Preventive Veterinary Medicine 246 (2026) 106710

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Abattoir surveillance: Identifying risk factors associated with bovine tuberculosis lesion detection in a low prevalence region

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ARTICLE INFO

Keywords:
Bovine tuberculosis
Cattle
Mycobacterium bovis
Postmortem
Slaughterhouse
Surveillance

ABSTRACT

Abattoir surveillance is a key component in control and eradication programs against bovine tuberculosis (bTB). In low-prevalence or officially tuberculosis-free (OTF) regions in which active surveillance is typically limited or non-existent, postmortem inspection at the abattoir constitutes one of the main diagnostic tool to guarantee the absence of disease transmission. Here, we evaluated the performance of abattoir postmortem inspection in Galicia, a low-prevalence region in Spain (now OTF). Between 2014 and 2019, 1,784,261 animals were culled in 41 abattoirs, of which a small proportion (0.74 %, n = 13,200) were reactors in bTB antemortem tests. Two mixed-effects logistic regression models assessed the risk of detecting bTB-compatible lesions adjusting for potential confounding factors (age, sex, breed, production type, herd size, location, year and season of slaughter, and antemortem bTB test results) while accounting for the lack of independence between animals from the same herd/slaughtered in the same abattoir. Lesions were detected in 0.013 % (n = 223 animals) and 2.3 % (n = 301 animals) of all the non-reactor and reactor slaughtered animals, respectively, and were confirmed through culture in 9.0 % and 29.9 % of the bTB-lesioned non-reactor and reactor animals. Probability of bTB-like lesion detection varied considerably between abattoirs and was influenced by several animal and farm-level factors: in non-reactors older beef cattle slaughtered in certain years were at higher risk, while in reactors beef cattle from certain provinces and years with a high skin fold thickness increase had a higher probability. These results provide important baseline data on the performance of passive surveillance in low-prevalence settings and offer valuable insights for enhancing bTB surveillance and control strategies.

1. Introduction

Even though the eradication of bovine tuberculosis (bTB) remains elusive in several countries worldwide, great efforts are made every year to conquer such achievement (Bezos et al., 2023; European Food Safety Authority (EFSA), 2024). In the case of Spain, the implementation of the bTB eradication program has led to 19 out of 50 provinces included in it, representing about 40 % of the cattle population of the country, reaching the officially bTB-free (OTF) status in recent years (MAPA, 2025).

As eradication is achieved, programs have to adapt the policies to a new situation with a lower risk of infection in which maintaining a very high specificity with an adequate sensitivity is key (e.g., increased time between testing, use of more specific but less sensitive techniques or cut-offs, increased importance of passive surveillance) (Boschiroli, 2023). In this context, abattoir surveillance, which consists of the postmortem inspection by qualified veterinarians for detection of lesions compatible with bTB followed by the laboratory analysis using culture, direct PCR or histopathology for a differential diagnosis of tissues collected at the slaughterhouse, is important and may play a crucial role in the maintenance of the OTF status in the cattle population (Corner et al., 1990; Foddai et al., 2015; Welby et al., 2012).

Nonetheless, postmortem inspection is not perfect, and several countries have recently evaluated the performance of their passive bTB surveillance programs to determine which factors impair their performance (Byrne et al., 2017; Frankena et al., 2007; Male Here et al., 2022; McKinley et al., 2018; O'Hagan et al., 2015; Pascual-Linaza et al., 2017). Among the results, certain similarities can be observed, such as the effect of age, breed, the results of previous antemortem bTB tests, the interval

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https://doi.org/10.1016/j.prevetmed.2025.106710
Received 16 June 2025; Received in revised form 29 September 2025; Accepted 5 October 2025

between the slaughter and the last bTB outbreak in the herd, and the herd status (i.e., OTF or bTB-infected) on the probability of detecting a lesion. Therefore, understanding which factors most influence both the detection and subsequent laboratory confirmation of a bTB-lesion is necessary to establish the reliability of this key tool in bTB eradication programs.

In Spain, two studies have evaluated the performance of abattoir surveillance. The first study, conducted in a high bTB prevalence region, identified risk factors linked with an increased probability of detection of bTB-compatible lesions at the animal, herd and abattoir levels (Pozo et al., 2021). The second study, which was performed in a low prevalence setting, found that the abattoir had a strong influence on the performance of postmortem surveillance. However, this second study did not evaluate risk factors and only considered data from skin test-positive herds (Garcia-Saenz et al., 2015). This aspect must be considered as it is known that training, experience, motivation, the number of technicians or the type of inspection (visual only or with palpation/incision) can modify the sensitivity of the postmortem inspection (Foddai et al., 2015; Garcia-Saenz et al., 2015; Gonçalves et al., 2022; Teklu et al., 2004).

Here, in a recently declared OTF region in Spain, Galicia (2021), we conducted an evaluation during the period leading up to OTF status, including data from all animals slaughtered over a six-year period (2014–2019). Animals were classified as reactors (those testing positive in the antemortem tests) or non-reactors (those testing negative) to assess whether abattoir surveillance performance was influenced by factors similar to as those identified in a high-risk region, and to determine its reliability and the between-abattoir variability in a low-prevalence setting.

2. Material and methods

2.1. Population of study

The study was conducted in Galicia, region in the Northeast of Spain (42°45’ N; 7°51’ W). The region is divided into four provinces (A Coruña, Lugo, Ourense and Pontevedra), which are further subdivided into 313 municipalities (Fig. 1). In January 2025, the cattle population in Galicia was 911,597 animals in 24,431 herds, representing 14.8 % of the total population of Spain (n = 6,159,439) and making it the region with the second largest cattle population in the country. Since 2014, the bTB prevalence has been consistently below 0.1 % (less than 20 herds per year), and in 2021 Galicia was declared OTF (MAPA, 2025).

