



Complementary roles of wild boar and red deer to animal tuberculosis maintenance in multi-host communities

Nuno Santos^{a,b,*}, Elisa Ferreras Colino^c, María Cruz Arnal^d, Daniel Fernández de Luco^d, Iker Sevilla^e, Joseba M. Garrido^e, Eliana Fonseca^f, Ana M. Valente^g, Ana Balseiro^{h,i}, João Queirós^{a,b}, Virgílio Almeida^{j,k}, Joaquín Vicente^c, Christian Gortázar^c, Paulo Célio Alves^{a,b,l,m}

^a CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Vairão, Portugal

^b BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Vairão, Portugal

^c Instituto de Investigación en Recursos Cinegéticos (IREC, CSIC-UCLM-JCCM), Ciudad Real, Spain

^d Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain

^e Animal Health Department, NEIKER-Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain

^f Instituto de Conservação da Natureza e das Florestas, I.P., Braga, Portugal

^g CESAM, Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal

^h Animal Health Department, Instituto de Ganadería de Montaña (IGM, CSIC-ULE), León, Spain

ⁱ Animal Health Department, Facultad de Veterinaria, Universidad de León, León, Spain, Instituto de Ganadería de Montaña (IGM, CSIC-ULE), León, Spain

^j Faculty of Veterinary Medicine, University of Lisbon, Campus da Ajuda, Lisboa, Portugal

^k CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Campus da Ajuda, Lisboa, Portugal

^l Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

^m Estação Biológica de Mértola (EBM), CIBIO, Mértola, Portugal

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ABSTRACT

The contribution of wildlife species to pathogen maintenance in multi-host communities has seldom been quantified. To assess the relative contribution of the main wildlife hosts of animal tuberculosis (TB) to its maintenance, we estimated the basic reproduction number (R_0) of *Mycobacterium tuberculosis* complex in wild boar and red deer at 29 sites in the Iberian Peninsula. Host abundance and true TB prevalence were estimated for each species at each site by sampling from distributions incorporating the uncertainty in the proportion of the population harvested each year, sensitivity, and specificity of the diagnostic methods, while excretion of mycobacteria was estimated using site-occupancy models. The distributions of these parameters were then used to estimate, at each site, the $R_{0,wild\ boar}$ (range 0.1 – 55.9, average 8.7, standard deviation 11.8), and the $R_{0,red\ deer}$ (0.1 – 18.9, 2.2, 3.9). Animal TB is maintained in epidemiological scenarios ranging from any single species acting as a maintenance host (the wild boar in 18 sites and the red deer in 5), to facultative multi-host disease (6 sites). The prevalence of TB in the red deer is likely an important driver of the epidemiology in multi-host communities. The wild boar was the main maintenance host of TB in most of the study sites and could have an epidemiological role linking the wildlife multi-host community and livestock.

1. Background

The relative contribution of wildlife species to pathogen maintenance in multi-host communities has seldom been quantified due to the difficulty of estimating relevant metrics (Corner, 2006; Delamater et al., 2019; Dobson, 2004; Fenton et al., 2015; Santos et al., 2020;

ENETWILD-consortium et al., 2020; Ryser-Degiorgis, 2013). One such metric is the basic reproduction number (R_0) of a pathogen, which can be defined as the number of secondary infections generated by an infected host in a naïve population (Diekmann et al., 1990). The concept of R_0 has been extended to pathogens maintained by host communities comprising multiple species (Dobson, 2004). When estimated over the

* Correspondence to: CIBIO, Universidade do Porto, Campus de Vairão 4485-661, Vairão, Portugal
 E-mail address: nuno.santos@cibio.up.pt (N. Santos).

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host community, R_0 (denoted $R_{0,tot}$) is a metric of the contribution of all host species to disease maintenance, which can then be partitioned by species-specific relative contributions (Fenton et al., 2015). The $R_{0,tot}$ depends on the composition of the community, the competence of each host species to transmit infection, and the rates of intra- and inter-species transmission (Fenton et al., 2015).

The estimation of R_0 requires comprehensive empirical information on the multi-host-pathogen system under study (Delamater et al., 2019; Fenton et al., 2015). Such wealth of information is seldom available for host-pathogen systems including wildlife (Corner, 2006; Dobson, 2004; Fenton et al., 2015; Santos et al., 2020; ENETWILD-consortium et al., 2020; Ryser-Degiorgis, 2013). Fenton et al., 2015 proposed a conceptual framework and analytical method for estimating R_0 for information-deficient multi-host-pathogen systems. The conceptual framework is a generalization of that proposed by Holt et al., 2003, which classifies multi-host-pathogen systems along a gradient of transmission dominated by a single host species (when only one host has $R_0 > 1$), obligatory multi-host (when no host has $R_0 > 1$), or facultative multi-host (when several hosts have $R_0 > 1$) (Fenton et al., 2015). The analytical method allows to directly quantify the contributions of each species to pathogen maintenance in multi-host communities based on metrics simpler to estimate in host-pathogen systems including wildlife: abundance, prevalence, excretion, and the overlap in intra- and inter-species transmission (Fenton et al., 2015). In this study, we apply this framework to the wild boar-red deer-animal tuberculosis system in the Iberian Peninsula.

Animal tuberculosis (TB) is a zoonotic disease caused by infection with *Mycobacterium bovis* and related members of the MTC, with a wide range of mammalian host species (Gortázar et al., 2012). Animal TB is an infectious disease of relevant economic impact on the livestock industry, a public health threat in some regions of the world, and a wildlife conservation issue (Gortázar et al., 2012; Zinsstag et al., 2006; Pérez-Morote et al., 2020). In the Iberian Peninsula, as elsewhere, animal TB is maintained by a complex multi-host community comprising livestock (cattle, goats, free-range pigs, and sheep) and wildlife (wild boar *Sus scrofa*, red deer *Cervus elaphus*, fallow deer *Dama dama*, and Eurasian badger *Meles meles*) (Santos et al., 2020). *Mycobacterium tuberculosis* complex is transmitted among these host species (Reis et al., 2020) and the dynamics of infection in one species influence infection in sympatric species (García-Jiménez et al., 2013). Infection can be transmitted either directly between hosts, requiring sustained close contact, or indirectly through environmental contamination with viable mycobacteria (Corner, 2006). Close inter-species contact between ungulates is rare in the Iberian Peninsula (Cowie et al., 2016; Triguero-Ocaña et al., 2020). Environmental contamination has been increasingly recognized as important for the inter-species transmission and maintenance of animal TB in multi-host communities (Murphy et al., 2021).

