors might play an important role in the BC, and probably on the impact of the disease on this wild reservoir.

Keywords

Animal tuberculosis, body condition, Kidney Fat Index, wild boar, wildlife management

1. Introduction

Animal body condition (BC), defined as the energy stored in tissues to keep functional normal life and to overcome periods of food shortages, may also be affected by the sublethal impact of pathogens. Immune response activity is energetically costly, particularly when hosts face chronic diseases over long periods (Graham et al., 2011; Lochmiller and Deerenberg, 2000; Yoshida and Delafontaine, 2015). The energy cost of mounting an immune response by vertebrates has been suggested to equal that of reproduction and growth (Graham et al., 2011). Ecological, behavioural and physiological differences support sex- and age-related differences in disease susceptibility in wildlife (Alexander and Stimson, 1988; Folstad and Karter, 1992; López-Olvera et al., 2015; Malo et al., 2009). Regarding the sex of the host, females of polygynous species invest more in self-maintenance than males, which prioritize mate attraction by diminishing the energetic resources available to immune function during the breeding season (Landete-Castillejos et al., 2004). Thus, males tend to show worse immune responses than females, a fact that is often exacerbated by the immunosuppressive effects of testosterone (Folstad and Karter, 1992). Consequently, males are often more susceptible to infection than females (López-Olvera et al., 2015), developing a more severe presentation of diseases (Alexander and Stimson, 1988; Malo et al., 2009). This male-biased infection has been reported in the literature for several diseases such as animal tuberculosis (TB), Chronic Wasting Disease (CWD) and many parasitic diseases in deer (De la Peña et al., 2020; Vicente et al., 2013), or cowpox in rodents (Burthe et al., 2008). Likewise, age is another key factor to understand host-pathogen relationships in wildlife. For chronic diseases such as TB (Vicente et al., 2013) or CWD (Miller and Conner, 2005), adults are more likely to be infected than their younger counterparts because of a higher chance of pathogen encounter during life. However, for pathogens with higher induced mortality (e.g. some genotypes of classical swine fever) as the infection period increases, young animals may show higher prevalence than adults, since older infected animals are likely to die at a faster rate (Cross et al., 2009). Age-related differences also occur regarding disease severity since young animals are more prone to suffer the impact of the disease because of the immaturity of their immune system and the limited prior exposure to pathogens stimulating adaptative immunity, together with the energetic costs of development and growth that they are experiencing (Lesage et al., 2001; Solomon, 1978; Vicente et al., 2013).

Despite the assumption of a negative relationship between infection and BC, a wide range of associations between both, from strongly positive to strongly negative, have been reported for wildlife populations (Sánchez et al., 2018). Host-pathogen coevolution implies selective pressure on the hosts, which have to elaborate more robust and diverse immune responses to cope with pathogen adaptations (manipulations in host defences and escaping in detection and elimination by the host immune system; Schmid-Hempel, 2009; Schulenburg et al., 2009). This immune activation increases the energetic demands of the infected host that uses their body reserves to compensate for the cost of infection (Graham et al., 2011; Lochmiller and Deerenberg, 2000). However, tolerance to infection would reduce such energetic demands minimising such cost of infection (e.g. Budischak et al., 2018; Medzhitov et al., 2012).

TB is a zoonotic, chronic and debilitating disease caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MTC) whose natural hosts are wild and domestic mammals (Gortázar et al., 2012). The main clinical sign exhibited by most infected species including humans is emaciation, especially in those individuals with severe disseminated TB (Renwick et al., 2007; Silva and Faccioli, 1988). As consequence, those infected individuals with impoverished BC are more susceptible to disease progression and experience higher pathogen load, further weakening their health status (Beldomenico et al., 2008; Beldomenico and Begon, 2010). In consequence, mortality due to TB infection ranges from 11% in African buffalo (*Syncerus caffer*; Jolles et al., 2005) [21] to 30% in Eurasian wild boar (*Sus scrofa*; Barasona et al., 2016).

TB severity is also positively related to a higher load of mycobacteria with the subsequent elevated MTC DNA shedding (Santos et al., 2015b). These individuals suffering from severe TB, also known as disseminated or widespread tuberculosis, show proliferative lesions in the lungs and in other organs.

The most frequent shedding routes for MTC include the oral and nasal route (through aerosols and exudates) and the faecal route in the most affected animals (Barasona et al., 2017; Santos et al., 2015a). Accordingly, the infection spreads through direct (aerosols or scavenging) and indirect routes (environmental contamination with exudates and faeces), the latter being key for the inter-species transmission (Caron et al., 2003; Morris et al., 1994). Thus, animals showing severe TB are expected to be “super-shedders”, being individuals with poor BC and exhibiting high mycobacteria load. These individuals, which excrete considerable amounts of mycobacteria for long periods (persistently infected) or through several routes simultaneously (Delahay et al., 2000), play a substantial role in the transmission and maintenance of TB in host populations (Santos et al., 2015a). Despite the well-known debilitating effect of TB, there are previous studies on ungulates that evidenced that BC was not statistically associated with the risks of being infected or testing positive to different shedding routes simultaneously (Barasona et al., 2017; Vicente et al., 2007). Both studies suggest that population factors somehow associated with BC may determine the spread of the MTC.