The first dataset encompassed information on all cattle slaughtered in Galicia between 2014 and 2019, including the herd of origin, the sex and breed of the animals, the dates of birth and slaughter, the abattoir where each animal was culled and the presence or absence of macroscopical bTB-like lesions at postmortem inspection. The second contained herd-level information of all herds in Galicia over the same period, including the number of animals per herd, their production type and their location (municipality). The third database included detailed information on all animals identified as reactors to antemortem bTB tests performed in Galicia [single or comparative intradermal tests (SIT/CIT) and interferon-gamma (IFN-γ) assay], with macroscopical lesions at the abattoir, or considered as high-risk cattle due to epidemiological reasons (because they were part of a herd subjected to depopulation due to bTB or came from a likely highly infected herd) by the official veterinary services; animals in this database were sampled in the abattoir and subjected to bacteriological culture regardless of the presence of lesions in the postmortem analysis. Thus, the database included information on the results of all antemortem diagnostic tests performed throughout the life of the animal as well as on the postmortem inspection and laboratory tests. Because complete information was only available for cattle located in farms in Galicia, the analysis was restricted to slaughtered animals which were sent to the abattoir in Galicia from a farm located in this Autonomous Region.

2.2. Statistical analysis

Variables available for the analysis at the animal level included sex (male or female), age (categorised as < 1, 1–2 and > 2 years old), breed [crossbreed, Holstein-Friesian, Rubia-Gallega (a native beef breed), and others], year (2014–2019) and season of slaughter. In addition, for the reactor cattle the diagnostic test on which the animal tested positive (SIT, CIT, IFN-γ, or both a skin test and IFN-γ) and the skin fold increase (in mm) at the bovine PPD inoculation site during the last skin test prior to culling were recorded. At the herd level, variables available were the province of the last herd in which the animal was located as well as the

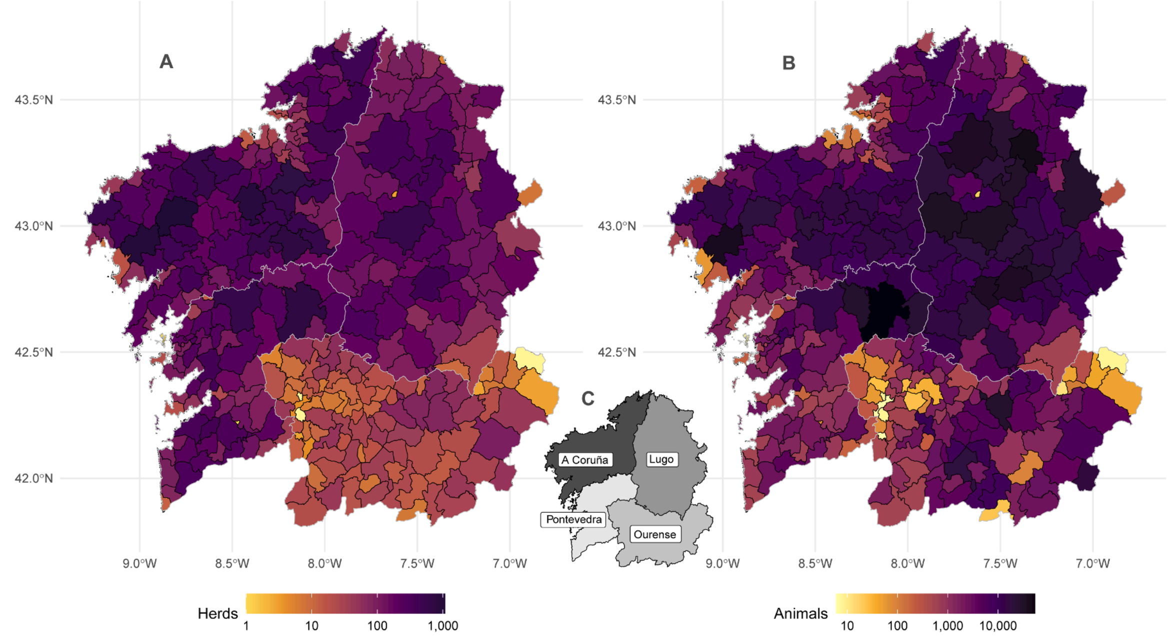

Fig. 1. Number of herds that submitted animals to the abattoir (A) and of animals 82 culled (B) per municipality in Galicia over the study period (2014-2019) and location of 83 the four provinces in the region (C).

production type (beef, dairy, fattening or mixed) and herd size (categorised in terciles as < 30, 30–100 and > 100 animals).

The crude probability of detection of lesions compatible with bTB (outcome variable) was first estimated for each abattoir. Then, logistic mixed-effects models were used to estimate the risk of detecting bTB-like lesions at postmortem inspection depending on animal and herd-level variables and accounting for the lack of independence between animals slaughtered in the same abattoir and/or coming from the same herd by including these variables as random effects. For this purpose, two models were built, one for animals that were reactors in their previous antemortem test (and were therefore subjected to a more thorough carcass examination due to the higher risk of infection in accordance with applicable regulations) and one for non-reactor animals.

The probability of detecting bTB-like lesions was modeled as:

$$logit\left(p_{ijk}\right) = \beta_0 + \sum \beta_1 X_{i1} + \ldots + \beta_n X_{in} + \alpha_j + \delta_k$$

where p_{ijk} is the probability of detecting TB-like lesions during postmortem inspection of animal i from herd j culled in abattoir k. $X_i \ldots X_n$ are the n fixed-effect available covariates (e.g., age, sex, production type, herd size, province, test, skinfold response), $\beta_i \ldots \beta_n$ the coefficients associated with those covariates, and α_j and δ_k are random intercepts for the herd of origin and the abattoir, respectively.

The probability of confirming the bTB infection by culture in animals with lesions was estimated using the same procedure through logistic mixed-effects models.

