

## Contribution of herd management, biosecurity, and environmental factors to the risk of bovine tuberculosis in a historically low prevalence region



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### ARTICLE INFO

#### Article history:

Received 11 October 2023

Revised 30 January 2024

Accepted 1 February 2024

Available online 8 February 2024

#### Keywords:

Cattle

Dairy

Epidemiology

Sheep

Wildlife

### ABSTRACT

Eradication of bovine tuberculosis (**bTB**) in certain historically low-prevalence regions remains elusive. A complete characterisation of the husbandry practices, biosecurity, and environment where farms are located is crucial to implement targeted in-farm risk mitigation protocols. Here, a detailed survey performed in 94 dairy cattle farms located in Navarra, a low-prevalence region of Spain between 2016 and 2020 was carried out. Data on 73 biosecurity, farm-, and environmental-level factors potentially associated with the risk of bTB occurrence were evaluated using an ordinal logistic regression model: farms were classified based on their prevalence index, a score linked to each farm to account for the severity and recurrence of bTB cases: 22.3% of the farms had a score of 1, 21.3% a score of 2, 26.6% a score of  $\geq 3$ , and 29.8% were negative herds. A statistically significant association between a higher prevalence index and the frequency of badger sightings along with the lease of pastures to sheep during Winter was identified. Farms that detected badgers on a monthly to daily basis in the surroundings and those that leased pastures for sheep flocks during Winter were four [odds ratio, 95% CI (4.3; 1.1–17.5)] and three (3.1; 1.0–9.9) times more likely to have the highest prevalence index, respectively (predicted probability<sub>prevalence index $\geq 3$</sub>  = 0.7; 95% CI 0.3–0.9). Conversely, farms that used a vehicle to transport animals from holdings to pastures were less likely (0.1; <0.1–0.3) to present higher levels of prevalence index compared with farms that used none (on foot). Results suggested that the combined effect of farm- and environmental-level risk factors identified here may be hampering disease eradication in Navarra, highlighting the need to implement targeted protocols on farms and grazing plots. An increased awareness of monitoring sheep and wildlife in direct or indirect contact with cattle herds in historically low bTB prevalence areas should be raised.

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### Implications

Not much is known about the mechanisms that impair the complete eradication of bovine tuberculosis in dairy cattle from the north of Spain, where disease prevalence has remained historically low. Identifying potential farm and environmental sources of the disease at the hunting-extensive livestock-wildlife interface is critical to inform farmers, stakeholders, and policymakers to implement on-farm mitigation protocols. Using an extensive survey that included data on > 70 specific risk points to the highest detail

from dairy farms, we suggest that certain biosecurity and husbandry practices, which may facilitate intra- and inter-species contact, were associated with the occurrence of bovine tuberculosis.

### Introduction

Animal tuberculosis (**TB**) is caused by several *Mycobacterium* species that belong to the *Mycobacterium tuberculosis* Complex (**MTC**). It is a multihost infection with a complex epidemiology (Martinez-Guijosa et al., 2020; Santos et al., 2022), where the environment plays a relevant role (Santos et al., 2015; Barasona et al., 2017). Furthermore, infection diagnosis in vivo remains challenging due to the limited (and variable) sensitivity of currently available tests, the disease stage of the infected animal, the effect of

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concurrent infections such as paratuberculosis caused by *Mycobacterium avium paratuberculosis* or the exposure to non-pathogenic mycobacteria, which can interfere with its diagnosis (Alvarez et al., 2009; Queiros et al., 2012; Broughan et al., 2016; Byrne et al., 2019; Bezos et al., 2023).

The prevalence of bovine TB (bTB) in Spain is unevenly distributed depending on the region and the type of production. Herd prevalence is much higher in the southwest and in extensive beef production systems (Martinez-Lopez et al., 2014; Bezos et al., 2023). These settings have received attention regarding the interaction and transmission between wildlife, mostly Eurasian wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*), and livestock (LaHue et al., 2016; Barasona et al., 2017; Triguero-Ocana et al., 2019). In comparison with Southwestern Spain, fewer studies have focused on bTB epidemiology in Northern Spain (Munoz-Mendoza et al., 2013; Pérez de Val et al., 2017) and specifically in relation to dairy cattle (Allepuz et al., 2011). On the British Isles, European badgers (*Meles meles*) can maintain MTC infection and eventually compromise cattle bTB control in dairy farming systems with pasture access or where badgers can enter farm buildings and contaminate cattle feed (Godfray et al., 2013; Broughan et al., 2016; Robertson et al., 2019). In Northern Spain, badgers do also contribute to the MTC maintenance community (Balseiro et al., 2013; Acevedo et al., 2019). Both in high-prevalence and low-prevalence regions, small ruminants and free-range pigs can locally contribute to MTC maintenance (Muñoz-Mendoza et al., 2016; Bezos et al., 2023).

Herd size, herd bTB history and local prevalence, cattle purchases, and the presence of infected hosts other than cattle are known risk factors for bTB in dairy cattle (Griffin et al., 1993; Skuce et al., 2012; Godfray et al., 2013; Broughan et al., 2016). Intensive production systems may be associated with higher rates of direct contact within cattle, which favours intra-herd transmission of pathogens (Johnston et al., 2011; Alvarez et al., 2012). In addition, the presence of stressors such as milking can decrease the immune status of the animal (Griffin et al., 1993). However, intensive systems allow biosecurity measures to be taken more effectively. In that sense, some studies claim that outbreaks in dairy herds are more easily controlled (Alvarez et al., 2012), although they are also associated with a shorter time interval between outbreaks (Doyle et al., 2014).

In this study, we visited 94 dairy farms in the region of Navarra, Northern Spain, to perform on-farm bTB risk assessments (Martínez-Guijosa et al., 2021). Despite the low bTB prevalence levels during the last decades (0.5% in 2002 and 0.2% in 2021), the region has so far been unable to reach the officially tuberculosis free status (OTF) and is still considered a low prevalence area [ $<1\%$  (Anon, 2023b)]. Our aim was to reveal the bTB risk factors associated with dairy farming in a historically low prevalence region.

## Material and methods

### Study population and data sources

The study was performed in the region of Navarra (centroid coordinates, UTM wgs84: 592425, 4743194), located in the north of Spain (Fig. 1) between the Pyrenees Mountain chain and the Ebro River valley. Navarra is characterised by a marked altitudinal gradient and a wide variety of climates and ecosystems. The Atlantic climate predominates in the north, with numerous rivers and tributaries, whereas the Mediterranean climate defines the orography in the south. Navarra holds 3.4% out of the total Spanish milk quota, with 253 993 tonnes in 2022 (Anon, 2022). There are 2 089 cattle herds (and  $> 120\ 000$  animals) in Navarra, of which 6.2% ( $n = 129$ ) are dairy herds (Anon, 2023a). The Spanish bTB eradica-