The wild boar constitutes the main reservoir of the MTC in the Mediterranean basin (Naranjo et al., 2008), where ungulates suffer from substantial inter-annual variations of in terms of food availability. Summer is the hardest season due to the drought and the subsequent reduced quality and quantity of food resources, leading to impoverish BC in ungulates (Bugalho and Milne, 2003; Rodriguez-Hidalgo et al., 2010). Furthermore, high densities of wild ungulates and free-ranging livestock coexist in these environments, exacerbating the impact of food restriction periods on the health status of wildlife (Gortázar et al., 2012). However, supplementary feeding is often provided to deal with these food shortages in private hunting estates of south-central Spain. This management strategy increases the carrying capacity of ecosystems and BC of animals (Vicente et al., 2007). So, populations fed with supplementary food are expected to show a better BC and an improved resistance against infections. However, the aggregation of animals around food may increase the transmission rates of pathogens (Acevedo et al., 2007; Laguna et al., 2018; Navarro-Gonzalez et al., 2013), thus promoting pathogen maintenance in the host community (Murray 2016). In wild boar, TB is characterized by high prevalence and generalization rates (Barroso et al., 2020; Vicente et al., 2013). Thus, a large proportion of wild boar becomes an important source of MTC contamination, especially adults showing disseminated lesions, since they possess the ability to excrete MTC bacteria through several routes simultaneously (Barasona et al., 2017). Cachectic moribund diseased wild boar roaming in the surrounding the farms and, to lesser extent individuals in good BC (especially dispersal young wild boar), may facilitate the spread of TB to wildlife and livestock from neighbouring areas since they cross roads, urban areas, open fields and croplands (Casas-Díaz et al., 2013; Hampton et al., 2004).

In this study, we explore the relationships between sex, age, TB severity and BC in hunted wild boar from south-central Spain. The specific aims of the work were to assess the relationship between sex, age and TB severity altogether on the BC of wild boar. Specifically, we tested the following hypothesis: as reported in previous studies and due to the trade-off related to the life history (Orłowska et al., 2013; Santos et al., 2013), adults and female wild boar should exhibit higher BC than juveniles and males, respectively. On the base of this premise, among wild boar showing tuberculosis-like lesions (TBL), juveniles and males are expected to be more sensitive to the emaciating effects of TB.

2. Methods

2.1 Study area

This study was carried out on seven big game estates located in Castilla-La Mancha, in south-central Spain (Figure 1). The study area includes the Toledo Mountains (MT), the Sierra Morena Mountains (SM), and the Guadiana River valley (GV; transition area between MT and SM). These regions have a Mediterranean climate (with Atlantic influence in the case of DNP), characterized by strong seasonality and higher inter-annual oscillations in rainfall levels (annual rainfall ranks between 300 and 700 mm). During the wet season (autumn-spring) most of the yearly rains occur, providing high-quality pastures. However, the drought season (summer) is the critical period for ungulates due to the shortage of food and water resources. Mediterranean vegetation is adapted to these circumstances, predominating Mediterranean woodlands of oak trees (mainly composed by *Quercus ilex*), scrublands with scattered pastures and small plots of croplands. In all the sampling sites included in this study, seasonal feeding provided to wild ungulates before hunting events is based on hay and alfalfa bales. Wild boar usually coexist with red deer (*Cervus elaphus*) in the sampling sites so as, in specific areas, with fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*).

2.2 Animal sampling

Between the hunting seasons, 2010/2011 and 2020/2021 (which typically occur from October to February), carcasses of 1,372 harvested wild boar were sampled in seven Spanish localities (sampling sites) belonging to four geographic areas (Figure 1). In big game hunting states, we arbitrarily selected a random age- and a sex-stratified subset of approximately 20 wild boar by season.

Necropsy examination and sample collection were performed in the field by qualified veterinarians. During the animals' necropsy, the right kidney with its surrounding fat was dissected (Santos et al., 2013) and the sex and age of the animals were determined. Animals (including 616 males and 756 females) were classified into three age classes based on the eruption of dentition patterns (Saenz de Buruaga et al., 2001): juveniles (<12 months; n= 269), yearlings (1-2 years; n= 306) and adults (>2 years; n= 797).

To obtain a BC index, the amount of surrounding fat was standardised based on the surface of the kidneys and the surplus fat was removed (Caughley and Sinclair, 1994). The right kidney was weighed both with fat and after removing the fat, on an electronic precision balance to the nearest 0.1 g. Thus, we got a single kidney mass (KM) and kidney fat (KF) values for each wild boar individual.

2.3 Tuberculosis diagnosis and severity assessment

The presence of TBL was recorded by macroscopic inspection of the head, thoracic, and mesenteric lymph nodes as well as abdominal and thoracic organs (Martín-Hernando et al., 2007; Vicente et al., 2006). This analysis routinely included submandibular lymph nodes and tonsils in the head, tracheobronchial and mediastinal lymph nodes and lungs in the thorax, and mesenteric lymph nodes and spleen in the abdomen. The positivity to MTC was confirmed by culture in a sample of positive individuals. To consider the TB severity, wild boar were classified into three categories: TB free (wild boar without TBL), mild (wild boar with a localized lesion pattern, observed in one location: head, thorax or abdomen) or severe (wild boar with a disseminated lesion pattern, observed in two or more locations).

2.4 Statistics

We calculated the Kidney Fat Index (KFI) according to Riney (1955), an index that measures the retroperitoneal fat stored around the kidneys. KFI was calculated by dividing KF by the KM and multiplying by 100, and it was used as an indicator of the BC for both descriptive statistics and statistical modelling. Along with the text, we will use BC rather than KFI to mention the energy stored by wild boar to support the costs of biological activities.