Among wildlife, wild boar and red deer stand out by the high prevalence, abundance, and widespread distribution attained and are considered the most important maintenance hosts (Gortázar et al., 2012). Despite this, the relative contribution of host species to TB maintenance has seldom been addressed. Recent molecular epidemiology studies employing Whole Genome Sequencing and phylodynamic models have demonstrated the close interconnectivity between host species in the Iberian Peninsula and elsewhere (Reis et al., 2021; van Tonder et al., 2021; Duault et al., 2022; Pozo et al., 2022). The TB-cattle-badger system in Central Europe and Britain has been the subject of more in-depth analysis, highlighting the preponderance of inter-specific transmission and higher badger-to-cattle transmission over the inverse (van Tonder et al., 2021; Duault et al., 2022; Crispell et al., 2019). Epidemiological dynamic models applied to the cattle-badger system in Britain support the importance of within-species transmission (Brooks-Pollock and Wood, 2015). Nevertheless, these methods have been applied either only locally (phylodynamic analysis) or fail to capture the localized nature of the epidemiological settings (dynamic models). This study aims to contribute to filling this gap by

analysing the TB-wild-boar-red deer system in the Iberian Peninsula through a large-scale disease ecology analysis.

While the analytical approach employed here facilitates improving knowledge of multi-host-pathogen systems including wildlife (Fenton et al., 2015), the required metrics are still challenging to obtain at a large scale for our system of interest. Abundance is effort-intensive to estimate in wild ungulate populations, particularly for the wild boar, and harvest data has been increasingly used for that purpose (Santos et al., 2020; ENETWILD-consortium et al., 2020). Apparent prevalence can be obtained from harvested animals but estimating the true prevalence requires integrating the sensitivity and specificity of the diagnostic method employed (Speybroeck et al., 2013). The detection of excretion of *Mycobacterium tuberculosis* complex (MTC) was reported for infected wild boar and red deer (Santos et al., 2015), but the true excretion probability, accounting for the intrinsically imperfect detectability of the method (Lachish et al., 2012), remains to be estimated.

Cross-sectional epidemiological studies have consistently identified risk factors for TB in wildlife, notably the high density and aggregation of wild ungulates in fenced game estates, widespread in the central-south-western Iberian Peninsula (Acevedo et al., 2007; Vicente et al., 2013). The presence of game fences is a proxy of the intensive management of wild ungulate populations, usually characterised by the absence of large predators, artificial feeding and medication, and captive breeding, leading to exceptionally high densities (Vicente et al., 2013; Macaulay et al., 2013), and associated with decreased genetic diversity (Queirós et al., 2016). Mediterranean ecosystems are markedly seasonal, and drought was shown to influence TB in wildlife through an increase in their aggregation, particularly at watering sites during the dry season (Triguero-Ocaña et al., 2020; Vicente et al., 2013; Abrantes et al., 2021). Several studies have identified a relationship between TB in wildlife and cattle (LaHue et al., 2016; Santos et al., 2018).

The goal of this study was to estimate the relative contribution of the two main wild hosts (wild boar and red deer) to TB maintenance in multi-host communities in the Iberian Peninsula. Furthermore, we addressed the effect of extrinsic disease determinants on the relative contribution of each host species. Specifically, we aimed to i) estimate the basic reproduction number of MTC in the wild boar and red deer at 29 study sites across the Iberian Peninsula; ii) assess the influence of each model parameter in the R_0 estimates for each species; and iii) assess the relationship between disease determinants (fencing, TB prevalence in cattle, wild boar, and red deer, aridity, and abundance of wild hosts) and the probability of TB being maintained as a single- or multi-host disease.

2. Materials and methods

2.1. Study sites

We estimated the R_0 of MTC in the wild boar and red deer in 29 study sites in Spain ($n = 18$) and Portugal ($n = 11$) (Fig. 1). The study sites were game management units such as national parks, national hunting zones, private game estates, or groups of nearby private game estates with similar characteristics. Sites were either fenced with a perimetral > 2 m-high fence constraining ungulate movements ($n = 11$), or unfenced ($n = 18$). Each site contributed with data on harvest and apparent TB prevalence for a variable number of years (1–5), all within the decade 2010–2019. When available, data from several years were averaged, otherwise, data for only 1 year was used (Table S2).

2.2. Abundance

First, abundance was estimated from the average annual harvest divided by the proportion of the population harvested each year (Santos et al., 2020).

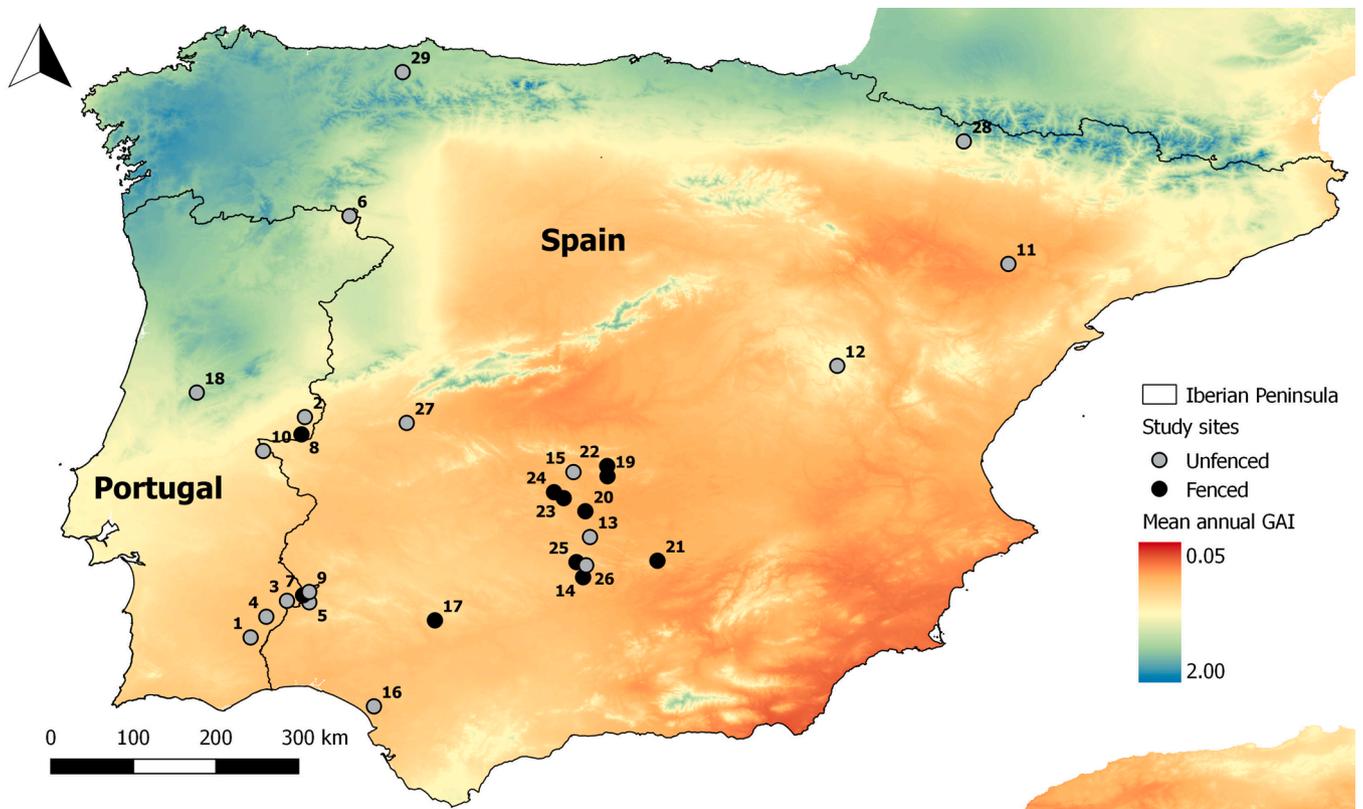


Fig. 1. Location of the study sites in the Iberian Peninsula. Global Aridity Index (GAI) map of the Iberian Peninsula with the location of the fenced (black) and unfenced (grey) study sites.

