



Evaluation of the performance of slaughterhouse surveillance for bovine tuberculosis detection in Castilla y Leon, Spain

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ABSTRACT

Post-mortem inspection (PMI) of routinely slaughtered cattle in abattoirs is an extremely valuable tool for detecting bovine tuberculosis (bTB) infected herds that can supplement active surveillance activities. However, its true performance is difficult to assess due to the multiple factors that may affect it. Here, we determined relative efficiencies in the detection of bTB-compatible lesions and probabilities of subsequent laboratory confirmation of abattoirs located in Castilla y Leon, one of the regions with the largest cattle population in Spain, between 2010 and 2017. The slaughtered animal population was split based on the results of the ante-mortem tests (reactors or non-reactors), and two generalized linear multivariable mixed models were fitted to each subpopulation to calculate the risk of lesion detection and laboratory confirmation per abattoir while accounting for the effect of potential confounding variables.

Throughout the 8-year period, ~30,000 reactors and >2.8 million non-reactor animals in the ante-mortem tests were culled in the abattoirs under study. Bovine TB compatible lesions were detected in 4,710 (16%) reactors and 828 (0.03%) non-reactor animals, of which >95% were confirmed as infected through bacteriology. The probability of disclosure of bTB-like lesions was associated with the animal subpopulation, type of source unit, the herd size, the year of slaughter, the breed and age of the animal, and/or the season of slaughter. The probabilities of detection of bTB-like lesions varied largely depending on the abattoir in both subpopulations, ranging from 603 to 3,070 per 10,000 animals for the reactors and 0.2–16.1 per 10,000 animals for the non-reactor animals.

Results obtained here will help to quantify the performance of PMI in abattoirs in Castilla y Leon and the between-abattoir variability, and to identify animals at increased risk of having bTB-like lesions detected during PMI based on animal- and farm-related factors.

1. Introduction

Bovine tuberculosis (bTB) is a chronic bacterial disease that mostly affects cattle with a complex epidemiology caused by members of the *Mycobacterium tuberculosis* complex (MTBC) that, despite the application of extensive control measures, is still prevalent in several regions worldwide (Good and Duignan, 2011). Disease eradication is challenging due to several factors including the limitations in the sensitivity

of available diagnostic techniques and the risk of reinfections of bTB herds due to the proximity to positive farms and contact with infected wildlife reservoirs (Pollock and Neill, 2002; Aranaz et al., 2004; de la Rua-Domenech et al., 2006; Gates et al., 2013). The post-mortem inspection (PMI) performed at the slaughterhouse is one of the main methods of surveillance for detection of bTB-infected cattle in officially tuberculosis free (OTF) countries. In non-OTF countries, PMI is also a major surveillance tool since it can help to detect unnoticed bTB infected

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animals (and herds) that were not detected at the ante-mortem tests typically used as part of the eradication programs.

However, and in spite of its importance, PMI has a limited sensitivity, similar to what has been described for ante-mortem tests such as the single intradermal test (SIT) and the interferon-gamma (IFN- γ) assay (de la Rua-Domenech et al., 2006). Bovine TB-like lesions may vary in size and location, and they may be overlooked during the routine PMI at the abattoir. Additionally, the proportion of bTB infected animals detected at the slaughterhouse PMI also depends on its thoroughness and other abattoir-related circumstances (Corner et al., 1990; Frankena et al., 2007). Failure to detect the infection at the abattoir in OTF infected herds may contribute to bTB spread in the herd, which implies particularly severe consequences for herds that are not subjected to frequent routine in-vivo testing, and may also favor the spread of infection to other OTF herds through animal movements. Therefore, estimating the sensitivity of PMI has great importance in the overall assessment of eradication/surveillance programs for bTB.