Then, we fit a set of linear mixed models (LMMs) to assess the relationship between sex, age class, disease severity and BC (as a response). In these models, sex, age class, the severity of the TB infection, and all their possible two-way interactions were included as fixed terms whereas the population (seven levels) and season of sampling (autumn: October-November; winter: December-February) as random-effect terms. LMMs were fitted with a normal error distribution and the identity link function (Zuur et al., 2009). Model selection was based on Akaike's Information Criterion (Akaike, 1974). We also estimated the Akaike weight (wi), which is the probability that a model is the best among those compared [52]. Models which differed in less than two AIC units ($\Delta AIC < 2$) were considered good candidates for explaining the observed BC variability (Burnham and Anderson, 2002). Once the best model/s was selected, we checked for normality and the absence of residual patterns in data variation. LMMs were fitted in the library "*lme4*" 1.1-21 version [53], and the library MuMIn 1.42.14 (Barton, 2019) was used to calculate the conditional and marginal coefficient of determination for LMMs (R^2). All statistical analyses were performed in the R 4.0.2 version (R Core Team, 2020).

3. Results

Mean ± standard error (SE), minimum and maximum KFI values, and sample size (n) according to sex, age class and TB status are shown in Table 1. For the three age classes considered, all the individuals with severe TB showed higher BC than TB free animals (difference of 5.92, 13.37 and 1.18 in % of KFI values in juveniles, yearlings, and adults, respectively, see Table 1). However, these differences were not statistically significant in models which include this interaction (models 4, 8 and 14). Concerning the sex, the wasting effect of the severe TB was only revealed in females, specifically, differences between TB free and severe TB in juvenile individuals were 2.67 % of KFI values (see Table 1). These differences were statistically significant in those models including this interaction (models 3, 6 and 9). However, these descriptive results should be interpreted with caution, accounting for possible cofconfoundingctors. Therefore, the results of the LMMs are interpreted below.

According to our LMM selection (Table 2), BC is driven by sex, age and TB (wi = 0.53, Table 3). The next candidate model (Δi =1.46) suggests that BC variability is only affected by sex and age differences (wi = 0.24, Table 3). BC was higher in females (82.99 ± 1.54) than in males (50.74 ± 2.03) and sexual differences in BC increase with age ($F_{1,\,1351}$ = 21.01, *p-value* <0.01, Figure 2a). For example, differences between females and males BC are 5.51 in juvenile (relative change in % of predicted KFI values), but 37.77 in adult wild boar (Figure 2a).

Regarding TB severity, no significant effect in the BC was revealed in the best model ($F_{1,\,1351}$= 2.44, *p*=0.09). Interestingly, in general, wild boar with severe TB showed higher BC than individuals without TBL (differences of 3.60 in % of predicted KFI values, *p-value* = 0.02, Figure 2b). Overall, higher BC was observed in females and adults, regardless of the TB severity status. Surprisingly, in both candidate models, the sampling population and season explained most of the variability in the BC (15.18% and 15.26% in models 1 and 2, respectively, see Table 2). The variability explained by fixed factors was 14.80% for model 1 (variability explained by both fixed and random factors: 29.98%) and 14.46% for model 2 (variability explained by both fixed and random factors: 29.71%).

4. Discussion

We describe the BC in a sample of more than one thousand wild boar, evidencing the age- and sex-dependent pattern, which is similar to those reported in previous studies on wild boar populations in Europe (Albrycht et al., 2016; Mocała et al., 2012; Orłowska et al., 2013). In general, the results show greater BC in females and older age classes, since the tendency to accumulate fat rises with the age of the animals (Risco et al., 2018). However, the most remarkable point is the tolerance of the wild boar to infections by TB, since its BC is not affected by the severity of the TB-related lesions, as has been reported in other species of ungulates (see Vicente et al. (2007) for red deer and Dejene et al. (2016) for cattle), which suggests that this species can act as a super-shedder of this pathogen.

Sex-related differences in susceptibility to infections have been previously reported (e.g. Alexander and Stimson, 1988; Schalk and Forbes, 1997; Vicente et al., 2007), showing males higher impact and rates of parasitism and infection as well as less effective immune responses than females (Moore and Wilson, 2002). The sexual differences in BC (more marked in adults), may also be associated with the relationship between BC and reproductive success in each sex, which response to different strategies (Festa-Bianchet et al., 1998; Gaillard et al., 2000). While adult females focus on achieving appropriate weight and BC for reproduction, males invest their resources in sexual competition during the breeding season (which takes place during the hunting seasons), as has been reported in polygynous ungulates (Festa-Bianchet et al., 1998). Hence, adult females of reproductive age are expected to show higher BC than younger females, even than yearlings that begin to reproduce, which sustain higher metabolic requirements derived from their growth and development processes (Albrycht et al., 2016; Lesage et al., 2001). By contrast, adult males are expected to be in worse BC than younger individuals around the breeding season, due to the immunosuppressive effect of the testosterone and its subsequent impact on BC, together with the sexual competition (Cizauskas et al., 2015; Ezenwa et al., 2012; Folstad and Karter, 1992; Malo et al., 2009). Moreover, from the second year of life onwards, males sustain high metabolic costs. These energetic demands are derived from the development of muscle tissue, skeletons and canines, which is more expensive metabolically than producing fat (Oftedal, 1983). During this age, males focus on increasing these tissues because heavier wild boar achieve greater reproductive success rates.