$$Hp = \frac{Hb}{P_{harv}} \tag{1}$$

where Hp = estimated host abundance, Hb = average number of animals harvested each year, and P_{harv} = estimated proportion of the population harvested each year.

Data on the average number of animals harvested each year was obtained for most study sites ($n = 25$) (Table S2). The P_{harv} parameter was estimated as a distribution incorporating variability and uncertainty, from published data for the Iberian Peninsula: one wild boar population studied by telemetry in Montes de Toledo, Spain (Barasona et al., 2016); and data from 25 red deer populations (Torres-Porras et al., 2014), plus two other populations (Camargo et al., 2021), all from Spain (Table S1). The uncertainty and variability in the P_{harv} estimates extracted from these references were incorporated as PERT distributions with mode=estimated proportion of the population harvested each year, minimum and maximum= ± 2 standard deviations from the mode (Vose, 2008). The PERT (Program Evaluation and Review Technique) distribution is a beta distribution with the α and β parameters obtained from ‘minimum’, ‘mode’ and ‘maximum’ estimates, subjectively determined by an estimator (Vose, 2008; MacCrimmon and Ryavec, 1964):

$$p \sim PERT(a, b, c) = Beta\left(\frac{(\mu - a) \times (2b - a - c)}{(b - \mu) \times (c - a)}, \frac{\alpha \times (c - \mu)}{(\alpha - a)}\right) \tag{2}$$

where a = minimum, b = mode, c = maximum, and $\mu = \frac{a+4b+c}{6}$.

The PERT distribution is widely used in quantitative risk analysis to represent the uncertainty of subjective estimates of a given quantity. We used the PERT distribution to specify distributions from published references where only a measure of central tendency (e. g. mode) and dispersion (standard deviation or confidence intervals) were available.

Several distributions of the proportion of the red deer population

harvested each year were extracted from the bibliography and weights were assigned to each distribution based on the author’s evaluation of the sample size, geographical scope, and quality of the data (Table S1 and Fig. S2). These distributions were then combined in a P_{harv} distribution through a probability tree implemented R 3.6.1 (R Development Core Team, 2008) using the package ‘mc2d’ (Pouillot and Delignette-Muller, 2010).

To estimate the distribution of Hp at each site, the P_{harv} distributions were sampled over 100,000 iterations in Markov Chain Monte Carlo (MCMC) simulations and convergence of the Hp estimates was assessed by the visual inspection of autocorrelation and trace plots and computation of Geweke test (Geweke, 1992). The Geweke test was used to assess the convergence of the MCMC simulations by comparing the mean of the first 10% iterations with the mean of the final 50% iterations (Geweke, 1992).

In 4 sites (sites 6, 16, 28, and 29), published estimates of red deer or wild boar abundance, obtained by distance sampling, fecal density counts, or direct census were used as prior information (Table S2). In these sites, population abundance was specified directly as PERT distributions (Vose, 2008).

2.3. True prevalence

Second, the true prevalence was estimated from the apparent prevalence, corrected by the sensitivity and specificity of the diagnostic test employed at each site [Eq. 3], using the package ‘prevalence’ (Develleschauwer et al., 2014) in R 3.6.1 (R Development Core Team, 2008), where:

$$Tp = \frac{Ap + Sp - 1}{Se + Sp - 1} \tag{3}$$

where Tp = true prevalence, Ap = apparent prevalence, Se = sensitivity of the diagnostic test, and Sp = specificity of the diagnostic test.

Apparent TB prevalence data was obtained for each site and consisted of the number of animals TB-positive/number of animals examined by gross pathology, bacteriological culture, or their combination in series (Table S4 and Fig. S3). Gross pathology consisted of the macroscopic examination of harvested animals, following described protocols (Martín-Hernando et al., 2007, 2010; Santos et al., 2010). Bacteriological culture followed a published protocol (Santos et al., 2010).

A beta(1,1) distribution was used as a flat prior for T_p , while the distributions of Se and Sp were computed as in Santos et al (Santos et al., 2020), with the inclusion of a more recent study evaluating the performance of TB diagnostic tests in the wild boar (Richomme et al., 2019) (Table S3). Published estimates of the sensitivity and specificity of TB diagnostic tests were specified in the model as PERT distributions [Eq. 2]. Raw data on the sensitivity and specificity of the diagnostic tests were specified as beta distributions (Vose, 2008):

$$p \sim \text{Beta}(s+1, n-s+1) \quad (4)$$

where s = number of true positives (for sensitivity) and true negatives (for specificity), and n = number of animals infected (for sensitivity) and not infected (for specificity).

When several distributions were available for the same parameter (e.g., several estimates of sensitivity or specificity of one diagnostic test), they were combined by a probability tree (Table S3), as described in the previous step for the P_{harv} parameter. To estimate the distribution of T_p at each site (Fig. S4), the distributions of Se and Sp were sampled over 100,000 iterations and convergence of the T_p estimates was assessed by the visual inspection of autocorrelation and trace plots and computation of the Geweke test (Geweke, 1992).

2.4. Excretion

Third, the probability of MTC excretion by infected wild boar and red deer was estimated by re-analyzing a dataset previously reported (Santos et al., 2015), this time under a site-occupancy framework (Lachish et al., 2012). Site-occupancy models were originally developed in ecological research to estimate the probability of one site being occupied by a given species, accounting for the imperfect detectability of the survey method (MacKenzie et al., 2006; Royle and Dorazio, 2008). They require replicated surveys of the sites to estimate the detection probability and correct the naïve occupancy estimates by the detection probability. They were also applied to epidemiological data and shown to be a valid alternative to estimate pathogen presence correcting for the sensitivity of the diagnostic test (Lachish et al., 2012). They were used in this study to obtain estimates of excretion correcting the naïve estimates by the imperfect sensitivity of the molecular detection method applied to several excretion routes.