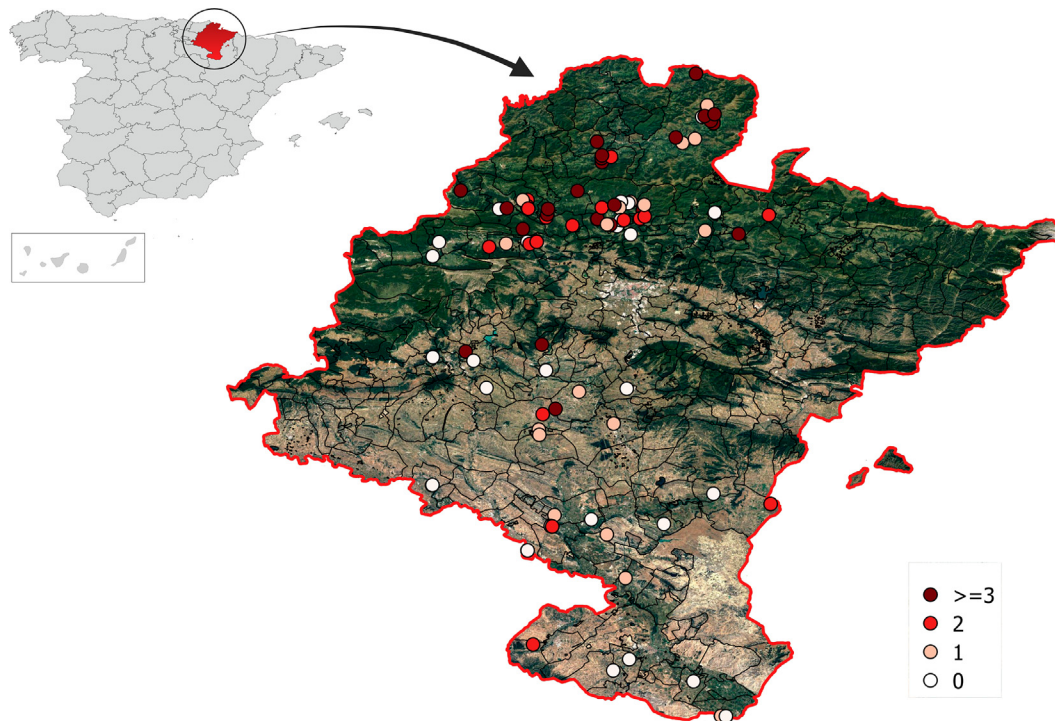
tion program in Navarra is based on test and slaughter surveillance and live animal annual testing using the single intradermal test (SIT) in all herds plus the interferon-gamma (IFN-g) assay as a complementary test in infected herds to maximise the diagnostic sensitivity (Anon, 2023b). Positive animals should be culled and subjected to postmortem inspection. Farms confirmed as infected are then subjected to follow-up tests using the SIT and IFN-g tests within the following 2–6 months until they recover the OTF status.

Here, a questionnaire survey was designed to collect detailed information on farm characteristics, farm management practices, and environmental factors in 94 (73% of the total dairy herds and 4.5% of the total number of herds in Navarra) farms with the highest census of dairy cattle in Navarra (see Results). These farms were selected by the Regional Veterinary Authorities as part of a biosecurity program to develop specific on-farm assessment of risks that would subsequently materialise in the form of a detailed report for each farm with the proposed mitigation actions. Farmers were provided with a final report of the findings regarding the critical risk points identified in the farm, recommendations, and proposals to improve biosecurity (data not shown). None of the farmers from the high-census farms from Navarra refused to take part in the study. We designed the questionnaire survey using an adapted protocol originally designed for beef cattle (Martínez-Guijosa et al., 2021) and visited the farms to complete the information collected by the survey between October and November 2020, as the validity of the questionnaire was not evaluated here. This on-farm evaluation and field reconnaissance aimed to verify farmer perceptions consisted of potential risk point characterisation (i.e., fencing, feeders, and watering points status, signs of wildlife) and risk scoring. Specifically, this survey collected data on several risk factors related to cattle (herd size, cattle purchases), farm management, hygiene, and biosecurity (water points, manure management, outdoor fencing), contact with other domestic species (i.e., presence of other livestock and domestic species inside the farm or in surroundings) and with the fauna and habitat (i.e., footprints, droppings, and frequency of sightings of red deer, roe deer, wild boar, and badger) as well as hunting-related factors (i.e., hunting area, density, hunting intensity, % of hunting harvest, etc.). The questionnaire included open (i.e., pasture area, number of cattle) and closed-ended questions based on two/multiple-choice (i.e., contact with other species), and rating scale (i.e., frequency of wildlife sightings categorized into 0 = never, 1 = sporadically, and  $>1$  = monthly to daily) questions. Complete information on the risk factors evaluated in this study and the scientific information supporting the questionnaire is accessible in [Supplementary Table S1](#). Data on bTB status (number of bTB positive/negative animals to the SIT test and lesioned/confirmed through bacteriological culture animals) of the analysed herds between 2016 and 2020 were extracted from the Spanish Bovine Tuberculosis Eradication Program provided by the Local Animal Health Service.

The outcome variable was defined as 'prevalence index', which was calculated out of the percentage of reactor animals to SIT in relation to the total number of animals tested per farm per year. Subsequently, this percentage was transformed into a value between 0 and 3, defined as 'SIT score', that was assigned to each farm each year ( $0\%_{\text{SIT-reactors}} = 0$ ;  $0\text{--}1\%_{\text{SIT-reactors}} = 1$ ;  $1\text{--}2\%_{\text{SIT-reactors}} = 2$ ;  $>2\%_{\text{SIT-reactors}} = 3$ ). The prevalence index of each farm represented the sum of the SIT score obtained each year, categorised into four categories ( $0$  to  $\geq 3$ ), and indicative of the degree of bTB persistence, recurrence, and diffusion within the farm throughout the 5-year span.

### Statistical analysis

Quantitative predictors were described through measures of central tendency [mean, median, and interquartile range (IQR)],



**Fig. 1.** Distribution of dairy cattle farms located in Navarra, Spain. Colours denote bovine tuberculosis (bTB) prevalence index (see Material and Methods).

while categorical independent variables were presented using absolute and relative frequency distribution. The association between each categorical variable and the outcome variable prevalence index was assessed using the Chi-square test, and the Fisher's exact test if there were few observations in the frequencies of the evaluated categorical variables (Supplementary Table S1). The relationship between quantitative predictors and the prevalence index was tested with the Kruskal-Wallis test followed by posthoc tests using Bonferroni corrections for multiple comparisons. Clustering of wildlife reservoir detection in the evaluated farms was assessed using the Bernoulli model of the spatial scan statistic, implemented using the SaTScan software (Kulldorff, 1997).

Finally, the effect of the explanatory variables on the ordinal form of the prevalence index was evaluated using an ordinal logistic regression model. Statistically significant variables at the level of 0.25 (univariable analyses) were used as covariates for stepwise regression. The likelihood ratio test was used to estimate the overall statistical significance of the models (Dohoo et al., 2003). Results were presented through odds ratio (OR) and 95% confidence interval (95% CI), while the Brant test was used to check the proportional odds assumption (Brant, 1990). Multicollinearity between the variables was tested using Cramer's V. The Lipsitz test was used to check the goodness of fit of the final model (Lipsitz et al., 1996). Finally, the predicted probabilities of each category of the prevalence index for the predictors included in the multivariable ordinal logistic regression model were calculated. The statistical analyses were performed using R software (R Core Team, 2023).