The low impact of TB on boar BC is in line with previous research concluding that BC is not linked to testing positive for TB or with the super-shedder condition in wild boar (Barasona et al., 2016; Vicente et al., 2007). Vicente et al. (2007) showed as in red deer BC was mainly dependent on the amount of supplemental food provided, but also on environmental conditions. However, our results contrast with other studies found in the literature which reported an impact of the infection progress on BC in other wild ungulates (Caron et al., 2003; Munyeme et al., 2010), as well as the consequences for immune response in wild boar (Gassó et al., 2016). The better BC found in wild boar showing severe TB, considered as super-shedders of MTC (Santos et al., 2015a), implies higher survival rates and the subsequent relevant epidemiological consequences for the spread and maintenance of TB in certain areas, thus acting as a TB reservoir (Naranjo et al., 2008; Risco et al., 2019). This may be mediated by the seasonal supplementary feeding practices carried out in these hunting estates. Providing wild boar with food could lead to an improved BC as well as a higher frequency of generalized TB due to a higher spatial aggregation and risk of TB transmission (Vicente et al., 2007, 2006). However, a great BC does not necessarily mean a better health and nutritional status, since this is conditioned by the type of supplement provided (i.e. corn-based supplementary fed wild boar have a great BC but they are malnourished (Murray et al., 2016)). Further studies are necessary to evaluate the relationship between BC, TB severity and the wild boar health status, including e.g. type of supplementary food or unspecific indicators of health status such as serum acute-phase proteins (Chen et al., 2003).

One possible explanation for our findings may be that wild boar hunted as presumably healthy are those that survive, whereas the cachectic and severely diseased wild boar maybe not be spotted or may die hidden in the dense scrubland (Barasona et al., 2016). However, the host-mycobacteria co-evolutionary process, in which mycobacteria and their hosts developed mechanisms to modulate the immune response, promotes the host survival with the subsequent benefits to the mycobacteria by increasing the probability of transmission. In this regard, López et al. (2016) reported a reduction in anaemia progression from adult wild boar exhibiting mild to severe TB, probably associated with this co-evolutionary phenomenon.

Our findings are consistent with those reported in managed (year-round fed) red deer populations, as compared to natural ones, in which the amount of supplemental food provided, as well as environmental factors in terms of habitat quality were positively associated with the BC (Rodriguez-Hidalgo et al., 2010; Santos et al., 2013; Vicente et al., 2007). These factors may include the supplementary feeding degree (classified by Laguna et al. (2022)), the nutritional quality of the food provided or the ground primary production.

According to our results, the sampling population and season explained most of the variability in the BC. In this sense, intrinsic features of each area, as well as the potential differences in food and water availability characteristic of each season, may drive BC (Laguna et al., 2022). Further research is warranted to elucidate the factors linked to population and season which drive differences in BC and the underlying ecological mechanism. Specifically, if the variability observed in BC between sampling populations is influenced by supplementary feeding degree, the nutritional quality of the food provided, the ground primary production, and population densities.

5. References

Acevedo, P., Vicente, J., Höfle, U., Cassinello, J., Ruiz-Fons, F., Gortazar, C., 2007. Estimation of European wild boar relative abundance and aggregation: A novel method in epidemiological risk assessment. Epidemiol. Infect. **135**, 519–527.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. **19**, 716–723.

Albrycht, M., Merta, D., Bobek, J., Ulejczyk, S., 2016. The demographic pattern of wild boars (*Sus scrofa*) inhabiting fragmented forest in North-Eastern Poland. Balt. For. **22**, 251–258.

Alexander, J., Stimson, W.H., 1988. Sex hormones and the course of parasitic infection. Parasitol. Today **4**, 189–193.

Barasona, J.A., Acevedo, P., Diez-Delgado, I., Queiros, J., Carrasco-García, R., Gortazar, C., Vicente, J., 2016. Tuberculosis-associated death among adult wild boars, Spain, 2009–2014. Emerg. Infect. Dis. **22**, 2178–2180.

Barasona, J.A., Torres, M.J., Aznar, J., Gortázar, C., Vicente, J., 2017. DNA Detection Reveals *Mycobacterium tuberculosis* Complex Shedding Routes in Its Wildlife Reservoir the Eurasian Wild Boar. Transbound. Emerg. Dis. **64**, 906–915.

Barroso, P., Barasona, J.A., Acevedo, P., Palencia, P., Carro, F., Negro, J.J., Torres, M.J., Gortázar, C., Soriguer, R.C., Vicente, J., 2020. Long-Term Determinants of Tuberculosis in the Ungulate Host Community of Doñana National Park. Pathogens **9**, 445.

Barton, K., 2019. MuMIn: Multi-model inference. R package 1.43.15. URL https://cran.r-project.org/web/packages/MuMIn/index.html (accessed 3.1.21).

Beldomenico, P.M., Begon, M., 2010. Disease spread, susceptibility and infection intensity: vicious circles? Trends Ecol. Evol. **25**, 21–27.

Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., Begon, M., 2008. Poor condition and infection: a vicious circle in natural populations. Proc. R. Soc. B Biol. Sci. **275**, 1753–1759.

Budischak, S.A., O'Neal, D., Jolles, A.E., Ezenwa, V.O., 2018. Differential host responses to parasitism shape divergent fitness costs of infection. Funct. Ecol. **32**, 324–333.

Bugalho, M.N., Milne, J.A., 2003. The composition of the diet of red deer (*Cervus elaphus*) in a Mediterranean environment: A case of summer nutritional constraint? For. Ecol. Manage. **181**, 23–29.

Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York.

Burthe, S., Telfer, S., Begon, M., Bennett, M., Smith, A., Lambin, X., 2008. Cowpox virus infection in natural field vole Microtus agrestis populations: Significant negative impacts on survival. J. Anim. Ecol. **77**, 110–119.