A wide variation in the performance of slaughterhouse surveillance between abattoirs has been previously described in Ireland (Frankena et al., 2007; Olea-Popelka et al., 2012), Great Britain (Shittu et al., 2013), and Northern Ireland (Pascual-Linaza et al., 2017). Reported differences in the reliability of slaughterhouse surveillance were attributed to animal/farm characteristics of the culled population (namely geographic origin, production type, bTB history, age), and/or factors associated to the abattoir PMI itself (size of the slaughtered population, line speed, personnel expertise).

Limited information on the efficiency of slaughterhouse surveillance in Spain is available. One study estimated the sensitivity of PMI as 31.4%, but the population analyzed was restricted to skin test positive animals from farms located in Catalonia, a low bTB prevalence region of the country (Garcia-Saenz et al., 2015), and therefore its validity for other areas, and in particular high-prevalence zones, may be limited. Given the diversity of the epidemiology of bTB in Spain, in order to assess the overall performance of passive surveillance in the frame of the eradication program it is necessary to include data from high prevalence areas. This study focuses on the evaluation of the performance of slaughterhouse surveillance in abattoirs in Castilla y Leon considering animals culled as part of the bTB eradication program (i.e., due to positive results in ante-mortem tests) or due to productive reasons. The specific aims of this study were I) to characterize the slaughtered population in abattoirs in Castilla y Leon during 2010–2017, and II) to obtain abattoir-specific estimates of the probabilities of detection of bTB-suspect lesions and of laboratory confirmation while adjusting for known and unknown animal, farm and abattoir specific characteristics.

2. Materials and methods

2.1. Study population and data sources

The target population of this study was the population of animals culled in abattoirs in Castilla y Leon. Although abattoirs in Castilla y Leon slaughter animals from farms located both within and outside the region, cattle culled in the region and whose farm of origin was also located in Castilla y Leon was considered the study population, since complete information on the bTB history and on the farm of origin of the animals was only available for this subgroup (see below).

Information available on animals slaughtered in Castilla y Leon during 2010–2017 included the identification, breed and age of the animal, and, for animals coming from farms in the region, the identification, location (coordinates), size and unit type (beef, bullfighting, dairy, fattening, breeding heifers or mixed type for herds; bullfighting arena, auctions and dealers, pastures, or others for non-herd locations) of the unit of origin. These data, together with information on cattle movement taking the animals to the abattoir (origin farm, destination abattoir, date of movement and batch size) and on the bTB status of each farm of origin (see below) were collected through the Spanish integral

traceability system (SITRAN) (Anon, 2020d). Census and movement data were used to describe the movement network connecting slaughterhouses (both in Castilla y Leon and in other regions in Spain) with the farms of origin of the slaughtered animals. Individual information on the animals culled in each abattoir was used to provide a descriptive analysis of the slaughtered cattle population in Castilla y Leon during the 8-year period. Here, the terms abattoir and slaughterhouse are used interchangeably to refer to the facility where animals were slaughtered. Only a certain number of abattoirs in Castilla y Leon are authorized to slaughter bTB-positive animals, and these are referred to as “authorized abattoirs” from now on.

2.2. Ante-mortem information

Briefly, according to the Spanish bTB eradication program all herds in Castilla y Leon are subjected to routine SIT testing with certain exceptions defined in national and international regulations (see below) (Anon, 1964, 2020b). Depending on the bTB prevalence of the local area, OTF herds are tested once or twice every year. Herds with a suspended or withdrawn OTF status are subjected to movement restrictions and, in case of confirmation of infection, the supplementary use of the IFN- γ assay in follow-up tests is repeated every 2–6 months (Anon, 1964, 2020b). The OTF status is recovered once negative results are obtained in two consecutive herds-tests separated by at least 60 days. For this study, information on the date of ante-mortem testing and number of tested and positive animals to the SIT and/or IFN- γ assay in farms in Castilla y Leon during the period 2010–2017 was also collected. Animals with a skin fold thickness increase in the SIT test >2 mm were considered positive based on severe interpretation. No information on test results prior to slaughter for animals originating from farms outside of Castilla y Leon was accessible. Additionally, no ante-mortem test results were available for a proportion of animals from farms in Castilla y Leon as testing was not compulsory for i) animals coming from closed fattening farms in areas with non-zero bTB prevalence and only moving to an abattoir, ii) animals coming from fattening farms that only introduced animals from OTF farms, or iii) bullfighting animals older than 24 months destined for bullfighting arena (Anon, 2020b). Therefore, animals with no information on ante-mortem tests were assumed to be non-reactors. Moreover, certain information such as herd size was not accessible for these animals since data on herd size was calculated as the median number of cattle subjected to herd testing.