## Results

Out of the 94 evaluated herds, 66 (70%) herds had at least one positive animal to the SIT test and were thus considered as bTB-positive herds, while 28 (30%) were negative over the study period. The mean number of animals tested to SIT per farm (after averaging

data collected in each farm over the 5-year period) was 208 animals (IQR = 141–370), whereas the mean percentage of reactors per farm per year ranged between 0.1% (max = 1.4) in 2020 and 1.1% in 2018 (max = 17, Fig. S1 A). Farms had an overall median SIT score (that is, the % of SIT reactors transformed into a 0–3 value) of 0 (IQR = 0–0, max = 3) during the study period, with a peak of 1 (IQR = 0–2, max = 3) in 2018 (Fig. S1 B). Out of the 66 bTB-positive farms, 36 farms (55%) tested positive (at least one reactor to SIT) for one year, 25 (38%) farms were positive for two years, whereas 5 (8%) farms were bTB-positive for at least three years (Table 1). Regarding the prevalence index, 21 (22%) herds had a score of 1, 20 (21%) a score of 2, 25 (27%) a score of  $\geq 3$ , whereas the remaining 28 (30%) negative herds obtained a score of 0 (Table 1). Eighty percent ( $n = 20/25$ ) of the farms with a prevalence index  $\geq 3$  were bTB positive for two or more years. The distribution of farms based on their prevalence index was very heterogeneous across the region of Navarra (Fig. 1).

Farms had a median herd size of 225 animals (IQR = 140–370). Most ( $n = 58/94$ ) farms used pastures, and around one-third (27/94) leased pastures to sheep flocks during Winter (Table 2, Supplementary Table S1). Out of them, 38% ( $n = 22/58$ ) and 52% ( $n = 14/27$ ) had prevalence indexes  $\geq 3$ , respectively. The disease was confirmed through bacteriological culture and/or lesioned animals were detected in 14% ( $n = 9/66$ ) of the positive farms. In fact, there was a statistically significant association between bTB confirmation and owning sheep as four out of the nine bTB confirmed farms reported sheep in the holding (OR = 4.8, 95% CI = 1.1–25.8,  $P < 0.05$ , Fisher's test). Furthermore, results of the univariable models revealed that, for farms that confirmed bTB through bacteriology and/or detected lesioned animals, the odds of a higher prevalence index was 15 times that of farms that did not confirm the disease (OR = 15.2, 95% CI = 3.5–106, Supplementary Table S1).

A statistically significant association between contact with other farming species in the farm and a higher prevalence index was observed, as for farms that reported contact with other farming species, the odds of scoring a higher prevalence index ( $\geq 3$ , 2, or

**Table 1**  
Prevalence index of the 94 dairy cattle farms included in the study and number of years that resulted in bovine tuberculosis (bTB) positive during 2016–2020.

bTB	Years positive	Prevalence index				Total (%)
		0	1	2	≥3	
Negative	0	28	0	0	0	28 (29.8)
Positive	1	0	21	10	5	36 (38.3)
	≥2	0	0	10	20	30 (31.9)
	Total (%)	28 (29.8)	21 (22.3)	20 (21.3)	25 (26.6)	94 (100.0)

**Table 2**  
Results of the final multivariable ordinal logistic model performed on 94 dairy cattle farms.

Variable	Level	Prevalence index					%	OR	95% Confidence interval		P-value <sup>a</sup>	P-value <sup>b</sup>
		0	1	2	≥3	Total			Multivariable	2.5%		
Leasing pastures for sheep flocks during Winter	No	25	15	16	11	67	71.3	NA	NA	NA	NA	0.05
	Yes	3	6	4	14	27	28.7	3.09	1.02	9.99	0.05	
Use of pastures	No	17	9	7	3	36	38.3	NA	NA	NA	NA	0.34
	Yes	11	12	13	22	58	61.7	1.99	0.48	8.47	0.34	
Frequency of badger sightings	0 (never)	21	10	9	6	46	48.9	NA	NA	NA	NA	0.03
	1 (sporadically)	6	9	8	13	36	38.3	3.03	1.12	8.51	0.03	
	>1 (monthly to daily)	1	2	3	6	12	12.8	4.34	1.13	17.48	0.03	
Solid fencing around farmyard	No	15	15	16	22	68	72.3	NA	NA	NA	NA	0.06
	Yes	13	6	4	3	26	27.7	0.42	0.16	1.04	0.06	
Movement to pastures	On foot	4	3	7	18	32	34.0	NA	NA	NA	NA	0.001
	None	17	9	9	4	39	41.5	0.57	0.12	2.74	0.48	
	Transport	1	2	2	1	6	6.4	0.49	0.08	2.89	0.42	
	Vehicle	6	7	2	2	17	18.1	0.09	0.02	0.31	0.001	
0   1	Intercept						0.43	NA	NA	0.33		
1   2	Intercept						1.56	NA	NA	0.61		
2   ≥3	Intercept						6.09	NA	NA	0.04		

Abbreviations: OR = odds ratio; NA = not applicable.

<sup>a</sup> Wald's test.

<sup>b</sup> Likelihood ratio test.

1 versus prevalence index of 0) was 3.7 times (OR = 3.7, 95% CI = 1.8–8) that of farms that reported no contact with other farming species. However, this effect was not observed when wildlife sightings other than badger (OR<sub>monthly to daily</sub> = 6.8; 95% CI = 2.1–24) and wild boar (OR<sub>presence of wild boar</sub> 2.1; 95% CI = 1.0–4.5) were evaluated (i.e., red deer, roe deer, and red fox, Supplementary Table S1). A statistically significant high-risk cluster of badger detection was observed in farms located in the north of Navarra, which included 32% (n = 30) of the evaluated farms (Fig. S2).

Regarding the risk factors related to farm biosecurity, only the variables solid fencing around farmyard (OR = 0.3; 95% CI = 0.1–0.6), and door closed (OR = 0.3; 95% CI = 0.1–0.7) were found to be related to the outcome variable. Farms with a prevalence index ≥ 3 detected and hunted a statistically significant (P < 0.05, Kruskal-Wallis test with Bonferroni corrections) higher number of wild boars and roe deers than negative (prevalence index = 0) farms. For every one unit increase in wild boar and roe deer sightings and/or hunting, the odds of scoring a higher prevalence index was multiplied 1.01–1.03 times (i.e., increased between 1 and 3%, Supplementary Table S1).

Overall, out of the 73 evaluated covariates, a set of 12 variables were statistically significant in the univariable models and thus selected for stepwise regression procedure in the multivariable model (Supplementary Table S1). The final model included the frequency of badger sightings, the use of pastures by sheep during Winter, the use of pastures by the herd, the type of movement to pastures, and the presence of solid fencing around the farmyard, based on lower Akaike information criterion (AIC). Results of the multivariable model revealed a statistically significant association between the frequency of badger sightings, the lease of pastures

for sheep flocks during Winter and a higher prevalence index, whereas farms that used a vehicle to transport animals from holdings to pastures were less likely to present higher levels of prevalence index (Table 2, Fig. S3 and S4). The distribution of the overall prevalence index and its associations with these covariates are presented in Table 2. Specifically, for farms that leased pastures to sheep during winter and those that detected the presence of badgers at least monthly over the study period, the odds of having a higher prevalence index (i.e., prevalence index ≥ 3, 2, or 1 versus prevalence index of 0) was 3.1 (95% CI = 1.0–9.9) and 4.3 (95% CI = 1.1–17.5) times that of farms that did not lease pastures to sheep during winter and those that did not detect badgers, respectively. The predicted probability of being in the highest category of the prevalence index (≥ 3) was 0.69 (95% CI 0.27–0.93) in farms that detected badgers in a monthly to daily basis, leased pastures for sheep flocks during Winter and moved animals to pastures on foot, with the remaining covariates held constant (i.e., use of pastures and solid fencing around farmyard, Fig. 2). Assumption of no multicollinearity was met as the independent variables were weakly correlated. The assumption of proportional odds was found satisfactory for the 5 risk factors included in the final model, while the overall proportionality assumption was not violated (P = 0.99). The Lipsitz likelihood-ratio test showed no statistically significant lack of fit (P = 0.19).