Caron, A., Cross, P.C., Du Toit, J.T., 2003. Ecological implications of bovine tuberculosis in African buffalo herds. Ecol. Appl. **13**, 1338–1345.

Casas-Díaz, E., Closa-Sebastià, F., Peris, A., Miño, A., Torrentó, J., Casanovas, R., Marco, I., Lavín, S., Fernández-Llario, P., Serrano, E., 2013. Recorded dispersal of wild boar (*Sus scrofa*) in Northeast Spain: Implications for disease-monitoring programs. Wildl. Biol. Pract. **9**, 19–26.

Caughley, G., Sinclair, A.R.E., 1994. Wildlife Ecology and Management. Backwell Science, Cambrige.

Chen, H.-H., Lin, J.-H., Fung, H.-P., Ho, L.-L., Yang, P.-C., Lee, W.-C., Lee, Y.-P., Chu, R.-M., 2003. Serum acute phase proteins and swine health status. Can. J. Vet. Res. **67**, 283–90.

Cizauskas, C.A., Turner, W.C., Pitts, N., Getz, W.M., 2015. Seasonal patterns of hormones, macroparasites, and microparasites in wild african ungulates: The interplay among stress, reproduction, and disease. PLoS One **10**, 1–29.

Cross, P.C., Drewe, J., Patrek, V., Pearce, G., Samuel, M.D., Delahay, R.J., 2009. Wildlife Population Structure and Parasite Transmission: Implications for Disease Management. In: Delahay, R.J., Smith, G.C., Hutchings, M.R. (Eds.), Management of Disease in Wild Mammals. Springer Japan, Tokyo, pp. 9–29.

De la Peña, E., Martín, J., Barja, I., Pérez-Caballero, R., Acosta, I., Carranza, J., 2020. The immune challenge of mating effort: steroid hormone profile, dark ventral patch and parasite burden in relation to intrasexual competition in male Iberian red deer. Integr. Zool. 1749-4877.12427.

Dejene, S.W., Heitkönig, I.M.A., Prins, H.H.T., Lemma, F.A., Mekonnen, D.A., Alemu, Z.E., Kelkay, T.Z., de Boer, W.F., 2016. Risk Factors for Bovine Tuberculosis (bTB) in Cattle in Ethiopia. PLoS One **11**, e0159083.

Delahay, R.J., Langton, S., Smith, G.C., Clifton-Hadley, R.S., Cheeseman, C.L., 2000. The spatio-temporal distribution of *Mycobacterium bovis* (bovine tuberculosis) infection in a high-density badger population. J. Anim. Ecol. **69**, 428–441.

Ezenwa, V.O., Stefan Ekernas, L., Creel, S., 2012. Unravelling complex associations between testosterone and parasite infection in the wild. Funct. Ecol. **26**, 123–133.

Festa-Bianchet, M., Gaillard, J.M., Jorgenson, J.T., 1998. Mass- and density-dependent reproductive success and reproductive costs in a capital breeder. Am. Nat. **152**, 367–379.

Folstad, I., Karter, A.J., 1992. Parasites, Bright Males, and the Immunocompetence Handicap. Am. Nat. **139**, 603–622.

Gaillard, J.M., Festa-Bianchet, M., Delorme, D., Jorgenson, J., 2000. Body mass and individual fitness in female ungulates: Bigger is not always better. Proc. R. Soc. B Biol. Sci. **267**, 471–477.

Gassó, D., Vicente, J., Mentaberre, G., Soriguer, R., Jiménez Rodríguez, R., Navarro-González, N., Tvarijonaviciute, A., Lavín, S., Fernández-Llario, P., Segalés, J., Serrano, E., 2016. Oxidative stress in wild boars naturally and experimentally infected with *Mycobacterium bovis*. PLoS One **11**, 1–17.

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. Mamm. Rev. **42**, 193–206.

Graham, A.L., Shuker, D.M., Pollitt, L.C., Auld, S.K.J.R., Wilson, A.J., Little, T.J., 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Funct. Ecol. **25**, 5–17.

Hampton, J.O., Spencer, P.B.S., Alpers, D.L., Twigg, L.E., Woolnough, A.P., Doust, J., Higgs, T., Pluske, J., 2004. Molecular techniques, wildlife management and the importance of genetic population structure and dispersal: a case study with feral pigs. J. Appl. Ecol. **41**, 735–743.

Jolles, A.E., Cooper, D. V., Levin, S.A., 2005. Hidden effects of chronic tuberculosis in African buffalo. Ecology **86**, 2358–2364.

Laguna, E., Barasona, J.A., Triguero-Ocaña, R., Mulero-Pázmány, M., Negro, J.J., Vicente, J., Acevedo, P., 2018. The relevance of host overcrowding in wildlife epidemiology: A new spatially explicit aggregation index. Ecol. Indic. **84**, 695–700.

Laguna, E., Carpio, A.J., Ortega, Z., Vicente, J., Acevedo, P., 2022. Disentangling the effects of supplementary feeding on population abundance and the aggregation of wild ungulates as drivers of the prevalence of tuberculosis in south-central Spain. Under Rev.

Landete-Castillejos, T., Gortázar, C., Vicente, J., Fierro, Y., Garcia, A., Gallego, L., 2004. Age-related foetal sex ratio bias in Iberian red deer (*Cervus elaphus hispanicus*): are male calves too expensive for growing mothers? Behav. Ecol. Sociobiol. **56**, 1–8.