Briefly, apparent excretion was assessed by 1–9 replicates of a molecular assay performed on different biological matrices (oronasal and bronchial washes, and feces) of MTC-infected wild boar ($n = 56$) and red deer ($n = 43$), as confirmed by bacteriological culture or molecular detection in tissues (Santos et al., 2015). A standard single-season occupancy model (MacKenzie et al., 2006; Royle and Dorazio, 2008) was fitted by maximum likelihood, with each animal considered a ‘site’ (in the standard occupancy meaning), and each replicate of the molecular assay for MTC in each excretion matrix as an observation (Lachish et al., 2012). The categorical covariates were the ‘excretion matrix’ (oronasal washes/bronchial washes/ feces), for the detection probability, and the ‘species’ (wild boar/red deer), for the occupancy probability. Estimates of occupancy were predicted for each species for the reference matrix (oronasal washes, which showed the highest detection probability: 0.751, CI_{95} 0.657 – 0.827). The estimated excretion probability for the wild boar (0.782, CI_{95} 0.593–0.898) and red deer (0.828, CI_{95} 0.630–0.932) (Fig. S4), were specified as PERT distributions common to all study sites [Eq. 2]. Site-occupancy models were fitted using the package ‘unmarked’ (Fiske and Chandler, 2011).

2.5. Basic reproduction number

Finally, the R_0 of MTC in each host species ($R_{0,wild\ boar}$ and $R_{0,red\ deer}$) was estimated following the approach proposed by Fenton et al., 2015. For environmentally transmitted parasites. Considering a pathogen maintained by 2 host species (i and j) in a system assumed to be at equilibrium, the R_0 can be directly estimated from the equation:

$$R_{0i} = \frac{1}{(1 - P_i)(\delta_{ij}\epsilon_{ij}\nu_{ij}\omega_{ij})} \quad (5)$$

where R_{0i} = basic reproduction number of the species i , P_i = prevalence in the host species i , $\delta_{ij} = \lambda_j / \lambda_i$ (λ_i being the MTC excretion of the host species i), $\epsilon_{ij} = H_j / H_i$ (H_i being the abundance of the host species i), $\nu_{ij} = P_j / P_i$, $\omega_{ij} = \beta_{ij} / \beta_{ii}$ (β_{ij} being a measure of transmission from species i to species j , and β_{ii} the intra-species transmission of host i), j being the other species in the 2-host system (Fig. 2).

This method is a generalization of the multi-species dynamic models of Funk et al., 2013 and Rudge et al., 2013 to allow direct (non-dynamic) estimation of species-specific R_0 relying on a limited set of standard epidemiological parameters (abundance, prevalence, excretion, and overlap in intra- and inter-species transmission). Assuming the system is at equilibrium removes the need for dynamic equations including parameters difficult to estimate in host-pathogen systems involving wildlife, such as the loss rate of infected individuals and infective forms in the environment, and uptake of infective forms from the environment (Fenton et al., 2015). This method incorporates heterogeneous sharing of infective stages, allowing the intra-species transmission to differ from the inter-species transmission, which is fundamentally realistic for the wildlife-TB system (Corner, 2006; Cowie et al., 2016; Triguero-Ocaña et al., 2020; Murphy et al., 2021).

The [Eq. 5] was implemented incorporating the variability and uncertainty in the parameter estimates: for H , the 100,000 estimated values of the abundance estimated for each species at each site (first step); for P , the 100,000 estimated values of the true prevalence of TB for the wild boar and red deer at each site (second step); for λ , the maximum likelihood estimates of excretion of MTC from infected wild boar and red deer specified as PERT distributions common to all study sites (third step); and for β , a flat distribution $\text{unif}(0,1)$ encompassing all the possible values of intra- and inter-species transmission of infection (Fig. 2). The R_0 was estimated by sampling 100,000 times from the distributions (λ and β) and the estimated values (H and P), using the package ‘mc2d’ (Pouillot and Delignette-Muller, 2010). The probability of TB being maintained at each study site by a single host species (only one species with an estimated $R_0 > 1$), as obligatory multi-host (both species with an estimated $R_0 < 1$), or as facultative multi-host (both species with an estimated $R_0 > 1$) were estimated as the proportion of the whole estimated distribution (100,000 estimates) falling into each of these categories (Table S6).

2.6. Statistical analysis

A sensitivity analysis was performed by estimating Spearman’s correlation (ρ) between the $R_{0,wild\ boar}$ and $R_{0,red\ deer}$ and each of the model parameters.

The relationships between the probability of each species being the single maintenance host (only 1 species with $R_0 > 1$) or TB being a multi-host disease ($R_{0,wild\ boar}$ and $R_{0,red\ deer} > 1$) at each site and the independent categorical variable ‘fence’ (reference category ‘unfenced sites’), and the continuous variables ‘cattle TB’ herd prevalence, ‘aridity’, ‘TB prevalence’ in wild boar and red deer, and ‘density’ of wild boar and red deer were assessed using generalised linear models with gaussian error function and identity link implemented in R (R Development Core Team, 2008). The fitness of the models was assessed using model diagnostic plots obtained from the package ‘boot’ (Canty and Ripley, 2021) (Fig. S3).