## Discussion

Multiple studies have provided knowledge for quantifying the relative role of the potential factors on bTB occurrence in Spain

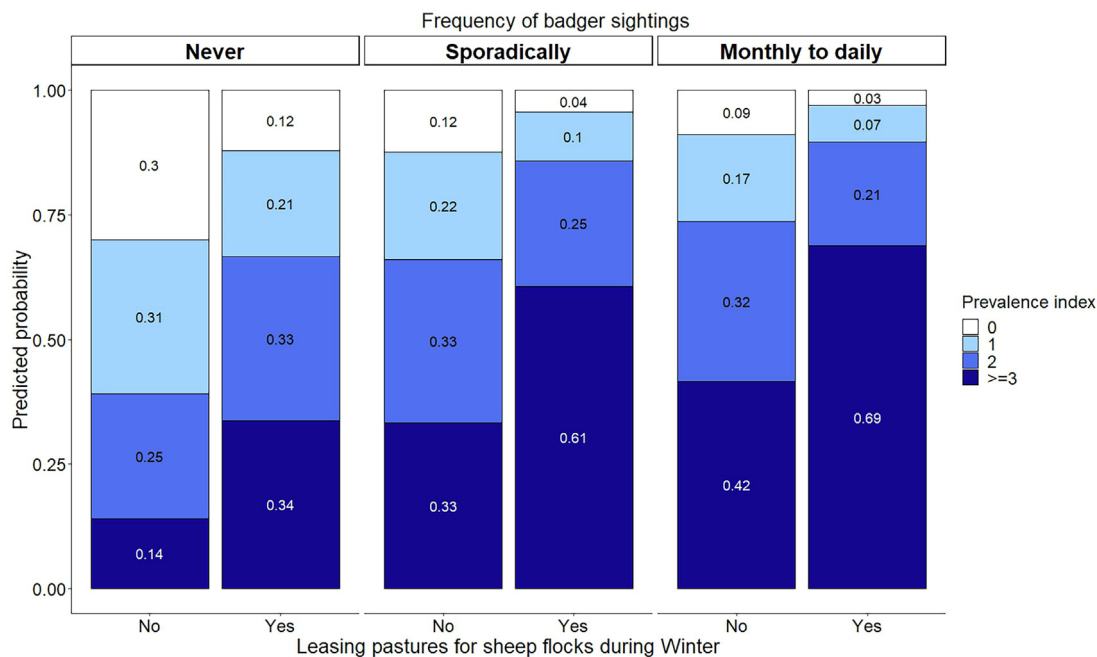


Fig. 2. Predicted probabilities of each category of the prevalence index for the predictors included in the multivariable ordinal logistic regression model.

(Guta et al., 2014; Martinez-Lopez et al., 2014; LaHue et al., 2016), but, to the best of our knowledge, none of them have been specifically assessed in dairy cattle, and none have evaluated the combined role of husbandry practices, biosecurity, wildlife sightings and hunting related factors on the risk of a herd having a more problematic bTB history. Here, we demonstrated that factors related to the ecosystems where the herds were located, the management practices and the direct and/or indirect contact with other domestic and wild reservoirs are responsible for the history of bTB severity in dairy herds in Navarra.

Bovine TB prevalence in Navarra has been historically low (< 1%). However, disease eradication remains elusive. In this context, we classified herds based on their prevalence index, a score that accounts for the number of SIT reactors disclosed per farm per year in order to adjust for the burden, intensity and recurrence of bTB in farms during the study period. Here, 66 (70%) farms were identified as bTB positive (at least one positive animal was disclosed in the SIT test). However, bTB was only confirmed through bacteriological culture in 9 of the 66 (14%) farms. This could be due to the limited period of time elapsed to detect early-stage infections and/or false positive reactions, as the priority in terms of performance of the SIT test in the region was to maximise Se (i.e., the likelihood of detecting bTB infected animals). Interestingly, the non-granulomatous lesions submission rate in Navarra in 2020, which evaluates the performance of slaughterhouse surveillance, was higher than the baseline target (0.46 vs 0.10 × 1 000 animals) established by the Spanish Ministry of Agriculture, Fisheries and Food, which suggest that sensitivity of abattoir surveillance in the region is adequate (Anon, 2020). More than a quarter (27%, n = 25) of the evaluated farms had a prevalence index ≥ 3 (and thus had at least one SIT reactor for one or more years), suggesting in fact that in spite of the overall low percentage of SIT reactors and the low SIT score (Fig. S1), the epidemiology of bTB in these problematic farms was different compared with the rest of the evaluated dairy farms. Despite the definition of the outcome variable may be seen as arbitrary, our aim here was to identify the risk factors that trigger the revision/suspension of the OTF status based on bTB positivity to the SIT test in the frame of the Spanish eradication program. Our results suggest that despite the low rate of bTB con-

firmation in the studied farms, the disease is controlled but its eradication does not occur due to the continuous re-entry of the pathogen as around half of the bTB-positive farms (46%, n = 30/66) tested positive for two or more years.

The presence of solid fencing in the farms was found to be a protective factor associated with the prevalence index in the univariable model. However, when other variables were considered in the multivariable model, the possibility that the observed effect on the prevalence index was due to chance could not be completely ruled out based on a 0.05 threshold for statistical significance (OR = 0.4, 95% CI = 0.2–1.0, P = 0.06). The absence of evidence of a statistically significant effect may be related to the low power due to the small sample size of herds with solid fencing with a prevalence index ≥ 2 (Table 2). Several studies demonstrated that the lack of perimeter fence is considered a risk factor for disease transmission in cattle and other species (Vercauteren et al., 2007; Dion et al., 2011; Mysterud and Rolandsen, 2019). Livestock fences can be effective with regard to controlling livestock movements but do not avoid nose-to-nose contact between cattle and may be permeable to wild ungulates and carnivores (O'Hagan et al., 2016). Thus, the use of solid perimeter fencing does not rule out the risk of direct and/or indirect MTC transmission (Barasona et al., 2013). In fact, out of the 26 herds with solid perimeter fencing around the farmyard, 42% (n = 11) declared the detection of badgers over the study period.