All analyses were conducted in R (R Core Team, 2023). Models were fitted using restricted maximum likelihood via ‘Template Model Builder’ using the ‘glmmTMB’ package (Brooks et al., 2017). First, a univariable analysis was performed and variables that were significant at the 0.05 level in a type II Wald chi-square test were selected for a step-backward selection procedure to identify the most parsimonious model following the same selection criteria (Fox and Weisberg, 2019). Multicollinearity between variables included in the multivariable models was evaluated using the variance inflation factor (VIF) through the package “performance”. Goodness-of-fit of the models was evaluated by performing a likelihood ratio test (LRT) between the selected model and the null and full model from package ‘lmtest’ (Zeileis and Hothorn, 2002). The performance of the models was compared through the area under the curve (AUC) obtained in ROC curves calculated using the package “pROC” (Robin et al., 2011).

3. Results

3.1. Data description

A total of 2,373,219 cattle were culled in the 41 registered abattoirs located in Galicia between 2014 and 2019. Of these, 1,784,261 cattle (75.2 %) from 48,185 herds in Galicia were included in this study (Fig. 1). The number of animals culled per abattoir over the study period varied widely, ranging from 2361 to 334,349 animals [median = 21,048, interquartile range (IQR) = 6716–38,157].

The province with the highest number of animals sent for slaughter was Lugo (n = 750,554; 42.1 %), followed by A Coruña (n = 547,149; 30.7 %), Pontevedra (n = 248,905; 14.0 %) and Ourense (n = 237,653; 13.3 %). Animals were culled in 41 abattoirs distributed unevenly across provinces: Lugo had the highest number of abattoirs (n = 15) followed by A Coruña (n = 12), Pontevedra (n = 9), and Ourense (n = 5). Around 50 % of the animals (n = 892,551) were slaughtered in a different province from the one in which their herd of origin was located. The distribution of animals according to their province of origin and the abattoir where they were slaughtered is shown in Fig. 2. Most slaughterhouses culled mainly animals less than 1 year old except for four slaughterhouses which culled mainly animals up to 2 years old, leading to a different distribution of the age of animals culled between slaughterhouses (Fig. 2).

Regarding the production type of herds, 47.9 % (n = 23,090) were beef herds, 23.9 % (n = 11,508) were fattening herds, 20.2 % (n = 9715) were dairy herds, and 8.0 % (n = 3872) were mixed herds. The animals belonged mainly to three different breeds, with crossbreed accounting for 49 % of all animals (n = 874,863), followed by Holstein-Friesian (28.9 %, n = 515,757) and Rubia-Gallega with 18.7 % (n = 333,926). The remaining animals (3.3 %, n = 59,715) were of 74 different breeds.

The median age of slaughtered animals was 0.83 years (IQR = 0.74–4.1 years), with dairy cattle being older (median = 5.0, IQR = 2.7–6.9) than beef cattle (median = 0.78, IQR = 0.67–0.9), fattening cattle (median = 0.82, IQR = 0.79–0.85) and mixed cattle (median = 0.79, IQR = 0.67–1.4).

The average herd size was 148 animals, with a median of 56 animals per herd (IQR = 22–144 animals). The size of the herds also varied according to the production type, with fattening herds being the largest (median = 133 animals per herd, IQR = 53–295), followed by dairy herds (median = 89, IQR = 41–200), beef herds (median = 32, IQR = 13–65) and mixed herds (median = 28, IQR = 13–53).

The number of animals culled per year remained relatively constant over the study period, ranging from 285,751 to 304,142 animals/year (median = 297,005, IQR = 293,835–301,551). Furthermore, the number of animals culled per season was also homogeneous, with a median of 75,094 animals culled (IQR = 73,504–75,908) across seasons.

The population was divided into two subpopulations based on the results of the antemortem test performed in their lifetime. Reactors comprised 13,200 cattle (0.75 % of all slaughtered animals from farms in Galicia) from 5797 herds (12.0 % of all herds included in the study) and were culled in one of the 16 abattoirs designated by the official veterinary services for reactor animals during the 2014–2019 period. Out of the 13,200 reactors, 11,318 were SIT positive, 1534 CIT positive, 256 IFN-γ positive and 92 were positive to both SIT and IFN-γ. The number of reactors slaughtered per abattoir also varied considerably, ranging from 10 to 4930 animals (median = 61, IQR = 36–1150).

3.2. bTB-like lesion detection risk analysis

A total of 524 animals (0.03 %) with bTB-like lesions were detected in 17 out of the 41 abattoirs (41.5 %). Of these, 223 (0.013 %, n = 1,771,261) were detected in non-reactor animals (with bTB infection confirmed by culture in 20 of them) and 301 (2.3 %, n = 13,200) in reactor animals (of which 90 were positive by culture) (Table 1). Furthermore, samples from 7767 non-reactor animals were subjected to bacteriological culture due to epidemiological reasons (see results) with positive results in four of them, while 12,826 reactor cattle were also analysed by bacteriology (with positive results in 73 animals) (Table 1).

In the non-reactor population, the univariable analyses identified that age, year of slaughter, herd production type and herd size were significantly associated ($p < 0.05$) with the detection of lesions at the abattoir. All variables that were associated in the univariable analyses were included in the final model (Table 2). There was a significant increase in the odds of detecting lesions with increasing age and in 2016 (OR = 1.8, 95 % CI 1.1–2.8; *p*-value = 0.017) and 2017 (OR = 1.6, 95 % CI 0.98–2.5; *p*-value = 0.063) compared with 2014, although the latter was only borderline significant, while the odds of lesions were lower in fattening (OR = 0.11, 95 % CI 0.01–0.88; *p*-value = 0.004) and dairy herds (OR = 0.52, 95 % CI 0.33–0.81; *p*-value = 0.037) compared with beef herds (Table 2).