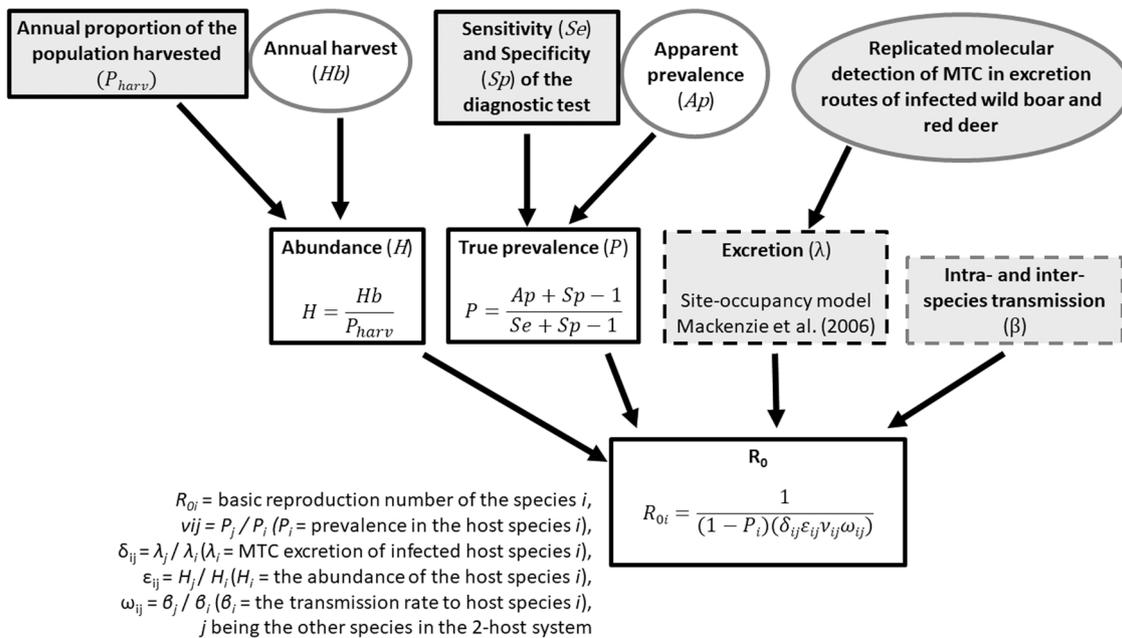


Fig. 2. Summary of the sequential workflow. Parameters used to estimate the basic reproduction number (R_0) of wild boar and red deer were estimated using Bayesian estimation for abundance and true prevalence (continuous black squares), maximum likelihood site-occupancy model for excretion (dashed black squares), or as a flat distribution (dashed grey squares). Data represented as grey circles. Parameters transversal to all study sites in light grey background, site-specific parameters in white background. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The presence of a perimetral > 2 m-high fence constraining the movements of wild ungulates is a reliable proxy for the intensive management of their populations as game species, which is a well-known risk factor for TB (Santos et al., 2018; Camargo et al., 2021). Cattle herd TB prevalence was extracted at the smallest geographical unit available (county in Portugal; ‘comarca ganadera’ in Spain) as the average of 2016 and 2019 Portuguese data (DGAV, 2017) and 2018–2019 Spanish data (MAPAMA, 2020). Each study site was assigned the herd prevalence of the geographical unit where it was located. Aridity was shown to be a risk factor for TB in wild ungulates in the markedly seasonal Mediterranean ecosystems of the Iberian Peninsula by influencing their aggregation, particularly at watering sites during the dry season (Triguero-Ocaña et al., 2020; Vicente et al., 2013; Abrantes et al., 2021). To assess the effect of this risk factor, the Global Aridity Index (GAI) was included as an independent variable in the model. The GAI is the ratio between precipitation and the Global Reference Evapotranspiration, thus representing rainfall over vegetation water demand, being higher in humid climates. The annual averages of the Global Aridity Index 1970–2000 data were obtained from the Consortium for Spatial Information (Trabucco and Zomer, 2018). Mean annual aridity was extracted from a 5 km buffer surrounding each study site, using QGIS version 2.18.0 (QGIS Development Team, 2021). Density was estimated by dividing the estimated abundance at each site by the area of the site. Prevalence was estimated as described previously.

Models including all the variables were compared under an information-theoretic approach (Burnham and Anderson, 2002) (Fig. S3) and those with $\Delta AIC_c < 2$ to the most supported model were averaged using the package ‘MuMin’ (Bartoni, 2015). All the independent variables showed Pearson correlations < 0.6 between them. One outlier observation influential (Cook’s distance > 1.5) in the multi-host probability model (site 15) (Fig. S3) was removed from the analysis. Graphics were produced using the package ‘ggplot2’ (Wickham, 2016) in R 3.6.1 (R Development Core Team, 2008).

3. Results

Across sites, the median estimates of the $R_{0,wild\ boar}$ ranged 0.1–55.9,

with an average value of 8.7 (standard deviation 11.8), and the median estimates of the $R_{0,red\ deer}$ ranged 0.1–18.9, with an average of 2.2 (standard deviation 3.9) (Fig. 3, Table S5 and Fig. S8). The $R_{0,wild\ boar}$ and $R_{0,red\ deer}$ were most correlated with the parameters true prevalence, intra- and inter-species transmission (Fig. 4). The true prevalence and abundance of the red deer were more correlated with both $R_{0,wild\ boar}$ and $R_{0,red\ deer}$ than the same parameters of the wild boar.

The average probability of TB being maintained by a single host species (only one species with an estimated $R_0 > 1$) was 0.528 ± 0.250 (mean \pm standard deviation) for the wild boar (single maintenance host in 18 sites) and 0.268 ± 0.228 for the red deer (single maintenance host in 5 sites). The average probability of TB being maintained as a multi-host disease (both species with an estimated $R_0 > 1$) was 0.204 ± 0.195 (6 sites) (Fig. 3, Table S6, and Fig. S8).

The probability of TB being maintained as a multi-host disease was significantly related to higher prevalence and density of the red deer, and with higher prevalence but lower density of the wild boar. Non-significant negative relationships with the GAI (lower GAI corresponds to higher aridity) and fencing were retained in the averaged model (Table 1 and Fig. 5).

The probability of the wild boar being the single maintenance host of TB was significantly related to lower prevalence in the red deer. The probability of the red deer as the single maintenance host of TB was significantly related to the prevalence in wild boar (negative relationship) and red deer (positive) (Table 1 and Fig. 5). A non-significant relationship with cattle TB herd prevalence was retained in the wild boar (positive relationship) and red deer averaged models (negative) (Table 1). The estimated density and prevalence of TB in the wild boar and red deer tend to be higher in the central-southwestern Iberian Peninsula, where are located the sites where TB was estimated to be maintained as a multi-host disease (Fig. 6).

4. Discussion

We estimated the R_0 of *Mycobacterium tuberculosis* complex infection in wild boar and red deer in 29 sites across the Iberian Peninsula. These wild ungulates are considered the most important maintenance hosts of