A statistically significant association between badger detection and the bTB prevalence index was observed, as farms that detected badgers at least sporadically were 3 times more likely to be scored with a higher prevalence index as compared with farms that did not report any badger holding all other variables constant (95% CI = 1.1–8.5). In fact, the predicted probabilities of being in the highest category of the prevalence index (≥3) increased by a range of 80–102% as the frequency of badger detection increased (i.e., 0.34 when no badgers were detected vs 0.61 and 0.69 in a sporadic and a monthly to daily detection, respectively, Fig. 2) in farms that leased pastures for sheep during Winter. As a result, farms with a higher frequency of badger sightings were likely to be more vulnerable to bTB. This is not surprising, as badger abundance has increased in Northern Spain in the last decades. A previous study

conducted in Navarra estimated a mean density of 0.9 badgers/km<sup>2</sup> (Martínez-Guijosa et al., 2020), but it can reach values of up to 3.8 adults/km<sup>2</sup> in other areas in the north of Spain where similar molecular profiles were shared between cattle and badger (Balseiro et al., 2013; Acevedo et al., 2014). Recent studies suggested that the bovine-badger infection pathway may be bidirectional (Blanco Vázquez et al., 2021). However, ours is the first study performed in the north of Spain that provides estimates of the contribution of badgers to bTB in dairy herds, as prior research was restricted to evidence of epidemiological links between badgers, wild boar, and cattle. Similar results though were obtained in a study conducted in Northern Ireland, where farms that reported the observation of live badgers had 2.5 (95% CI = 1.1–5.5) times higher risk to develop bTB breakdowns compared with farms without recent breakdown (O'Hagan et al., 2016). With the results obtained, the importance of taking the badger into account in Northern Spain when carrying out biosecurity protocols can be confirmed (Balseiro et al., 2013). The results obtained for wild boar and red deer indicate a lower importance of these species in Navarra compared to other regions such as Southwestern Spain (Naranjo et al., 2008). Red deer are present only in 20% of Navarra, and there are no records of MTC infection in red deer in this region. Wild boars are widespread and abundant, but their MTC infection prevalence is low as compared to Southern Spain, where the prevalence can be as high as 77% (Barroso et al., 2020; Martínez-Guijosa et al., 2020). In a study performed in the Basque Country, the region adjacent to Navarra, 17% of hunted wild boars showed antibodies against MTC (Varela-Castro et al., 2020), while 8.8% of the wild boars hunted during 2015–2016 were confirmed by culture in Navarra (Martínez-Guijosa et al., 2020). Our results agree with those obtained by other authors, who indicated a less relevant epidemiological role of these species in Northern Spain (Gortazar et al., 2017). However, the potential role of wild boar in bTB maintenance in Navarra should not be neglected. In this way, the epidemiology of bTB in the north of the Iberian Peninsula may be more similar to the situation in Great Britain and the Republic of Ireland with the badger as the most important wild species (Vial et al., 2011; Byrne et al., 2015). Additionally, a high-risk cluster of herds that reported badger presence was detected in the north of Navarra (Fig. S2), the area where most of the grazing-based herds were located.

The cattle management system in Navarra is highly influenced by a marked seasonal variation in climate conditions, temperatures, and rainfall. Grazing-based farming systems are common, with herds being more likely to be in contact with other cattle, sheep and/or wildlife offering potential chances of disease transmission (Campbell et al., 2020). Here, out of the 58 grazing-based herds, 81% (n = 47) were bTB positive at least once during the study period, and around half of them (47%, n = 22) had a prevalence index  $\geq 3$ , suggesting that grazing may be linked to bTB maintenance (Table 2). Additionally, the nature of transport from farms to pasture areas was associated with a higher prevalence index. Herds which used owned vehicles to transport animals to pastures had lower odds of an increased bTB severity compared to those that used none (on foot). The movement of cattle on foot is linked to a higher possibility of contact with cattle from other herds and other animal species (direct contacts) or access to shared water points (indirect contacts) during the journey. In fact, out of the farms that move cattle on foot, 94% reported contact with other cattle (n = 30/32), 72% detected badgers in the surroundings (n = 23/32), and 69% reported wild boar sightings (n = 22/32). These results suggest that movement to pastures on foot may be a proxy for bTB transmission between domestic and/or wildlife reservoir, which concurs with other studies conducted in Northern (Martínez-Guijosa et al., 2020) and Northwestern (Gortazar et al., 2017) Spain.

In this line, our study also suggested that herds with characteristics related to the presence of, or potential (in)direct contact with, other farming species in general, and with sheep in particular, presented a higher risk of increased prevalence index. Direct (own sheep) or indirect (sheep grazing during Winter) contact with sheep was associated with the outcome in the univariable analyses, but the final model suggested a stronger effect of the latter. In fact, around half of the farms that confirmed bTB owned sheep and reported leasing pastures for sheep flocks. Sheep were previously identified as a bTB risk for beef and dairy cattle in Northwestern Spain (Muñoz-Mendoza et al., 2016) and for beef cattle in Navarra (Martínez-Guijosa et al., 2020). In this study, we further confirmed the importance of sheep in dairy herds as well.

Statistically significant association between variables related to the purchase of animals (i.e., in-degree, the % of external replacement, the number of animals purchased from France) and the prevalence index became inconclusive in the univariable analyses. Although animal purchase has been recurrently identified as a relevant factor in the introduction of infectious diseases, and specifically of bTB (Milne et al., 2019; Pozo et al., 2019; Ciaravino et al., 2021), its relationship with the prevalence index could not be evaluated (lack of convergence of the in-degree model) or could not be demonstrated (OR<sub>% of external replacement</sub> = 1.1, 95% CI = 0.9–1.1, P = 0.12; OR<sub>number of animals purchased from France</sub> = 1.0, 95% CI = 0.9–1.0, P = 0.11, Supplementary Table S1). This may be due to differences in the study population and/or the origin of the purchased animals.

Results suggested that the combined effect of farm- and environmental-level risk factors identified here may be hampering disease eradication in the region, which mainly hinted re-infection from sources outside the farm (wildlife, sheep, and other cattle herds). However, within-herd bTB transmission cannot be ruled out. The use of whole genome sequencing of strains circulating in the region (including those recovered from the bTB-confirmed farms) may be highly informative to evaluate potential epidemiological links between hosts and decipher the sources of (re-) infection, but it was not performed here as it was beyond the scope of this study.

Findings obtained here should be interpreted with caution, as associations shown here may not reflect causation (Bello and Renter, 2018). Conversely, statistically non-significant results do not imply a lack of association with the prevalence index, but rather these results may be compatible with alternative hypotheses (Greenland et al., 2016). One of the weaknesses of our research was the selection of herds: a non-probabilistic convenience sampling strategy was applied so that only dairy farms with the highest cattle census in Navarra, which were considered more prone to remain active for longer periods of time than smaller farms thus allowing the application of measures, were included. These farms were part of a biosecurity research project aimed at evaluating risks for livestock-wildlife interaction and disease transmission to subsequently develop On-farm Risk Mitigation Protocols. Although our selection could lead to some selection bias, potentially leading to inflated overall effect sizes and/or inaccurate estimates, we believe the subset of evaluated farms acted as a proxy of the dairy production system in Navarra: all large farms accepted to take part in the study, which represented ~73% of the total number of dairy farms in Navarra. The validity of the questionnaire provided to the farmer was not evaluated, and thus, potential error variance and noise in data may have been introduced into the analyses. Nevertheless, the information evaluated here included data gathered by authors through on-farm evaluations and field reconnaissance to corroborate and verify farmer perceptions (Elangovan and Sundaravel, 2021). Moreover, the wide 95% confidence intervals obtained in the final model (i.e., OR<sub>Frequency of badger sightings 1 (sporadically)</sub> = 3, 95% CI = 1.1–8.5 and OR<sub>Leasing pastures for sheep flocks</sub> = 3.1,

95% CI = 1–9.9) suggested an inherent uncertainty in our estimates, and thus further studies with an increased sample size are needed to support our inferences. Although significance tests and 95% confidence intervals do not by themselves provide evidences about the presence or absence of an effect, epidemiological features (and the direction of the effects) at the wildlife-livestock interface identified here agree with those obtained in other studies performed in the north of Spain in general (Muñoz-Mendoza et al., 2016), and in Navarra in particular (Martínez-Guijosa et al., 2020).