In the reactor population year of slaughter, herd production type, herd size, province of origin of the herd, breed, test on which animals were positive and skin fold increase were associated with the detection of lesions in the univariable analyses and were included in the multivariable analysis (Table 3). In the multivariable model, herd size and test defining the reactor status were excluded due to lack of significance and breed was discarded because it was highly correlated with production type (VIF = 3.35 and 3.59 for breed and production type, respectively),

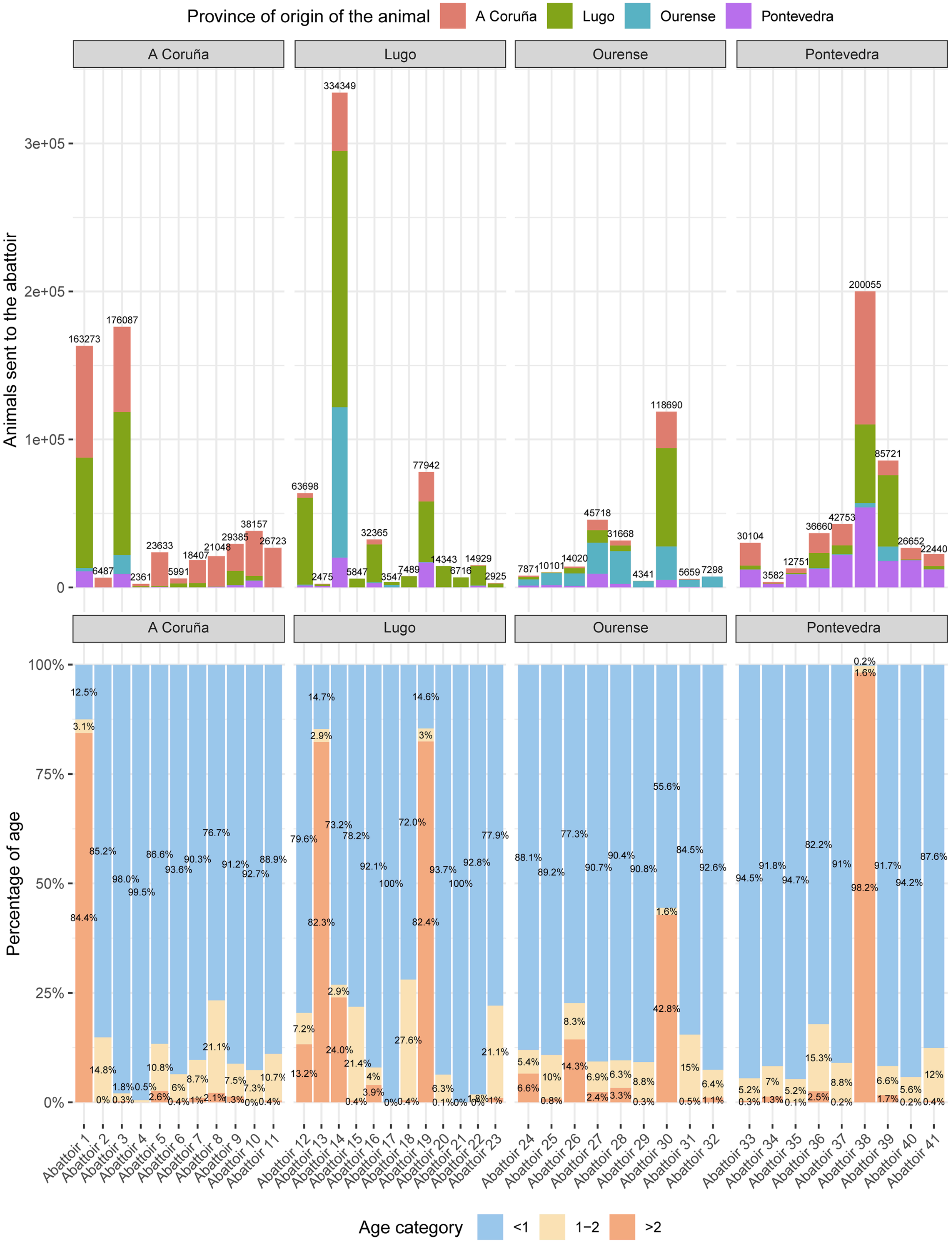

Fig. 2. A. Distribution of animals sent to slaughter by province of origin and abattoir location. **B.** Distribution of the age of the animals sent to slaughter by abattoir.

Table 1
Number of animals with positive and negative results in the antemortem tests, postmortem inspection, and bacteriological culture.

Reactor	Visible lesions	bTB-culture positive	bTB-culture negative	Not analysed by culture	Total
Negative	Yes	20	203	-	223 (0.013 %)[a]
Negative	No	4	7763	1,763,071	1,770,838
Positive	Yes	90	211	-	301 (2.3 %)[b]
Positive	No	73	12,826	-	12,899

[a] Percentage of the non-reactor population.

[b] Percentage of the reactor population.

and the model including the latter was better (LRT; $p < 0.05$). Thus, the final model included the year of slaughter, production type, province of origin and the increase in skinfold at the bovine-PPD inoculation site (Table 3). There was a decreasing trend in the odds of finding lesions over the years, with all years being significantly different from 2014. As in non-reactors, the odds of lesion detection in animals were lower in animals from dairy herds (OR = 0.40, 95 % CI 0.28–0.58; p-value < 0.001) compared with beef herds (Table 3). It was not possible to include fattening herds in the multivariable analysis as only four animals from the fattening herds tested positive to the tests. Animals from herds located in Pontevedra had significant lower odds of bTB-lesion detection than the reference (A Coruña) (OR = 0.42, 95 % CI 0.24–0.74; p-value = 0.017). Finally, the odds of finding a lesion increased by 1.12 (95 % CI 1.08–1.17; p-value = < 0.001) for each mm of increase in skinfold in the skin test prior to the slaughter of the animal.

The variability associated with the abattoir and herd random effects was higher for the non-reactor subpopulation (variance = 0.33 and 8.14 for the abattoir and herd effect, respectively) than for the reactor population (variance = 0.18 and variance = 2.85).

Multivariable models to estimate the probability of lesion confirmation adjusting by covariates did not converge due to the limited number of lesioned animals.

The crude rate of lesion detection over the study period was 12.6 per 100,000 animals for non-reactors and 22.8 per 1000 animals for reactors. Abattoir-crude lesion detection rate for non-reactors ranged from 0 to 46.9 per 100,000 animals (median = 0, IQR = 0–3.4) while for reactors ranged from 0 to 100 per 1000 animals (median = 24.4, IQR = 14.8–42.6).