Lesage, L., Crête, M., Huot, J., Ouellet, J.P., 2001. Evidence for a trade-off between growth and body reserves in northern white-tailed deer. Oecologia **126**, 30–41.

Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos **88**, 87–98.

López-Olvera, J.R., Serrano, E., Armenteros, A., Pérez, J.M., Fandos, P., Carvalho, J., Velarde, R., Cano-Manuel, F.J., Ráez, A., Espinosa, J., Soriguer, R.C., Granados, J.E., 2015. Sex-biased severity of sarcoptic mange at the same biological cost in a sexually dimorphic ungulate. Parasit. Vectors **8**, 583.

López, V., Villar, M., Queirós, J., Vicente, J., Mateos-Hernández, L., Díez-Delgado, I., Contreras, M., Alves, P.C., Alberdi, P., Gortázar, C., de la Fuente, J., 2016. Comparative Proteomics Identifies Host Immune System Proteins Affected by Infection with *Mycobacterium bovis*. PLoS Negl. Trop. Dis. **10**, 1–22.

Malo, A.F., Roldan, E.R.S., Garde, J.J., Soler, A.J., Vicente, J., Gortazar, C., Gomendio, M., 2009. What does testosterone do for red deer males? Proc. R. Soc. B Biol. Sci. **276**, 971–980.

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.

Medzhitov, R., Schneider, D.S., Soares, M.P., 2012. Disease Tolerance as a Defense Strategy. Science. **335**, 936–941.

Miller, M.W., Conner, M.M., 2005. Epidemiology of chronic wasting disease in free-ranging mule deer: spatial, temporal, and demographic influences on observed prevalence patterns. J. Wildl. Dis. **41**, 275–290.

Mocała, P., Merta, D., Pomykacz, M., Frąckowiak, W., 2012. Winter diet of wild boars (*Sus scrofa*) inhabiting forest and forest-farmland environments in Poland. 9th Int. Symp. Wild Boar other Suids - B. Abstr. **63**, 43–44.

Moore, S.L., Wilson, K., 2002. Parasites as a Viability Cost of Sexual Selection in Natural Populations of Mammals. Science. **297**, 2015–2018.

Morris, R.S., Pfeiffer, D.U., Jackson, R., 1994. The epidemiology of *Mycobacterium bovis* infections. Vet. Microbiol. **40**, 153–177.

Munyeme, M., Muma, J.B., Siamudaala, V.M., Skjerve, E., Munang'andu, H.M., Tryland, M., 2010. Tuberculosis in Kafue lechwe antelopes (*Kobus leche kafuensis*) of the Kafue Basin in Zambia. Prev. Vet. Med. **95**, 305–308.

Murray, M.H., Becker, D.J., Hall, R.J., Hernandez, S.M., 2016. Wildlife health and supplemental feeding: A review and management recommendations. Biol. Conserv. **204**, 163–174.

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.

Navarro-Gonzalez, N., Fernández-Llario, P., Pérez-Martín, J.E., Mentaberre, G., López-Martín, J.M., Lavín, S., Serrano, E., 2013. Supplemental feeding drives endoparasite infection in wild boar in Western Spain. Vet. Parasitol. **196**, 114–123.

Oftedal, O.T., 1983. Wildlife feeding and nutrition. Academic Press, New York.

Orłowska, L., Rembacz, W., Florek, C., 2013. Carcass weight, condition and reproduction of wild boar harvested in north-western Poland. Pest Manag. Sci. **69**, 367–370.

R Core Team, 2020. R: A language and environment for statistical computing.

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.

Riney, T., 1955. Evaluating condition of free-ranging red deer (*Cervus elaphus*) with special reference to New Zealand. New Zeal. J. Sci. **36**, 429–463.

Risco, D., Gonçalves, P., Mentaberre, G., Navarro-González, N., Casas-Díaz, E., Gassó, D., Colom-Cadena, A., Fernández-Aguilar, X., Castillo-Contreras, R., Velarde, R., Barquero-Pérez, O., Fernández-Llario, P., Lavín, S., Fonseca, C., Serrano, E., 2018. Biometrical measurements as efficient indicators to assess wild boar body condition. Ecol. Indic. **88**, 43–50.

Risco, D., Martínez, R., Bravo, M., Fernández Llario, P., Cerrato, R., Garcia-Jiménez, W.L., Gonçalves, P., García, A., Barquero-Pérez, Ó., Quesada, A., Hermoso de Mendoza, J., 2019. Nasal shedding of *Mycobacterium tuberculosis* in wild boar is related to generalised tuberculosis and concomitant infections. Vet. Rec. **185**, 629–629.

Rodriguez-Hidalgo, P., Gortazar, C., Tortosa, F.S., Rodriguez-Vigal, C., Fierro, Y., Vicente, J., 2010. Effects of density, climate, and supplementary forage on body mass and pregnancy rates of female red deer in Spain. Oecologia **164**, 389–398.

Saenz de Buruaga, M., Lucio-Calero, A., Purroy, F.J., 2001. Reconocimiento de sexo y edad en especies cinegéticas, 1st ed. Edilesa, Madrid, Spain.

Sánchez, C.A., Becker, D.J., Teitelbaum, C.S., Barriga, P., Brown, L.M., Majewska, A.A., Hall, R.J., Altizer, S., 2018. On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. Ecol. Lett. **21**, 1869–1884.