Disease control and eradication often face challenges related to the farmers' reticent attitudes towards the success of the surveillance program, especially in historically low prevalence regions as Navarra. In this context, the use of questionnaire surveys in data collection provides an integrative disease management at the hunting-extensive livestock-wildlife interface in which the contribution of the farmer's perspective is integrated. Our findings highlighted the need to implement and improve targeted biosecurity measures and protocols on farm and grazing plots [i.e., shorter testing intervals in farms with high prevalence index, wildlife population control actions such as active removal, impermeable/selective physical barriers (Boadella et al., 2012; Barasona et al., 2013)], together with an increased awareness of monitoring sheep in direct or indirect contact with cattle herds. Control efforts should also be focused on a better understanding of farmers' motivations through social science research and biosafety communication (Martínez-Guijosa et al., 2021; Preite et al., 2023). In this sense, the characterisation of farms based on the burden of reactors along a 5 year-span, even at very low prevalences, provides valuable information to develop On-farm Risk Mitigation Protocols to implement targeted measures in dairy herds located in Navarra. Additionally, farm characterisation using the risk scoring system developed here may be adaptable to dairy herds located in similar epidemiological scenarios where bTB remains historically at low prevalence, which is highly demanded in order to reduce the costs associated to bTB-related problems.

### Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2024.101105>.

### Ethics approval

Ethical review and approval were not required for the animal study because all data on bovine tuberculosis were collected as part of authorised regulatory surveillance in the framework of the Spanish National Eradication Program for Bovine Tuberculosis. Written informed consent for participation was obtained from the premises owners for this study.

### Data and model availability statement

None of the data were deposited in an official repository. The models that support the study findings are available from the authors upon request, whereas the data are confidential in the framework of the Spanish National Eradication Program for Bovine Tuberculosis.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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### CRediT authorship contribution statement

**P. Pozo:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Resources, Validation. **J. Isla:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Visualization. **A. Asiain:** Writing – review & editing, Validation. **D. Navarro:** Writing – review & editing, Validation. **C. Gortázar:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

### Declaration of interest

None.

### Acknowledgements

We thank the farmers and veterinary officers that agreed to participate in the study.

### Financial support statement

This study is a contribution to the Ministerio de Economía y Competitividad (MINECO) Plan Nacional (PID2022-141906GB-C21) and the project Ganadería con una sola salud: monitoreo ambiental y mitigación de riesgos para la producción ganadera segura y sostenible y la conservación de la biodiversidad (PLEC2021-008113) funded by MCIN/AEI /10.13039/501100011033 and by European Union NextGeneration EU/ PRTR. P. Pozo is a recipient of a Juan de la Cierva Formación grant (FJC2021-046805-I) funded by the Ministerio de Ciencia e Innovación and Agencia Estatal de Investigación (MCIN/AEI/10.13039/501100011033) and by European Union NextGenerationEU/PRTR.

### References

- Acevedo, P., González-Quirós, P., Prieto, J.M., Etherington, T.R., Gortázar, C., Balseiro, A., 2014. Generalizing and transferring spatial models: a case study to predict Eurasian badger abundance in Atlantic Spain. *Ecological Modelling* 275, 1–8. <https://doi.org/10.1016/j.ecolmodel.2013.12.011>.
- Acevedo, P., Prieto, M., Quirós, P., Merediz, I., de Juan, L., Infantes-Lorenzo, J.A., Triguero-Ocaña, R., Balseiro, A., 2019. Tuberculosis epidemiology and badger (*Meles meles*) spatial ecology in a hot-spot area in atlantic spain. *Pathogens* 8, 292. <https://doi.org/10.3390/pathogens8040292>.
- Allepez, A., Casal, J., Napp, S., Saez, M., Alba, A., Vilar, M., Domingo, M., Gonzalez, M. A., Duran-Ferrer, M., Vicente, J., Alvarez, J., Munoz, M., Saez, J.L., 2011. Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006–2009). *Preventive Veterinary Medicine* 100, 44–52. <https://doi.org/10.1016/j.prevetmed.2011.02.012>.
- Alvarez, J., de Juan, L., Bezos, J., Romero, B., Saez, J.L., Marques, S., Dominguez, C., Minguez, O., Fernandez-Mardomingo, B., Mateos, A., Dominguez, L., Aranaz, A., 2009. Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd with a mixed infection using interferon-gamma detection assay. *Veterinary Microbiology* 135, 389–393. <https://doi.org/10.1016/j.vetmic.2008.09.060>.
- Alvarez, J., Perez, A.M., Bezos, J., Casal, C., Romero, B., Rodriguez-Campos, S., Saez-Llorente, J.L., Diaz, R., Carpintero, J., de Juan, L., Dominguez, L., 2012. Eradication of bovine tuberculosis at a herd-level in Madrid, Spain: study of within-herd transmission dynamics over a 12 year period. *BMC Veterinary Research* 8, 100. <https://doi.org/10.1186/1746-6148-8-100>.