According to the final models, the median predicted probability of lesion detection in the non-reactor and reactor populations was 1.2×10^{-5} (IQR = 3.7×10^{-6}–7.3×10^{-5}) and 0.008 (IQR = 0.003–0.018, max = 0.76) respectively. The abattoir-adjusted lesion detection rate for non-reactor animals ranged from 1.6 to 46.9 per 100,000 animals culled (median = 3.4 per 100,000 animals, IQR = 2.1–6.8). In contrast, the abattoir-adjusted lesion detection risk for reactor animals ranged from 6.8 to 59.9 per 1000 animals culled (median = 27.9 per 1000 animals, IQR = 19.3–39.9). The crude and adjusted estimates for lesion detection by abattoir and population are reported in Supplementary material 1.

The proportion of laboratory-confirmed lesioned animals was lower in non-reactors (20/223; 9.0 %) than in reactors (90/301; 29.9 %), with an abattoir-crude risk of confirmation for non-reactors ranging from 0 % to 19.4 % (median = 0 %, IQR = 0–2.8 %), while for reactors it ranged from 0 % to 100 % (median = 32.9 %, IQR = 14.5–39.8 %).

4. Discussion

After years of implementation of the national bTB eradication programme several regions in Spain have either achieved the OTF status or are already in the process of achieving it (MAPA, 2025). In this context, in which the pressure of antemortem testing is usually lightened, the reliability of passive surveillance becomes even more important for the maintenance of the OTF status (Foddai et al., 2015; Sergeant et al., 2017). A previous study performed in a high-prevalence region of Spain identified important differences in the detection rates depending on factors associated with the population culled but also on the abattoirs, therefore suggesting that the sensitivity of postmortem inspection is not homogeneous (Pozo et al., 2021). Here, we performed a similar analysis but focusing this time on a low prevalence region in order to determine whether the same or different factors were associated with the probability of lesion detection and whether detection rates were also dependent on the abattoirs where inspection took place. While only animals originating from herds in Galicia could be included in the study due to availability of data, these represented 75 % of the total population of cattle slaughtered in this region and thus our results are based on a very large sample of the target population.

For this assessment, the population was divided into animals sent to slaughter because they were reactors to antemortem diagnostic tests (skin tests and/or IFN-γ) and therefore suspected of being infected, and those that were culled as part of the normal husbandry system. This was done since these two groups likely belong to different subpopulations in terms of their risk of having bTB-like lesions, as also suggested by the very different raw proportions of animals with lesions among non-reactors (0.013 %) and reactors (2.3 %).

For non-reactors, there was an increase in the odds of detecting bTB-like lesions as age increased. This finding is consistent with the results of other studies (Frankena et al., 2007; Male Here et al., 2022; McKinley et al., 2018; Olea-Popelka et al., 2012; Pascual-Linaza et al., 2017; Pozo et al., 2021), with only one exception (Byrne et al., 2017), although this study was conducted in herds with an ongoing bTB breakdown. Presence of bTB-compatible lesions among non-reactors could be due to well-established infections that had been however missed by antemortem tests due to a lack of sensitivity (e.g., in old anergic animals) (Pollock and Neill, 2002) or due to infection with other microorganisms leading to granulomatous lesions in adult animals, such as *Rhodococcus equi*, *Corynebacterium pseudotuberculosis*, *Actinomyces bovis*, *Actinobacillus lignieresii, Nocardia spp.*, non-tuberculous mycobacteria (NTM), or even to the presence of carcinomas (Elbert et al., 2021; Jubb et al., 2016; Nuru et al., 2017; Zachary, 2017). This increased risk of lesions in older animals could be related to a weakened immune system as animals age (regardless of the cause) and/or to a longer period since exposure allowing the development of bTB-like lesions due to bacteria other than bTB. Involvement of processes other than bTB in causing lesions in older animals is also suggested by the relatively low proportion of lesioned animals confirmed by bacteriology among non-reactors (9 %, Table 2) and by the fact that this association between age and presence of lesions was not observed among reactor cattle (Table 3), much more likely to be bTB-infected and among which the confirmation risk was three times higher. Nevertheless, when a similar analysis was performed in a high prevalence setting in Spain an increasing risk of detecting lesion as age increased was also found among reactors, what could indicate that the dynamics of bTB infection could be different and affect different subpopulations (Pozo et al., 2021).

The prevalence of bTB in the study region, Galicia, experienced a gradual decrease over the years of study [from 0.11 % in 2014 to less than 0.03 % in 2019 (MAPA, 2024)], reaching the OTF status in 2021. In this context of reduced infectious pressure, reactor animals are more likely to be not infected (and thus lack lesions) or, if infected, they may be still in the very early stages of infection so lesions would not have developed yet. That could explain the decreasing odds of lesion detection in the postmortem inspection observed in reactor animals over the 2014–2019 period (Table 3).

We observed a significant association between the production type and the odds of detecting lesions in both non-reactor and reactors, with dairy cattle having lower risk than beef animals. Production type is often mentioned among the most important factor influencing the risk of bTB in cattle, mainly due to differences in animal management (i.e., highly productive dairy herds vs. free-range suckler herds) (Downs et al.,

Table 2
Univariable and multivariable model results for abattoir lesion detection in non-reactor animals.