2.3. Post-mortem information

As part of the eradication program, all SIT and/or IFN- γ positive animals are culled and subjected to an exhaustive abattoir inspection for the detection of bTB-like macroscopic lesions. In addition, all animals routinely slaughtered are also subjected to a visual inspection and incision of relevant lymph nodes and organs for detection of lesions (EFSA Panel on Biological Hazards, 2013). Most detected bTB-like lesions are subsequently submitted to the laboratory to perform bacteriological culture and confirm the infection. Information on presence of bTB-like lesions and bacteriology results (for those submitted to the laboratory), along with the animal identification, breed, date of birth and date of slaughter was available for all animals slaughtered in abattoirs in Castilla y Leon (Anon, 2020b).

2.4. Statistical analyses

First, the Castilla y Leon slaughterhouse movement network considering all movements going to abattoirs in the region, regardless of the location of the farm of origin, was constructed in a social network analysis framework as previously described in the region (Pozo et al., 2019). The geographic distance covered by the movements from farms in Castilla y Leon to the abattoirs was calculated using the Haversine formula. The distributions of the movement distances going to the

different abattoirs were compared using the Kruskal-Wallis test followed by post-hoc tests with Bonferroni corrections for multiple comparisons, whereas the association between the province of the farm and the province of the connected abattoir(s) was assessed using the chi-square test (χ^2). The correlation between the median age and the total number of slaughtered animals, and between the number of incoming movements and number of animals slaughtered at the abattoir were assessed using Spearman's rho correlation test.

Second, information on the animal and its farm of origin, together with bTB history and post-mortem data, was merged in a single database. Slaughterhouse-specific crude probabilities of detection (the proportion of animals with detected bTB-like lesions among those culled in each slaughterhouse) and laboratory confirmation (proportion of animals in which infection was confirmed among those from which samples were submitted to the laboratory from each slaughterhouse) were estimated.

The slaughtered animal population was then split into two subpopulations based on the information available on the ante-mortem tests: a subpopulation containing all positive animals (reactor animals) and a subpopulation including animals with negative results and those for which no results of ante-mortem tests were present in the database (non-reactors or animals for routine slaughter). Subsequently, two generalized linear multivariable mixed models were fitted to each subpopulation to calculate the adjusted risk of lesion detection and of laboratory confirmation per abattoir accounting for the effect of potential confounding variables, as described elsewhere (Frankena et al., 2007). Briefly, the model

$$\ln\left(\frac{p_{i,j,k}}{1-p_{i,j,k}}\right) = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \dots + \beta_n X_{in} + \alpha_j + \gamma_k$$

was fitted, where $p_{i,j,k}$ is the probability of detecting a bTB-like lesion (model 1) or of confirming the infection through bacteriological culture (model 2) in animal i from farm j culled in slaughterhouse k . Then, $X_1 \dots X_n$ are the covariates included in the model, $\beta_1 \dots \beta_n$ are the coefficients for those covariates, and α_j and γ_k represent the farm- and abattoir-specific effects, respectively. Two random effects were thus considered in the model to account for the lack of independence of animals coming from the same farm and culled at the same abattoir.