- Anon. 2020. Informe sobre las tasas de remisión de granulomas en animales sacrificados de rutina en matadero (Programa de vigilancia de la tuberculosis bovina en matadero) 2020. Retrieved on 02 March 2023 from [https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/tasaderemisiondegranulomas2020\\_tcm30-579968.pdf](https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/tasaderemisiondegranulomas2020_tcm30-579968.pdf).
- Anon. 2022. El sector del vacuno de leche en cifras. Principales indicadores económicos. Retrieved on 27 May 2023 from: [https://www.mapa.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/indicadores2022\\_tcm30-655886.pdf](https://www.mapa.gob.es/es/ganaderia/temas/produccion-y-mercados-ganaderos/indicadores2022_tcm30-655886.pdf).
- Anon. 2023a. Informe Sistema Integral de Trazabilidad Animal (SITRAN). Retrieved on 01 May 2023 from: <https://www.mapa.gob.es/es/ganaderia/temas/trazabilidad-animal/registro/default.aspx>.
- Anon. 2023b. Programa Nacional de Erradicación de Tuberculosis Bovina 2023 (Infección por el complejo *Mycobacterium tuberculosis*). Retrieved on 10 September 2023 from: [https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/programatb2023\\_tcm30-640045.pdf](https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/programatb2023_tcm30-640045.pdf).
- Balseiro, A., Gonzalez-Quiros, P., Rodriguez, O., Francisca Copano, M., Merediz, I., de Juan, L., Chambers, M.A., Delahay, R.J., Marreros, N., Royo, L.J., Bezos, J., Prieto, J. M., Gortazar, C., 2013. Spatial relationships between Eurasian badgers (*Meles meles*) and cattle infected with *Mycobacterium bovis* in Northern Spain. *Veterinary Journal* (London, England : 1997) 197, pp. 739–745. <https://doi.org/10.1016/j.tvjl.2013.03.017>.
- Barasona, J.A., VerCauteren, K.C., Saklou, N., Gortazar, C., Vicente, J., 2013. Effectiveness of cattle operated bump gates and exclusion fences in preventing ungulate multi-host sanitary interaction. *Preventive Veterinary Medicine* 111, 42–50. <https://doi.org/10.1016/j.prevetmed.2013.03.009>.
- Barasona, J.A., Vicente, J., Díez-Delgado, I., Aznar, J., Gortazar, C., Torres, M.J., 2017. Environmental presence of *Mycobacterium tuberculosis* complex in aggregation points at the wildlife/livestock interface. *Transboundary and Emerging Diseases* 64, 1148–1158. <https://doi.org/10.1111/tbed.12480>.
- Barroso, P., Barasona, J.A., Acevedo, P., Palencia, P., Carro, F., Negro, J.J., Torres, M.J., Gortazar, C., Soriguer, R.C., Vicente, J., 2020. Long-term determinants of tuberculosis in the ungulate host community of Doñana national park. *Pathogens* 9, 445. <https://doi.org/10.3390/pathogens9060445>.
- Bello, N.M., Renter, D.G., 2018. Invited review: reproducible research from noisy data: revisiting key statistical principles for the animal sciences. *Journal of Dairy Science* 101, 5679–5701. <https://doi.org/10.3168/jds.2017-13978>.
- Bezós, J., Sáez-Llorente, J.L., Álvarez, J., Romero, B., Díez-Guerrier, A., Domínguez, L., de Juan, L., 2023. Bovine tuberculosis in Spain, is it really the final countdown? *Irish Veterinary Journal* 76, 13. <https://doi.org/10.1186/s13620-023-00241-0>.
- Blanco Vázquez, C., Barral, T.D., Romero, B., Queipo, M., Merediz, I., Quirós, P., Armenteros, J., Juste, R., Domínguez, L., Domínguez, M., Casais, R., Balseiro, A., 2021. Spatial and temporal distribution of *Mycobacterium tuberculosis* complex infection in Eurasian badger (*Meles meles*) and cattle in Asturias, Spain. *Animals* (Basel) 11, 1294. <https://doi.org/10.3390/ani11051294>.
- Boadella, M., Vicente, J., Ruiz-Fons, F., de la Fuente, J., Gortazar, C., 2012. Effects of culling Eurasian wild boar on the prevalence of *Mycobacterium bovis* and *Aujeszky's disease virus*. *Preventive Veterinary Medicine* 107, 214–221. <https://doi.org/10.1016/j.prevetmed.2012.06.001>.
- Brant, R., 1990. Assessing proportionality in the proportional odds model for ordinal logistic regression. *Biometrics* 46, 1171–1178. <https://doi.org/10.2307/2532457>.
- Broughan, J.M., Judge, J., Ely, E., Delahay, R.J., Wilson, G., Clifton-Hadley, R.S., Goodchild, A.V., Bishop, H., Parry, J.E., Downs, S.H., 2016. A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland. *Epidemiology and Infection* 144, 2899–2926. <https://doi.org/10.1017/S095026881600131x>.
- Byrne, A.W., Kenny, K., Fogarty, U., O'Keefe, J.J., More, S.J., McGrath, G., Teeling, M., Martin, S.W., Dohoo, I.R., 2015. Spatial and temporal analyses of metrics of tuberculosis infection in badgers (*Meles meles*) from the Republic of Ireland: trends in apparent prevalence. *Preventive Veterinary Medicine* 122, 345–354. <https://doi.org/10.1016/j.prevetmed.2015.10.013>.
- Byrne, A.W., Graham, J., Milne, G., Guelbenzu-Gonzalo, M., Strain, S., 2019. Is There a relationship between bovine tuberculosis (bTB) herd breakdown risk and *Mycobacterium avium* subsp. *paratuberculosis* Status? an Investigation in bTB Chronically and Non-chronically Infected Herds. *Frontiers in Veterinary Science* 6, 30. <https://doi.org/10.3389/fvets.2019.00030>.
- Campbell, E.L., Menzies, F.D., Byrne, A.W., Porter, S., McCormick, C.M., McBride, K.R., Scantlebury, D.M., Reid, N., 2020. Grazing cattle exposure to neighbouring herds and badgers in relation to bovine tuberculosis risk. *Research in Veterinary Science* 133, 297–303. <https://doi.org/10.1016/j.rvsc.2020.09.032>.
- Ciaravino, G., Laranjo-González, M., Casal, J., Sáez-Llorente, J.L., Allepuz, A., 2021. Most likely causes of infection and risk factors for tuberculosis in Spanish cattle herds. *The Veterinary Record* 189, e140.
- Dion, E., VanSchalkwyk, L., Lambin, E.F., 2011. The landscape epidemiology of foot-and-mouth disease in South Africa: a spatially explicit multi-agent simulation. *Ecological Modelling* 222, 2059–2072. <https://doi.org/10.1016/j.ecolmodel.2011.03.026>.
- Dohoo, I., Martin, W., Stryhn, H., 2003. *Veterinary epidemiologic research*. AVC Inc., Charlottetown, Prince Edward Island, Canada.
- Doyle, L.P., Gordon, A.W., Abernethy, D.A., Stevens, K., 2014. Bovine tuberculosis in Northern Ireland: risk factors associated with time from post-outbreak test to subsequent herd breakdown. *Preventive Veterinary Medicine* 116, 47–55. <https://doi.org/10.1016/j.prevetmed.2014.06.010>.
- Elangovan, N., Sundaravel, E., 2021. Method of preparing a document for survey instrument validation by experts. *MethodsX* 8, <https://doi.org/10.1016/j.mex.2021.101326>.
- Godfray, H.C.J., Donnelly, C.A., Kao, R.R., Macdonald, D.W., McDonald, R.A., Petrokofsky, G., Wood, J.L.N., Woodroffe, R., Young, D.B., McLean, A.R., 2013. A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain<sup>1</sup>. *Proceedings of the Royal Society B: Biological Sciences* 280, 20131634. <https://doi.org/10.1098/rspb.2013.1634>.
- Gortazar, C., Fernandez-Calle, L.M., Collazos-Martinez, J.A., Minguez-Gonzalez, O., Acevedo, P., 2017. Animal tuberculosis maintenance at low abundance of suitable wildlife reservoir hosts: a case study in northern Spain. *Preventive Veterinary Medicine* 146, 150–157.
- Greenland, S., Senn, S.J., Rothman, K.J., Carlin, J.B., Poole, C., Goodman, S.N., Altman, D.G., 2016. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. *European Journal of Epidemiology* 31, 337–350. <https://doi.org/10.1007/s10654-016-0149-3>.
- Griffin, J.M., Haehes, T., Lynch, K., Salman, M.D., McCarthy, J., Hurley, T., 1993. The association of cattle husbandry characteristics, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. *Preventive Veterinary Medicine* 17, 145–160. [https://doi.org/10.1016/0167-5877\(93\)90025-0](https://doi.org/10.1016/0167-5877(93)90025-0).
- Guta, S., Casal, J., Napp, S., Saez, J.L., Garcia-Saenz, A., Perez de Val, B., Romero, B., Alvarez, J., Allepuz, A., 2014. Epidemiological investigation of bovine tuberculosis herd breakdowns in Spain 2009/2011. *PLoS One* 9, e104383.
- Johnston, W.T., Vial, F., Gettinby, G., Bourne, F.J., Clifton-Hadley, R.S., Cox, D.R., Crea, P., Donnelly, C.A., McInerney, J.P., Mitchell, A.P., Morrison, W.I., Woodroffe, R., 2011. Herd-level risk factors of bovine tuberculosis in England and Wales after the 2001 foot-and-mouth disease epidemic. *International Journal of Infectious Diseases* 15, e833–e840. <https://doi.org/10.1016/j.ijid.2011.08.004>.
- Kulldorff, M., 1997. A spatial scan statistic. *Communications in Statistics - Theory and Methods* 26, 1481–1496. <https://doi.org/10.1080/03610929708831995>.
- LaHue, N.P., Banos, J.V., Acevedo, P., Gortazar, C., Martinez-Lopez, B., 2016. Spatially explicit modeling of animal tuberculosis at the wildlife-livestock interface in Ciudad Real province, Spain. *Preventive Veterinary Medicine* 128, 101–111. <https://doi.org/10.1016/j.prevetmed.2016.04.011>.
- Lipsitz, S.R., Fitzmaurice, G.M., Molenberghs, G., 1996. Goodness-of-fit tests for ordinal response regression models. *Journal of the Royal Statistical Society Series C (Applied Statistics)* 45, 175–190. <https://doi.org/10.2307/2986153>.
- Martínez-Guijosa, J., Lima-Barbero, J.F., Acevedo, P., Cano-Terriza, D., Jiménez-Ruiz, S., Barasona, J.A., Boadella, M., García-Bocanegra, I., Gortazar, C., Vicente, J., 2021. Description and implementation of an on-farm wildlife risk mitigation protocol at the wildlife-livestock interface: tuberculosis in mediterranean environments. *Preventive Veterinary Medicine* 191, <https://doi.org/10.1016/j.prevetmed.2021.105346>.
- Martínez-Guijosa, J., Romero, B., Infantes-Lorenzo, J.A., Díez, E., Boadella, M., Balseiro, A., Veiga, M., Navarro, D., Moreno, I., Ferreres, J., Domínguez, M., Fernández, C., Domínguez, L., Gortazar, C., 2020. Environmental DNA: a promising factor for tuberculosis risk assessment in multi-host settings. *PLoS One* 15, e0233837.
- Martínez-Lopez, B., Barasona, J.A., Gortazar, C., Rodríguez-Prieto, V., Sánchez-Vizcaino, 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. *Preventive Veterinary Medicine* 116, 268–278. <https://doi.org/10.1016/j.prevetmed.2013.11.002>.
- Milne, G., Graham, J., Allen, A.R., Lahuerta-Marin, A., McCormick, C.M., Presho, E., Skuce, R.A., Byrne, A., 2019. Characteristics of Northern Irish cattle herds without bovine tuberculosis infection. *The Veterinary Record* 184, 772. <https://doi.org/10.1136/vr.105225>.
- Munoz-Mendoza, M., Marreros, N., Boadella, M., Gortazar, C., Menendez, S., de Juan, L., Bezos, J., Romero, B., Copano, M.F., Amado, J., Saez, J.L., Mourelo, J., Balseiro, A., 2013. Wild boar tuberculosis in Iberian Atlantic Spain: a different picture from Mediterranean habitats. *BMC Veterinary Research* 9, 176. <https://doi.org/10.1186/1746-6148-9-176>.
- Muñoz-Mendoza, M., Romero, B., Del Cerro, A., Gortazar, C., García-Marín, J.F., Menéndez, S., Mourelo, J., de Juan, L., Sáez, J.L., Delahay, R.J., Balseiro, A., 2016. Sheep as a potential source of bovine TB: epidemiology, pathology and evaluation of diagnostic techniques. *Transboundary and Emerging Diseases* 63, 635–646. <https://doi.org/10.1111/tbed.12325>.
- Mysterud, A., Rolandsen, C.M., 2019. Fencing for wildlife disease control. *Journal of Applied Ecology* 56, 519–525. <https://doi.org/10.1111/1365-2664.13301>.
- 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. *Veterinary Microbiology* 127, 1–9. <https://doi.org/10.1016/j.vetmic.2007.10.002>.
- O'Hagan, M.J., Matthews, D.I., Laird, C., McDowell, S.W., 2016. Herd-level risk factors for bovine tuberculosis and adoption of related biosecurity measures in Northern Ireland: A case-control study. *Veterinary Journal* (London, England : 1997) 213, pp. 26–32. <https://doi.org/10.1016/j.tvjl.2016.03.021>.
- Pérez de Val, B., Napp, S., Velarde, R., Lavín, S., Cervera, Z., Singh, M., Allepuz, A., Mentaberre, G., 2017. Serological follow-up of tuberculosis in a wild boar population in contact with infected cattle. *Transboundary and Emerging Diseases* 64, 275–283. <https://doi.org/10.1111/tbed.12368>.
- Pozo, P., VanderWaal, K., Grau, A., de la Cruz, M.L., Nacar, J., Bezos, J., Perez, A., Minguez, O., Alvarez, J., 2019. Analysis of the cattle movement network and its association with the risk of bovine tuberculosis at the farm level in Castilla y Leon, Spain. *Transboundary and Emerging Diseases* 66, 327–340. <https://doi.org/10.1111/tbed.13025>.