Variable	Exposure level	Observations (%)	Lesioned (%)	Confirmed bTB (%)	Univariable Adjusted OR (95 % CI)	REML *p*-value	Wald *p*-value	LRT *p*-value	Multivariable Adjusted OR (95 % CI)	REML *p*-value	Wald *p*-value
Non-reactors		1,771,061	223 (0.013 %)	20 (9.0 %)							
Age	< 1 year	1,147,768	17 (0.001 %)	0 (0 %)	REF[a]		< 0.001	< 0.001			< 0.001
	1–2 years	84,718	12 (0.014 %)	1 (8.3 %)	7.5 (3.4–16.6)	< 0.001			8.2 (3.7–18.3)	< 0.001	
	> 2 years	538,575	194 (0.036 %)	19 (9.8 %)	19.3 (10.4–35.8)	< 0.001			20.8 (11.2–38.7)	< 0.001	
Year of slaughter	2014	291,315	38 (0.013 %)	14 (36.8 %)	REF		< 0.001	< 0.001			< 0.001
	2015	301,088	38 (0.013 %)	3 (7.9 %)	1.2 (0.7–2.0)	0.50			1.1 (0.7–1.9)	0.62	
	2016	304,142	56 (0.018 %)	1 (1.8 %)	1.9 (1.2–3.0)	0.01			1.8 (1.1–2.8)	0.017	
	2017	295,083	56 (0.019 %)	0 (0 %)	1.63 (1.0–2.7)	0.05			1.6 (0.98–2.5)	0.063	
	2018	293,682	17 (0.006 %)	1 (5.9 %)	0.64 (0.35–1.2)	0.17			0.59 (0.32–1.1)	0.094	
	2019	285,751	18 (0.006 %)	1 (5.6 %)	0.70 (0.38–1.3)	0.26			0.65 (0.35–1.2)	0.16	
Production type	Beef	737,785	93 (0.013 %)	12 (12.9 %)	REF		0.03	< 0.001			0.008
	Dairy	499,049	113 (0.023 %)	2 (1.8 %)	1.0 (0.64–1.6)	0.90			0.52 (0.33–0.81)	0.004	
	Fattening	423,763	1 (0.000 %)	0 (0 %)	0.04 (0–0.32)	0.003			0.11 (0.01–0.88)	0.037	
	Mixed	110,464	16 (0.015 %)	6 (37.5 %)	1.1 (0.45–2.4)	0.90			0.86 (0.39–1.9)	0.71	
Herd size	< 30 animals	586,208	47 (0.008 %)	5 (10.6 %)	REF		0.06	0.70			0.02
	30–100 animals	582,641	87 (0.015 %)	14 (16.1 %)	1.7 (1.0–2.8)	0.049			1.8 (1.1–2.9)	0.012	
	> 100 animals	602,212	89 (0.015 %)	1 (1.1 %)	1.0 (0.6–1.7)	0.95			1.1 (0.65–1.8)	0.76	
Province of origin	A Coruña	542,494	84 (0.015 %)	13 (15.5 %)	REF		0.34	0.78			
	Lugo	744,613	96 (0.013 %)	7 (7.3 %)	0.73 (0.45–1.21)	0.22					
	Ourense	237,007	11 (0.005 %)	0 (0 %)	0.50 (0.19–1.32)	0.16					
	Pontevedra	246,947	32 (0.013 %)	0 (0 %)	0.62 (0.32–1.21)	0.16					
Sex	Female	707,583	186 (0.026 %)	19 (10.2 %)	REF		0.35				
	Male	21,194	6 (0.028 %)	0 (0 %)	0.47 (0.10–2.3)	0.35					
	NA	1,042,284	31 (0.003 %)								
Breed	Crossbreed	872,554	52 (0.006 %)	9 (17.3 %)	REF		0.093	0.07			
	Holstein-Friesian	506,930	134 (0.026 %)	5 (3.7 %)	1.8 (1.1–3.0)	0.013					
	Rubia-Gallega	332,410	29 (0.009 %)	5 (17.2 %)	1.2 (0.68–1.9)	0.61					
	Other	59,167	8 (0.014 %)	1 (12.5 %)	1.2 (0.50–2.7)	0.71					
Season	Spring	450,613	58 (0.013 %)	3 (5.2 %)	REF		0.15	0.99			
	Summer	463,046	60 (0.013 %)	2 (3.3 %)	1.0 (0.71–1.5)	0.84					
	Fall	446,014	64 (0.014 %)	6 (9.4 %)	0.91 (0.62–1.4)	0.64					
	Winter	411,388	41 (0.01 %)	9 (22.0 %)	0.64 (0.41–1.0)	0.048					

[a] Reference category.

Table 3
Univariable and multivariable model results for abattoir lesion detection in reactor animals.