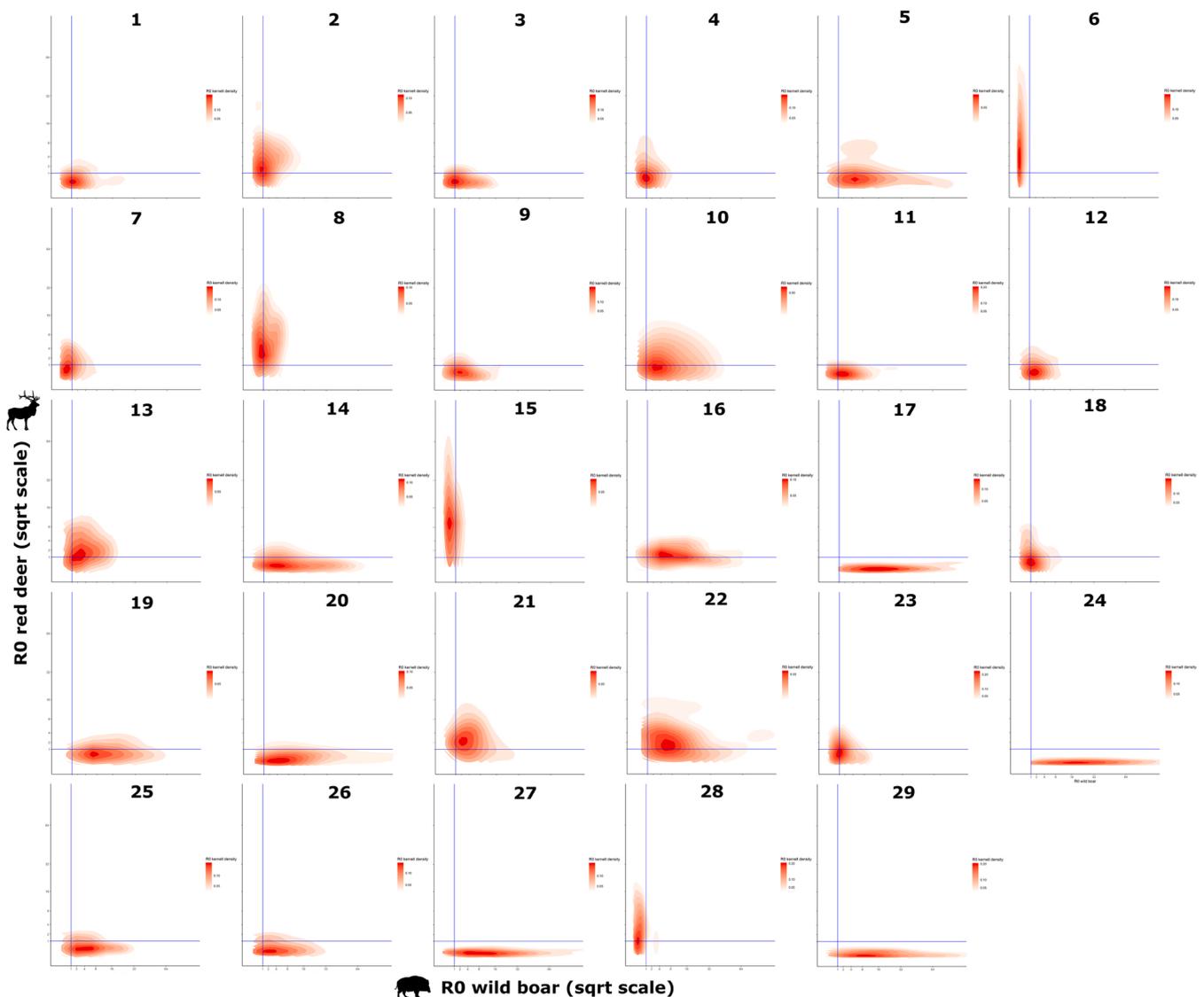


Fig. 3. Basic reproduction number estimated for the wild boar and red deer in 29 study sites. Kernel density of 100 estimates of the R_0 of *Mycobacterium tuberculosis* complex in the wild boar and red deer drawn randomly from the posterior distribution. Axes in square root scale, $R_0 = 1$ as blue horizontal and vertical lines. Individual plots are reported in more detail in Fig. S4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

animal TB across much of the Iberian Peninsula (Gortázar et al., 2012), nevertheless, the quantification of their relative contributions to disease maintenance was lacking. Our results support that both species can maintain animal TB in different epidemiological scenarios, ranging from any species acting as a single maintenance host to a facultative multi-host situation (Fenton et al., 2015).

Sites where TB was maintained as a facultative multi-host disease showed high true TB prevalence, particularly in the wild boar (range 0.781–0.945) but also the red deer (0.088–0.477) (Table 1 and Fig. 5). Notable examples include Doñana National Park (site 15) and several sites in Castilla-la Mancha, Spain (sites 13, 21, 22), and eastern central-south Portugal (sites 8, 10) where TB was shown to persist at high prevalence for more than a decade (Vicente et al., 2013; Santos et al., 2010; Gortázar et al., 2009). A persistent high-prevalence setting is the definition of hyper-endemicity. While in these hyper-endemic sites TB can be maintained by any single host species, both greatly contribute to the $R_{0, tot}$. In some of these sites, such as in Doñana National Park, other host species including cattle and fallow deer further contribute to the TB maintenance community (Barroso et al., 2020). Spill-over from livestock was possible as TB was present in cattle in these sites with a local herd

prevalence of up to 28.6% and has also been reported in the fallow deer (Santos et al., 2020; Triguero-Ocaña et al., 2020; Martín-Hernando et al., 2010).

The significantly lower density of wild boar at the sites where TB was maintained as a multi-host disease (Table 1 and Fig. 5) could be a consequence of disease-induced mortality in this species (Barasona et al., 2016). This result suggests that hyper-endemic TB could be regulating wild boar populations, even causing local declines in abundance, supporting the predictions of dynamic models (Tanner et al., 2019). Furthermore, the prevalence of TB in the wild boar strongly increased with the probability of multi-host disease, suggesting that this species might be acting as an amplifier host in these hyper-endemic epidemiological scenarios. The multi-host probability increased non-significantly with aridity (lower GAI) and in unfenced populations (Table 1 and S7). These sites tend to lie in the central-south-western Iberian Peninsula (Fig. 6), a region characterized by moderately high aridity (Fig. 1), where host aggregation at watering points was shown to be a risk factor for TB (Triguero-Ocaña et al., 2020; Vicente et al., 2013; Abrantes et al., 2021).

The wild boar was the most important maintenance host for TB in

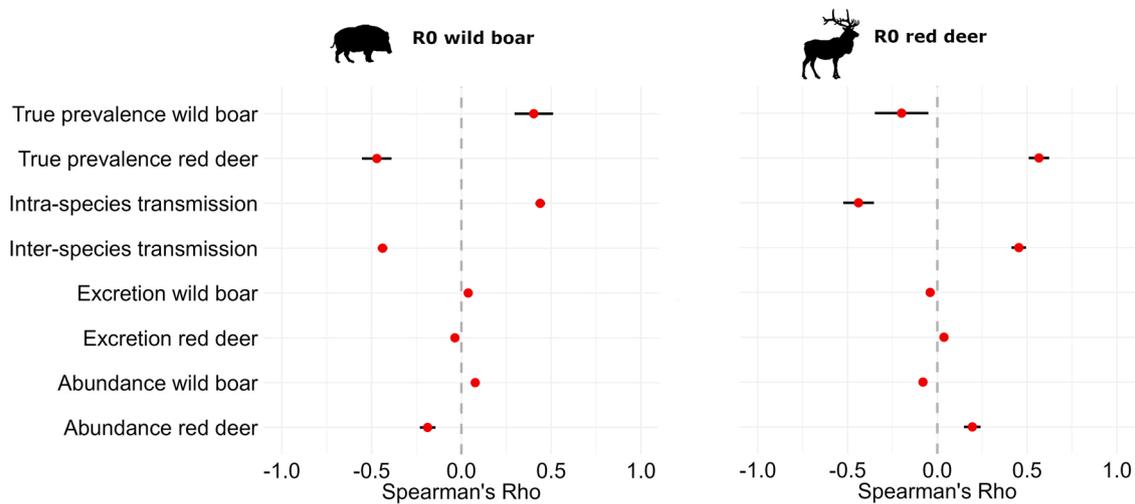


Fig. 4. Sensitivity analysis of the estimated R_0 in the wild boar and red deer. Spearman's correlation (Rho) between the parameters and the estimated R_0 of *Mycobacterium tuberculosis* complex in the wild boar and red deer across the 29 study sites, with standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Summary of the averaged model of the probability of animal tuberculosis being a single or multi-host disease. Models with $\Delta AICc < 2$ from the most supported model were included in the conditional averaged model. Significant relations highlighted in bold.