- Preite, L., Barroso, P., Romero, B., Balseiro, A., Gortázar, C., 2023. Struggling to improve farm biosecurity: do free advice and subsidies hit the target? *Preventive Veterinary Medicine* 212. <https://doi.org/10.1016/j.prevetmed.2023.105839> 105839.
- Queiros, J., Alvarez, J., Carta, T., Mateos, A., Ortiz, J.A., Fernández-de-Mera, I.G., Martín-Hernando, M.P., Gortázar, C., 2012. Unexpected high responses to tuberculin skin-test in farmed red deer: implications for tuberculosis control. *Preventive Veterinary Medicine* 104, 327–334. <https://doi.org/10.1016/j.prevetmed.2011.11.009>.
- R Core Team, 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Robertson, A., Judge, J., Wilson, G.J., Vernon, I.J., Delahay, R.J., McDonald, R.A., 2019. Predicting badger visits to farm yards and making predictions available to farmers. *PLoS One* 14, e0216953.
- Santos, N., Santos, C., Valente, T., Gortázar, C., Almeida, V., Correia-Neves, M., 2015. Widespread environmental contamination with *Mycobacterium tuberculosis* complex revealed by a molecular detection protocol. *PLoS One* 10, e0142079.
- Santos, N., Colino, E.F., Arnal, M.C., de Luco, D.F., Sevilla, I., Garrido, J.M., Fonseca, E., Valente, A.M., Balseiro, A., Queiros, J., Almeida, V., Vicente, J., Gortazar, C., Alves, P.C., 2022. Complementary roles of wild boar and red deer to animal tuberculosis maintenance in multi-host communities. *Epidemics* 41. <https://doi.org/10.1016/j.epidem.2022.100633> 100633.
- Skuce, R.A., Allen, A.R., McDowell, S.W., 2012. Herd-level risk factors for bovine tuberculosis: a literature review. *Veterinary Medicine International* 2012. <https://doi.org/10.1155/2012/621210> 621210.
- Triguero-Ocana, R., Barasona, J.A., Carro, F., Soriguer, R.C., Vicente, J., Acevedo, P., 2019. Spatio-temporal trends in the frequency of interspecific interactions between domestic and wild ungulates from Mediterranean Spain. *PLoS One* 14, e0211216.
- Varela-Castro, L., Alvarez, V., Sevilla, I.A., Barral, M., 2020. Risk factors associated to a high *Mycobacterium tuberculosis* complex seroprevalence in wild boar (*Sus scrofa*) from a low bovine tuberculosis prevalence area. *PLoS One* 15, e0231559.
- Vercauteren, K., Lavelle, M., Seward, N., Fischer, J., Phillips, G., 2007. Fence-line contact between wild and farmed white-tailed deer in michigan: potential for disease transmission. *The Journal of Wildlife Management* 71, 1603–1606. <https://doi.org/10.2193/2006-179>.
- Vial, F., Johnston, W.T., Donnelly, C.A., 2011. Local cattle and badger populations affect the risk of confirmed tuberculosis in British cattle herds. *PLoS One* 6, e18058.