Variable	Exposure level	Observations	Lesioned (%)	Confirmed bTB (%)	Univariable Adjusted OR (95 % CI)	REML *p*-value	Wald *p*-value	LRT *p*-value	Multivariable Adjusted OR (95 % CI)	REML *p*-value	Wald *p*-value
Reactors		13,200[a]	301 (2.28 %)	90 (29.9 %)							
Age	< 1 year	372	11 (2.96 %)	6 (54.6 %)	REF[c]		0.46	0.53			
	1–2 years	472	19 (4.03 %)	2 (10.5)	1.6 (0.68–4.0)	0.27					
	> 2 years	12,356	271 (2.19 %)	82 (30.3 %)	1.2 (0.57–2.4)	0.69					
Year of slaughter	2014	1609	137 (8.51 %)	46 (33.6 %)	REF		< 0.001	< 0.001	REF	-	< 0.001
	2015	1833	57 (3.11 %)	15 (26.3 %)	0.27 (0.18–0.41)	< 0.001			0.28 (0.18–0.43)	< 0.001	
	2016	1633	45 (2.76 %)	18 (40.0 %)	0.30 (0.19–0.47)	< 0.001			0.31 (0.2–0.49)	< 0.001	
	2017	2358	37 (1.57 %)	4 (10.8 %)	0.20 (0.13–0.31)	< 0.001			0.2 (0.13–0.31)	< 0.001	
	2018	2887	15 (0.52 %)	3 (20.0 %)	0.07 (0.04–0.13)	< 0.001			0.07 (0.04–0.12)	< 0.001	
	2019	2880	10 (0.35 %)	4 (40.0 %)	0.05 (0.03–0.10)	< 0.001			0.05 (0.02–0.09)	< 0.001	
Production type	Beef	3603	147 (4.08 %)	68 (46.3 %)	REF		< 0.001	< 0.001	REF	-	< 0.001
	Dairy	8483	121 (1.43 %)	12 (9.9 %)	0.10 (0.29–0.56)	< 0.001			0.40 (0.28–0.58)	< 0.001	
	Fattening	4[b]	0 (0 %)	0 (0 %)	-	-			-	-	-
	Mixed	1110	33 (2.97 %)	10 (30.3 %)	0.73 (0.43–1.3)	0.26			0.74 (0.42–1.3)	0.32	
Herd size	< 30 animals	3847	92 (2.39 %)	34 (37.0 %)	REF		0.031	0.47			
	30–100 animals	6339	152 (2.4 %)	38 (24.7 %)	0.78 (0.55–1.1)	0.16					
	> 100 animals	3014	55 (1.82 %)	18 (32.7 %)	0.52 (0.31–0.85)	0.009					
Province of origin	A Coruña	4655	109 (2.34 %)	43 (39.5 %)	REF		0.035	0.01	REF	-	0.008
	Lugo	5941	137 (2.31 %)	30 (21.9 %)	1.1 (0.78–1.7)	0.50			1.1 (0.76–1.6)	0.62	
	Ourense	646	27 (4.18 %)	15 (55.6 %)	1.9 (0.98–3.6)	0.056			0.97 (0.51–1.8)	0.92	
	Pontevedra	1958	28 (1.43 %)	2 (7.1 %)	0.62 (0.35–1.1)	0.11			0.42 (0.24–0.74)	0.003	
Sex	Female	12,990	290 (2.23 %)	84 (29.0 %)	REF		0.44				
	Male	85	4 (4.71 %)	2 (50.0 %)	1.6 (0.48–5.5)	0.44					
Breed	Crossbreed	2309	93 (4.03 %)	45 (48.4 %)	REF		< 0.001	< 0.001			
	Holstein-Friesian	8827	140 (1.59 %)	16 (11.4 %)	0.50 (0.35–0.71)	< 0.001					
	Rubia-Gallega	1516	49 (3.23 %)	17 (34.7 %)	0.90 (0.58–1.4)	0.64					
	Other	548	19 (3.47 %)	12 (63.2 %)	1.1 (0.63–2.1)	0.66					
Season	Spring	3501	93 (2.66 %)	31 (33.3 %)	REF		0.085	0.24			
	Summer	3544	57 (1.61 %)	15 (26.3 %)	0.64 (0.43–0.95)	0.027					
	Fall	3715	84 (2.26 %)	23 (27.4 %)	0.92 (0.64–1.3)	0.67					
	Winter	2440	67 (2.75 %)	21 (31.3 %)	1.1 (0.72–1.6)	0.78					
Test	CIT	1534	20 (1.30 %)	1 (5.0 %)	REF		0.038	0.01			
	SIT	11,318	253 (2.24 %)	71 (28.1 %)	1.4 (0.84–2.4)	0.19					
	IFN-γ	256	10 (3.91 %)	4 (40.0 %)	0.99 (0.39–2.5)	0.98					
	SIT-IFN-γ	92	18 (19.6 %)	14 (77.8 %)	4.6 (1.8–11.8)	0.001					
Skin fold increase (mm)	Continuous	13,200	301 (2.28 %)	90 (29.9 %)	1.1 (1.03–1.14)	0.0014	0.0014	< 0.001	1.12 (1.08–1.17)	< 0.001	< 0.001

[a] At the multivariable analysis it was 13,196 observations.
[b] Not included in the analysis.
[c] Reference category.

2016). Furthermore, a variation in susceptibility to bTB infection has been linked to dairy vs. beef production (Richardson et al., 2014; Wright et al., 2013), which could also have an impact on lesion development and/or early detection in agreement with previous studies (Byrne et al., 2017; Male Here et al., 2022; O'Hagan et al., 2015; Pozo et al., 2021). Nonetheless, in the study region not all dairy herds are managed intensively and conversely not all beef herds are subjected to an extensive management. Among non-reactors, animals from fattening herds had even lower odds of harboring lesions than dairy cattle as also previously described for a high prevalence bTB setting (Pozo et al., 2021). This could not be assessed for the reactor population due to the very low sample size in this cohort. The high number of new entries into fattening herds represents a significant risk factor for the introduction of bTB into a herd (Doyle et al., 2020; Gates et al., 2013; Pozo et al., 2019). However, these herds have a high replacement rate and animals are purchased young, so they stay in their herd of origin for a short time. In addition, fattening cattle are typically slaughtered within their first year of life, what limits the time for animals to get infected and for lesions to develop.

The location from which an animal originates is often identified as a factor influencing the risk of observing lesions, generally due to differences in local bTB prevalence (Frankena et al., 2007; McKinley et al., 2018; Pascual-Linaza et al., 2017). In the case of this low prevalence region, no differences were observed in the non-reactor population but a lower risk of detecting lesions among reactor animals from the province of Pontevedra was found. This area was the first to be declared OTF in Spain in 2019, and therefore our finding may be linked to a lower bTB infection pressure, which could also explain the lower proportion of confirmed animals in this province (7.1 % of lesioned reactor animals with 39.5 %, 21.9 % and 44.4 % for the other three provinces).

An increase in herd size has been linked to a decreased risk of lesions found at the abattoir in other studies (Clegg et al., 2016; Male Here et al., 2022). In our study, the association was not linear for non-reactors, with a significantly higher risk in animals from medium-sized herds (30–100 animals) compared to small herds ($<$ 30 animals) while no differences were found for animals from larger herds ($>$ 100 animals), what may reflect differences in herd management or exposure dynamics.

The final risk factor identified in the reactor population was increased an skin fold at the site of bovine-PPD inoculation, consistent with findings from previous studies (Byrne et al., 2017; O'Hagan et al., 2015). This indicates a correspondence between lesion severity and the intensity of the cell-based immune response in immunocompetent animals. Supporting this, animals that reacted to two diagnostic tests (SIT and IFN-γ) showed an increased risk of showing lesions in the univariable analysis, indicating a possible association between a stronger immune response and disease progression. However, this variable was only borderline significant in the multivariable model ($p = 0.07$) and was excluded from the final model. In bTB, the size and spread of lesions are typically indicative of an active, advanced stage of disease (Wangoo et al., 2005), and are generally associated with an increased humoral rather than cell-mediated response (Lyashchenko et al., 2020). However, lesions may develop as early as the first 3–6 months after infection (Palmer et al., 2007), when the performance of skin test is optimal, thus explaining the observed association (Holder et al., 2024; Pollock et al., 2006).