The effect of the characteristics of the animal (age at slaughter, breed and season and year of slaughter) and herd (province of origin, herd size and unit type) on the risk of bTB detection and confirmation was first evaluated using univariable logistic regression models with the presence of bTB-like lesion (yes/no) and laboratory confirmation (yes/no) as the outcome variables. Breed was categorized into crossbreed, Holstein Friesian, bullfighting and others, whereas unit type was grouped into beef, fattening, dairy/mixed, bullfighting and others. Age was categorized into three categories based on terciles (≤ 1 , $1-2$, and > 2 years), while season of slaughter was categorized into trimesters (January-March, April-June, July-September and October-December). The variable year of slaughter was tested in the models as a categorical variable (2010–2017). Herd size was calculated as the median number of animals tested in routine/follow-up herd tests during the study period and was categorized into quartiles. Significant variables based on a liberal p -value threshold ($p \leq 0.2$) were then included in multivariable models. The final models were built using a backward selection procedure based on a likelihood ratio test ($p \geq 0.05$). Results of multivariable models were expressed as odds ratio (OR) and 95 % confidence intervals (CI). To select the optimal random effects structure, two different multivariable generalized linear mixed models per subpopulation (reactors/animals for routine slaughter) including farm or farm and abattoir random effects were compared via the likelihood ratio test using the restricted maximum likelihood estimation (REML) as seen elsewhere (Zuur et al., 2009).

Estimated crude and adjusted detection/confirmation risks derived from the multivariable logistic regression models were compared

between abattoirs, and the correlation between abattoir-specific detection and confirmation risks was assessed using Spearman's rho correlation test. The coefficients extracted from the farm-level random effect were also compared across province of origin, unit types and predominant breed in the farm.

Diagnostics and goodness-of-fit evaluation of the final mixed models were performed using a simulation-based approach to create scaled residuals from each fitted model as described elsewhere (Hartig, 2020b). If the fitted model is correct, the scaled residuals based on a number (250 by default) of synthetic datasets simulated from the fitted model would follow a uniform distribution (Hartig, 2020a). The Kolmogorov-Smirnov (KS) test was used for testing overall uniformity of the residuals.

Multiple comparisons, network analyses, graphics and model fitting were conducted using PMCMRplus (Pohlert, 2020), igraph (Csardi and Nepusz, 2006), ggplot2 (Wickham, 2009), ggspatial (Dunnington, 2018), dplyr (Wickham et al., 2019), rgdal (Bivand et al., 2013), sp (Pebesma and Bivand, 2005), lme4 (Bates et al., 2015), glmmTMB (Brooks et al., 2017), and DHARMA (Hartig, 2020b) packages for R version 3.6.2 software (R Core Team, 2019).

3. Results

3.1. Castilla y Leon slaughterhouse movement network

During 2010–2017, there were 933,981 movements to slaughterhouses (4,489,665 animals) involving a) farms in Castilla y Leon as origin and/or b) abattoirs in Castilla y Leon, which altogether formed the total Castilla y Leon slaughterhouse movement network. There were 60 abattoirs located in Castilla y Leon, of which six were excluded from further analyses as no information of the number of culled animals was available (32 movements). After the exclusion of these abattoirs and when the movements to destinations outside the region were removed, the remaining 54 abattoirs received 83.2% ($n = 776,999/933,981$) movements (3,487,890 animals) out of the total slaughterhouse movement network (Table 1 and Supplementary Text 1)

Annually, the total number of movements to slaughterhouses in Castilla y Leon ranged between 84,601 (in 2017) and 111,161 (in 2011) whereas the number of culled animals varied between 397,268 (in 2014) and 475,935 (in 2012), respectively. A slight decrease in the number of

Table 1

Number of movements, slaughtered animals and abattoirs in Castilla y Leon (CyL) during 2010–2017.