Santos, J.P.V., Fernández-De-Mera, I.G., Acevedo, P., Boadella, M., Fierro, Y., Vicente, J., Gortázar, C., 2013. Optimizing the sampling effort to evaluate body condition in ungulates: A case study on red deer. Ecol. Indic. **30**, 65–71.

Santos, N., Almeida, V., Gortázar, C., Correia-Neves, M., 2015a. Patterns of *Mycobacterium tuberculosis*-complex excretion and characterization of super-shedders in naturally-infected wild boar and red deer. Vet. Res. **46**.

Santos, N., Santos, C., Valente, T., Gortázar, C., Almeida, V., Correia-Neves, M., 2015b. Widespread Environmental Contamination with *Mycobacterium tuberculosis* Complex Revealed by a Molecular Detection Protocol. PLoS One **10**, e0142079.

Schalk, G., Forbes, M.R., 1997. Male Biases in Parasitism of Mammals: Effects of Study Type, Host Age, and Parasite Taxon. Oikos **78**, 67.

Schmid-Hempel, P., 2009. Immune defence, parasite evasion strategies and their relevance for ‘macroscopic phenomena’ such as virulence. Philos. Trans. R. Soc. B Biol. Sci. **364**, 85–98.

Schulenburg, H., Kurtz, J., Moret, Y., Siva-Jothy, M.T., 2009. Introduction. Ecological immunology. Philos. Trans. R. Soc. B Biol. Sci. **364**, 3–14.

Silva C.L., Faccioli L.H., R.G.M., 1988. The role of cachectin/TNF in the pathogenesis of tuberculosis. Brazilian J. Med. Biol. Res. **21**, 489–492.

Solomon, J.B., 1978. Immunological milestones in ontogeny. Dev. Comp. Immunol. **2**, 409–424.

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**, 92–103.

Vicente, J., Höfle, U., Fernández-De-Mera, I.G., Gortazar, C., 2007. The importance of parasite life history and host density in predicting the impact of infections in red deer. Oecologia **152**, 655–664.

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.

Yoshida, T., Delafontaine, P., 2015. Mechanisms of Cachexia in Chronic Disease States. Am. J. Med. Sci. **350**, 250–256.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. GLM and GAM for Count Data. In: Environmental Forum. pp. 209–243.

Authors contributions

E.S. and C.G. participated in the study design. P.B., E.S. and A.C. performed the experiments. P.B., E.S., C.G., and A.C. completed the analysis. P.B. and E.S. drafted the manuscript. J.V., P.A., C.G., A.C., and E.S. supervised the analysis and critically revised the manuscript. All authors provided substantial intellectual contributions and approved the final version of the manuscript.

Funding

This research was funded by the Ministerio de Economía y Competitividad (MINECO; AEI/FEDER, UE; AGL2016-76358-R) and the EU/FEDER grant WILD DRIVER (CGL2017-89866), and PAIDI-RNM 118 Junta de Andalucia. E. Serrano was supported by the Spanish Ministerio de Economía y Competitividad (MINECO) through a Ramón y Cajal agreement (RYC-2016-21120). A.J. Carpio is supported by a Juan de la Cierva contract (FJCI-2017-33114) from MINECO-UCLM.

Conflict of interest

The authors declare no potential conflict of interest.

Tables

Table 1. Average body condition (Kidney Fat Indexes ± standard error) by sex, age classes and TB status. The range is indicated in brackets and the total sample size (N) is also shown

Age class			**Sex**	
			Female	Male
Juveniles			55.30 ± 2.98 (2.38-183.78) N: 142	47.75 ± 2.96 (4.35-158.33) N: 127
	TB status	No visible lesions	55.04 ± 3.94 (2.38-183.78) N: 97	44.25 ± 3.85 (4.35-158.33) N: 78
		Mild	58.42 ± 5.50 (15.63-115.00) N: 26	48.66 ± 5.68 (9.38-117.14) N: 28
		Severe	52.37 ± 6.18 (20.00-108.33) N: 19	59.56 ± 7.31 (16.67-134.29) N: 21
Yearlings			77.13 ± 3.94 (4.41-402.27) N: 178	56.40 ± 3.73 (4.20-200.00) N: 128
	TB status	No visible lesions	73.22 ± 4.95 (4.41-249.21) N: 104	50.88 ± 5.07 (4.20-190.38) N: 65
		Mild	79.00 ± 10.32 (5.66-402.27) N: 39	59.15 ± 6.79 (11.94-200.00) N: 37
		Severe	86.67 ± 7.28 (22.50-206.90) N: 35	66.32 ± 9.12 (9.23-185.00) N: 26

Adults			94.40 ± 2.83 (4.17-390.91) N: 436	49.79 ± 2.01 (1.49-263.01) N: 361
	TB status	No visible lesions	88.37 ± 4.14 (4.17-390.91) N: 192	51.61 ± 3.30 (1.49-263.01) N: 159
		Mild	102.31 ± 5.44 (11.98-346.67) N: 141	44.14 ± 3.19 (3.09-157.89) N: 94
		Severe	94.78 ± 5.31 (6.41-261.11) N: 103	52.01 ± 3.71 (6.13-214.58) N: 108

Table 2. Model selection for body condition, defined as the Kidney Fat Index, to sex, age class, and TB severity in 1,372 wild boar from seven sampling areas of South-central Spain. The models were fitted using the population and season as random effect factors. Age of animals was classified into three categories: juveniles, yearlings and adults. TB severity was expressed as free, mild or severe. Best model highlighted in bold font. The asterisk "*" means interaction between terms; K number of parameters, including both the intercept and the error terms; AIC Akaike's Information Criterion; Δi difference of AIC with respect to the best model; *wi* Akaike weight