Variables	Multi-host disease			Wild boar single maintenance host			Red deer single maintenance host		
	β	Standard error	95% confidence interval (β)	β	Standard error	95% confidence interval (β)	β	Standard error	95% confidence interval (β)
Intercept	-3.223	0.538	-4.308, -2.138	0.657	0.258	0.128, 1.187	-0.579	0.288	-1.170, 0.013
Fence	-0.211	0.193	-0.610, 0.189	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Fenced									
Cattle TB	n. a.	n. a.	n. a.	3.884	2.419	-1.097, 8.866	-4.012	2.649	-9.479, 1.455
Global Aridity Index (GAI)	-2.570	1.523	-5.728, 0.589	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Density WB	-0.142	0.033	-0.209, -0.074	n. a.	n. a.	n. a.	0.056	0.055	-0.057, 0.168
Density RD	0.022	0.007	0.008, 0.037	-0.031	0.019	-0.070, 0.008	0.051	0.025	-0.0003, 0.102
Prevalence WB	3.154	0.361	2.410, 3.897	n. a.	n. a.	n. a.	-4.665	1.250	-7.240, -2.090
Prevalence RD	2.605	0.626	1.326, 3.884	-3.992	1.339	-6.741, -1.243	5.922	1.639	2.543, 9.300

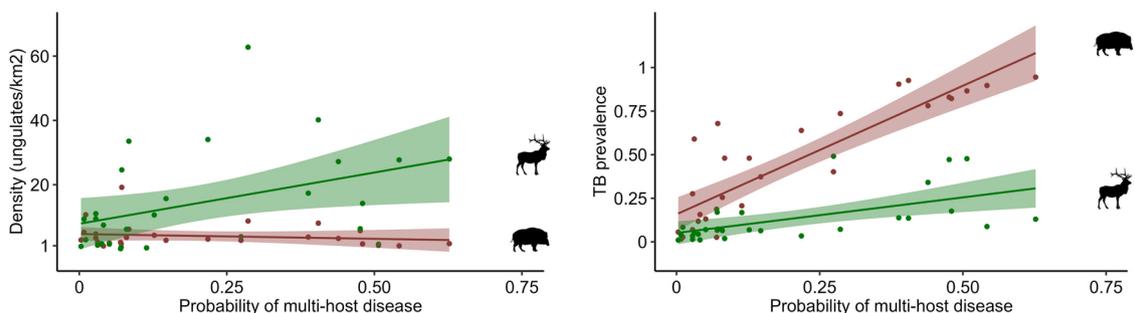


Fig. 5. Estimated probability of animal tuberculosis being single-or multi-host disease at each site according to the wild boar and red deer density and prevalence. Probability of animal tuberculosis being a single-host (only 1 species with $R_0 > 1$) or multi-host disease (both species with $R_0 > 1$) estimated from the 100,000 simulations of the R_0 of *Mycobacterium tuberculosis* complex at each site for the wild boar (red) and red deer (green). Linear relationship with 95% confidence intervals as function of the estimated density and animal tuberculosis prevalence in wild boar (red) and red deer (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

most sites, and the red deer at five sites (Fig. 6 and Table S6). This observation supports previous studies concluding that both species can act as maintenance hosts for TB (Gortázar et al., 2012; Vicente et al., 2013). Interestingly, the prevalence of TB in the red deer was more related to the probability of the wild boar being a maintenance host than the prevalence in the wild boar itself, as supported by the sensitivity analysis and GLM (Table 1, Fig. 4 and Table S7). These results suggest that the prevalence in the red deer could be an important driver of the

epidemiology of TB in multi-host communities, as suggested for the Brotonne forest in France (Zanella et al., 2012), despite the fact this species was identified as the main host in a minority of our study sites.

The cattle herd TB prevalence was retained in the averaged models of the probability of wild boar and red deer being maintenance hosts, with contrasting but non-significant effects. The cattle herd TB prevalence tended to decrease with the increasing probability of the red deer being a maintenance host, suggesting weak epidemiological links between