Item	Number of movements	%	Number of animals	%	
Total CyL slaughterhouse movement network	933,981	100	4,489,665	100	
Movements to abattoirs in CyL^a	776,999	83.2	3,487,890	77.7	
Movements from farms outside CyL^a	131,877	14.1	645,740	14.4	
Movements to abattoirs outside CyL	156,950	16.8	1,001,775	22.3	
Origin farm and destination abattoir in CyL^a	645,122	100	2,842,150	100	
	Avila (9) ^b	70,791	11	362,153	12.7
	Burgos (6)	39,644	6.1	152,768	5.4
	Leon (6)	89,645	13.9	303,812	10.7
	Palencia (6)	43,554	6.8	111,384	3.9
Province of destination abattoir	Salamanca (7)	158,798	24.6	577,857	20.3
	Segovia (6)	54,319	8.4	433,102	15.2
	Soria (2)	4,848	0.8	10,889	0.4
	Valladolid (6)	98,394	15.3	516,118	18.2
	Zamora (6)	85,129	13.2	374,067	13.2

^a After the exclusion of six abattoirs with no complete information of the culled animals.

^b Number of slaughterhouses in each province.

movements to abattoir over the study period was identified, and a similar seasonal pattern was observed for all years, with the highest and lowest number of both movements and culled animals taking place during summer and winter periods, respectively (Fig. 1).

When only movements coming from farms located within Castilla y Leon were considered ($n = 645,122$, 69.1% out of the total slaughterhouse movement network), abattoirs located in Salamanca were the most common destination of both movements (24.6%, $n = 158,798$) and animals (20.4%, $n = 577,857$) (Table 1, Fig. 2). Further details of the movement characterization are included in Supplementary Text 1. As expected, there was a high significant positive correlation between the number of incoming movements to an abattoir in Castilla y Leon and the animals it received ($\rho = 0.96$, $p < 0.001$).

3.2. Slaughterhouse surveillance data in abattoirs in Castilla y Leon

A total of 2,842,150 animals culled in Castilla y Leon abattoirs (81.5% of all animals culled in the region) included a source located in the region (Tables 1 and 2). This was the population used to assess the efficiency of bTB passive surveillance in abattoirs in Castilla y Leon as complete information on the animals originating from outside the region (645,740 animals) was not available for this study.

Out of the 54 Castilla y Leon abattoirs with demographic information on the culled animals, 19 were authorized to slaughter reactors in ante-mortem bTB tests (authorized abattoirs). During the study period, 2,496,044 animals coming from Castilla y Leon herds were culled in these authorized abattoirs (Table 2). There was information on the results of ante-mortem bTB tests of 1,674,507 animals, of which 1.7% ($n = 29,382/1,674,507$) were slaughtered due to positive ante-mortem (SIT and/or IFN- γ test) test results. The remaining non-authorized abattoirs ($n = 35$) culled 346,106 animals for routine slaughter during the eight-year time span (Table 2).

At this point, the study population (cattle slaughtered in abattoirs in Castilla y Leon from farms in Castilla y Leon) was split into two subsets, namely those positive to the ante-mortem tests and culled only in authorized abattoirs (reactors, $n = 29,382$), and those either negative or not subjected to ante-mortem testing (non-reactors or animals for routine slaughter, $n = 2,812,768$), which were culled in both authorized or non-authorized abattoirs (Table 2). Median age of culled animals was 1.2 years (IQR = 1–1.4) (Figure S1), and culled animals due to positive

results to the ante-mortem tests were significantly older (median = 4.9, IQR = 2.4–8.8) than those slaughtered for reasons other than bTB (median = 1.2, IQR = 1–1.4, same as in the overall population) ($p < 0.001$, Mann-Whitney test). Overall, a moderate positive correlation ($\rho = 0.36$, $p < 0.05$) between age and number of slaughtered animals per abattoir was observed. Additionally, there were four abattoirs that culled animals with an overall median age higher than 2 years, whose slaughtered animal population accounted for between 0.9% and 4.3% of all culled animals (compared to the overall median of 0.5% per abattoir, IQR = 0.2–2.2). No trend in the number of slaughtered cattle was observed over the 8-year period in both animal subpopulations. Median number of culled animals per year was 3,914 (IQR = 3,325–4,027) for the reactors and 349,082 (IQR = 342,052–367,598) for the non-reactor subpopulation.