Reference	Biological models	K	AIC	Δi	w_i
1	**Sex*age class + TB severity**	**11**	**668.31**	**0.00**	**0.53**
2	Sex*age class	8	669.77	1.46	0.26
3	Sex*age class + sex*TB severity	14	670.58	2.07	0.19
4	Sex* age class + age class*TB severity	17	674.45	6.14	0.02
5	Sex + TB severity + age class	10	705.92	37.61	0.00
6	Sex*TB severity + age class	11	706.07	37.76	0.00
7	Sex + age class	7	706.63	38.32	0.00
8	Age class*TB severity + sex	13	711.10	42.79	0.00
9	Sex*TB severity	8	727.56	59.25	0.00
10	Sex + TB severity	7	727.60	59.29	0.00
11	Sex	4	732.32	64.01	0.00
12	Age class	5	886.91	218.60	0.00
13	Age class + TB severity	8	887.34	219.03	0.00
14	Age Class*TB severity	11	893.12	224.81	0.00
15	TB severity	5	908.88	240.57	0.00
16	Null model	2	911.93	243.62	0.00

Table 3. Summary of the best two Linear Mixed Model candidates examining the relationship between body condition, sex, age class and TB severity, including the population and season as random factors. Reference categories were female for sex, juveniles for age class and TB free for TB severity. The screenplay “-” means interaction between terms

Model 1: Sex-age class + TB severity	**F** **df**	**Estimate ± SD**	***p***
Sex	204.34 (1, 1351)	Male: -0.05 ± 0.04	<0.01
Age Class	15.43 (2, 1351)	Yearling: 0.17 ± 0.03** Adult: 0.23 ± 0.03**	<0.01
Sex-age class	21.01 (2, 1351)	Male-Yearling: -0.12 ± 0.05* Male-Adult: -0.27 ± 0.04**	<0.01
TB severity	2.44 (2, 1351)	Mild: 0.03 ± 0.02 Severe: 0.06 ± 0.02*	0.09
Model 2: Sex-age class	**F** **df**	**Estimate ± SD**	***p***
Sex	203.86 (1, 1353)	Male: -0.05 ± 0.04	<0.01
Age Class	15.39 (2, 1353)	Yearling: 0.18 ± 0.03** Adult: 0.24 ± 0.03**	<0.01
Sex-age class	20.66 (2, 1353)	Male-Yearling: -0.12 ± 0.05* Male-Adult: -0.27 ± 0.04**	<0.01

*$p>0.05$, **$p>0.01$

Figures

Figure 1. Map of South Central Spain showing the location of the seven sampling sites. The shaded area represents the two Spanish provinces in which the sampling areas were located: Toledo and Ciudad Real

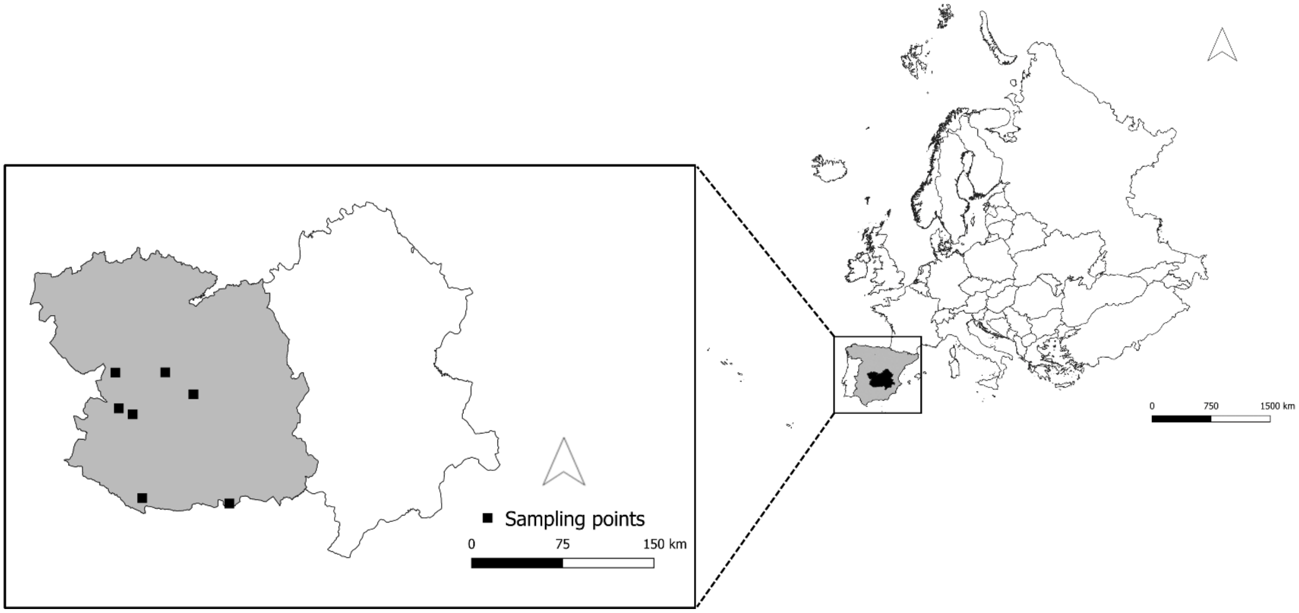

Figure 2. Mean predicted body condition (Kidney Fat Index ± standard error represented by the error bars) depending on (a) the sex and age classes, and (b) the TB severity (no visible lesions (free), mild and severe)