The heterogeneity in the risk of lesion detection depending on the abattoir is in agreement with previous studies and may indicate that, in addition to the characteristics of the animals slaughtered in each abattoir and of their herds of origin considered in the multivariable models, there may be other factors influencing the performance of postmortem inspection acting at the abattoir level (Frankena et al., 2007; Garcia-Saenz et al., 2015; Male Here et al., 2022; Pozo et al., 2021). Training of staff working at the abattoir and working conditions (e.g., lighting, speed of the chain) are described in the literature as having a significant influence (Foddai et al., 2015; Garcia-Saenz et al., 2015; Teklu et al., 2004). Here, the abattoir-adjusted lesion detection risk for non-reactors was lower than in another Spanish study in a high prevalence setting (Pozo et al., 2021) (median of 3.4 vs. 8.5 lesions per 100,000 animals), and significantly lower than the crude national submission rate of granulomatous lesions, which has remained in between 23 and 29 lesions per 100,000 animals over the years of the study (MAPA, 2022). These results are generally lower than those reported elsewhere (Frankena et al., 2007; Male Here et al., 2022; Olea-Popelka et al., 2012; Pascual-Linaza et al., 2017), but estimates available in the literature often combine reactor and non-reactor animals, making comparisons challenging. The influence of the abattoir in the probability of lesion detection was lower in the reactor subpopulation, which could be due to the application of a more standardized and careful inspection procedure in animals sent for slaughter in the frame of the eradication program regardless of the abattoir.

The number of confirmed lesions was low partially due to the very limited number of animals exhibiting lesions, which did not allow us to conduct a detailed risk analysis for identification of factors associated with confirmation rates by bacteriology. Nonetheless, the proportion of laboratory-confirmed animals among those with lesions was lower here than in the study carried out in a high-prevalence setting in Spain (with 99 % confirmation for reactors and 79.5 % for non-reactors vs. 29.9 % and 9.0 % here) (Pozo et al., 2021) and lower than estimates from other countries with varying infection rates, such as Ireland (Frankena et al., 2007; Male Here et al., 2022; Olea-Popelka et al., 2012), Great Britain (O'Hagan et al., 2015; Pascual-Linaza et al., 2017; Shittu et al., 2013) and Portugal (Gonçalves et al., 2022), where values ranged from 40.7 % to 68.5 %. This may suggest that the underlying infection pressure was lower in our study, and/or that submission protocols in other regions are more biased towards the selection of the most obvious TB-like lesions for confirmation.

The detection of lesions compatible with bTB is subject to limitations, primarily due to the human factor, particularly when conducted visually. In this region, since 2017, the National Eradication Program has included the term "follow-up animal," referring to reactor animals not slaughtered due to suspicion of cross-reactions or animals from farms with a history of bTB without depopulation. These animals appear to be the future of slaughterhouse surveillance in free regions, serving as a basic complement to the passive surveillance currently in place. However, during the study period, their number was still very insignificant. Future evaluations will determine whether follow-up animals are a key element in the detection of infected animals in low-prevalence and OTF areas. If this is confirmed, these animals will serve as a valuable management tool, facilitating compensation for the loss of diagnostic sensitivity caused by increasing the interval between tests and reducing pressure on the field by directing it to abattoir sampling.

5. Conclusions

Abattoir surveillance constitutes a fundamental component of bTB control and eradication programs, particularly in regions transitioning to or already OTF. An analysis of data from a low bTB prevalence setting revealed that factors influencing lesion detection varied depending on the population evaluated. For non-reactors, age (older animals at higher risk) and production type (dairy and fattening herds at lower risk) were related to the probability of detection of lesions. In reactor animals, risk of lesion detection was lower in dairy herds compared to beef herds, higher in animals with stronger skinfold responses at the bovine-PPD inoculation site, and was also influenced by geographical origin. The observed decline trend in lesion detection over the study period was found in both populations, likely reflecting the overall reduction in bTB prevalence in the region. This study highlights the importance of evaluating abattoir surveillance under different epidemiological conditions, as evidenced by the observed differences compared to high-prevalence regions. By establishing a baseline for passive surveillance performance in low-prevalence regions, this study provides a framework for future evaluations, including the integration of emerging tools such as "follow-up" animals. These insights will enhance the adaptability of bTB control and eradication programs, ensuring their effectiveness as regions progress toward and maintain OTF status.

CRediT authorship contribution statement

Julio Alvarez: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Beatriz Romero:** Writing – review & editing, Supervision, Resources. **Jorge Mourelo:** Writing – review & editing, Resources. **Marta Alvarez-Fidalgo:** Writing – review & editing, Resources, Data curation. **Marta Muñoz-Mendoza:** Writing – review & editing, Resources, Data curation. **Pilar Pozo:** Writing – review & editing, Methodology, Investigation. **Roxana Triguero-Ocaña:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Alberto Gomez-Buendia:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation.

Funding

This work was funded by project Integrated Strategies for Tuberculosis Control and Eradication in Spain (ERATUB) (RTI2018-096010-BC22, Ministerio de Ciencia, Innovación y Universidades, MICINN) and the Spanish Ministry of Agriculture, Fisheries and Food. Alberto Gomez-Buendia holds a PhD fellowship (Reference CT58/21-CT59/21) from the Universidad Complutense de Madrid and Banco Santander.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julio Alvarez reports financial support was provided by Spain Ministry of Science and Innovation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the collaboration and support of the EU-RL for Bovine Tuberculosis, and the Spanish Ministry of Agriculture, Fisheries and Food.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106710.

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