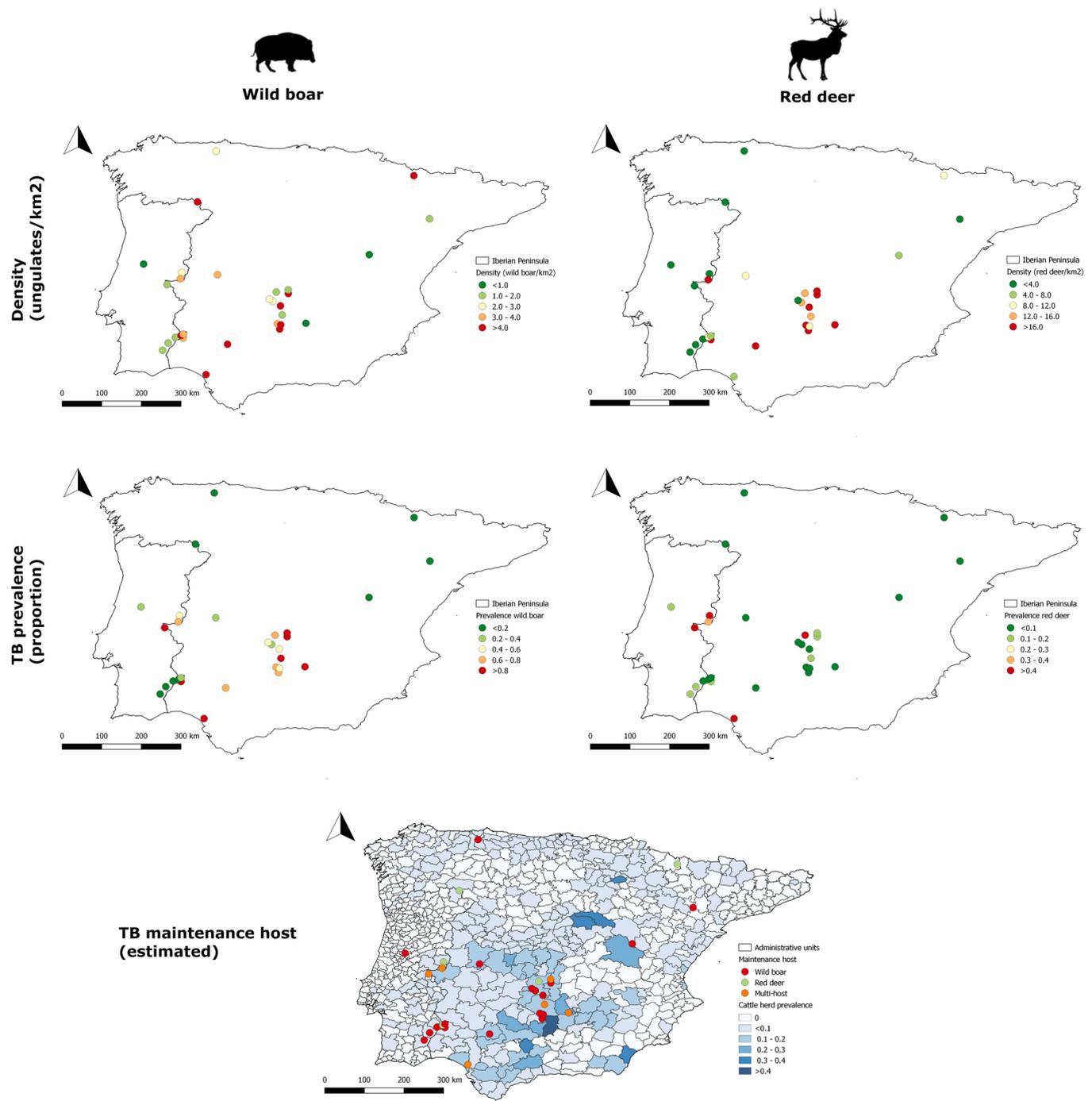


Fig. 6. Estimated density and prevalence of wild boar and red deer and probability of maintenance host status for the wild boar and red deer at each site. Maintenance host map: cattle herd tuberculosis prevalence of the administrative units of the Iberian Peninsula: county (Portugal: average of 2016 and 2019 data) and *comarca ganadera* (Spain: average of 2018 and 2019 data). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these species (Table 1). On the other hand, cattle herd TB prevalence tended to increase with the probability of the wild boar being a maintenance host, supporting a possible role for this species as a bridge host connecting the multi-host wildlife community and cattle (Caron et al., 2015). While the results show that TB was maintained by wild boar in the absence of infection in cattle, they also support an epidemiological link between these host species, as suggested by other studies in the Iberian Peninsula (Reis et al., 2020; LaHue et al., 2016; Santos et al., 2018; Barasona et al., 2014). Whether this link is dominated by spillover from wild boar to cattle or the inverse is yet to be conclusively

determined. However, the high prevalence of TB in wild boar at fenced hunting estates in the absence of contact with cattle strongly supports the former hypothesis (Gortázar et al., 2017).

The unprecedented perspective on the epidemiology of TB in wildlife multi-host communities obtained in this study was possible by bringing together concepts and methods from a range of disciplines: ecology (i. e. partitioning the contribution of host species to the $R_{0,tot}$, and site-occupancy models for excretion data) (Dobson, 2004; Fenton et al., 2015; Lachish et al., 2012; MacKenzie et al., 2006), epidemiology (i. e. estimation of true prevalence from apparent prevalence) (Speybroeck

et al., 2013), and risk analysis (i. e. the Bayesian framework to directly estimate the R_0 incorporating the variability and uncertainty in the parameters) (Vose, 2008). This set of inter-disciplinary tools could be applied to other regions where TB is maintained by multi-host communities, provided relevant data is available. Better-quality data, particularly regarding the parameters true prevalence, intra- and inter-species transmission of infection (Fig. 4) will be essential to estimate the R_0 of each species with lower uncertainty. The authors recognize that this study is a simplification of a complex system, as other maintenance hosts, not included in the analysis due to lack of data, may significantly contribute to the $R_{0,tot}$ (Santos et al., 2020). Wildlife examples are the fallow deer (Triguero-Ocaña et al., 2020) and the Eurasian badger (Blanco-Vázquez et al., 2021). Most importantly, livestock such as cattle, goats, sheep, and pigs can maintain TB in the absence of spillover from wildlife (LaHue et al., 2016; Napp et al., 2013; Ciaravino et al., 2021). Livestock species, including cattle, could play a relevant role in the epidemiology of TB in multi-host communities (Santos et al., 2020), and future studies are required to estimate the R_0 of MTC in multi-host communities including livestock. Animal tuberculosis is a chronic endemic disease, and the host species are long-lived, so the time frame of its epidemiological processes is relatively long. Nevertheless, annual variations in prevalence have been reported (Vicente et al., 2013; Barroso et al., 2020), and future studies should address the dynamics of the R_0 of different host over time.

5. Conclusions

We show that animal tuberculosis is maintained by wildlife communities across the Iberian Peninsula in single-host or multi-host epidemiological scenarios. While any of the wildlife host species investigated can maintain TB, the prevalence of TB in the red deer is likely an important driver of the multi-host epidemiology due to its influence on the probability of any of the species being maintenance host. The wild boar was the single maintenance host of TB in most of the study sites and our results suggest this species could have an epidemiological role in linking the wildlife multi-host community and livestock.

Ethics

No animals were harmed or killed for this study and no ethical approval was necessary.

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CRediT authorship contribution statement

Conceptualization: N.S., J.Q., V.A., J.V., C.G., and P.C.A.; Data curation: N.S., E.F.C., M.C.A., D.F.L., E.F., A.M.V., A.M.V., and J.Q.; Formal analysis: N.S. and E.F.C.; Funding acquisition: D.F.L., I.S., A.B., J.V., C.G., P.C.A.; Investigation: N.S., E.F.C., M.C.A., D.F.L., I.S., J.M.G., E.F., A.M.V., J.Q., A.B., J.V., C.G.; Methodology: N.S., V.A., J.V., C.G., P.C.A.; Project administration: C.G. and P.C.A.; Resources: I.S., J.M.G., A.B., J.V., C.G., P.C.A.; Supervision: V.A., C.G., P.C.A.; Validation: N.S., M.C.A., D.F.L., I.S., J.M.G., V.A., A.B., J.V., C.G., P.C.A.; Visualization: N.S.; Writing – original draft: N.S., E.F.C., A.B., V.A., C.G., P.C.A. All authors assisted with the interpretation of the data and revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in

the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability

The datasets supporting this article have been reported in the supplementary material Tables S1–S4.

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Author statement

This study did not involve any procedure that could be considered animal experimentation. All data and samples were collected from animals harvested for recreational or management purposes. No animal was harmed for the purpose of this study.

Data accessibility

The datasets supporting this article are reported in the supplementary material Tables S1–S4.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.epidem.2022.100633](https://doi.org/10.1016/j.epidem.2022.100633).

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