The characteristics of the animals included in the analyses are shown in Table 3 and Table S1. Fattening was the main unit type of origin among the culled animals in Castilla y Leon abattoirs (63.1%, $n = 1,794,079$, Table 3). Among bTB reactors the predominant unit type was beef (78.5%, $n = 23,055/29,382$). This could be partly due to the high proportion of fattening animals among the animals for routine slaughter (53.3%, $n = 956,985$). Salamanca was the province that accounted for the largest proportion of animals in both the bTB-reactors (39.6%, $n = 11,621/29,382$) and the animals for routine slaughter (30.3%, $n = 852,168$, Table 3). This was expected since it is the province home to the largest cattle population within Castilla y Leon. Crossbreed was the predominant breed in both the reactors (54.3%, $n = 15,961$) and the animals for routine slaughter (67.4%, $n = 1,896,364$), followed by a miscellaneous breed category (others, 31.8% and 19.6%, respectively) and Holstein Friesian cattle (10.3% and 11.3%, respectively) (Table 3). Out of the animal subpopulation with ante-mortem tests results prior to slaughter ($n = 1,674,507$), there were 12,826 reactors to the SIT, 17,551 animals positive to the IFN- γ assay and 995 positives to both. Reactors to the SIT test and the IFN- γ assay were mainly beef (80.3%, $n = 799$) animals and originated from Salamanca (41.3%, $n = 411$) and Avila (23.5%, $n = 234$). Data on ante-mortem test results was not accessible for 1,167,643 animals which were considered as animals for routine slaughter (further information is provided in Supplementary Text 2). Additionally, since information on herd size was calculated as the median number of animals tested in routine/follow-up herd tests, no information on this variable was available for 1,827 reactors and

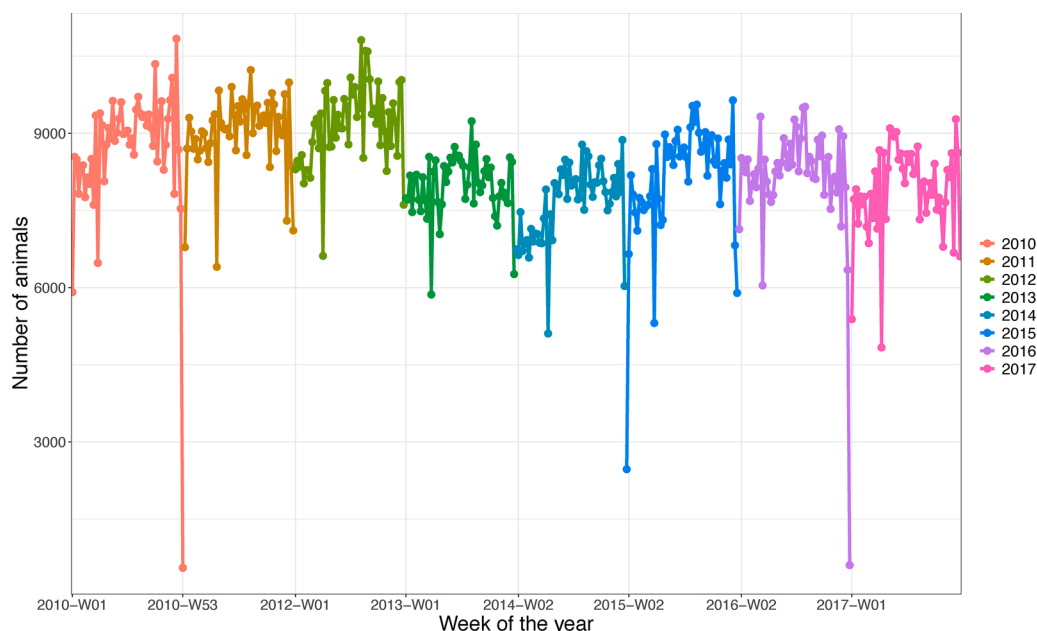


Fig. 1. Total number of animals slaughtered per week in Castilla y Leon abattoirs during 2010–2017.

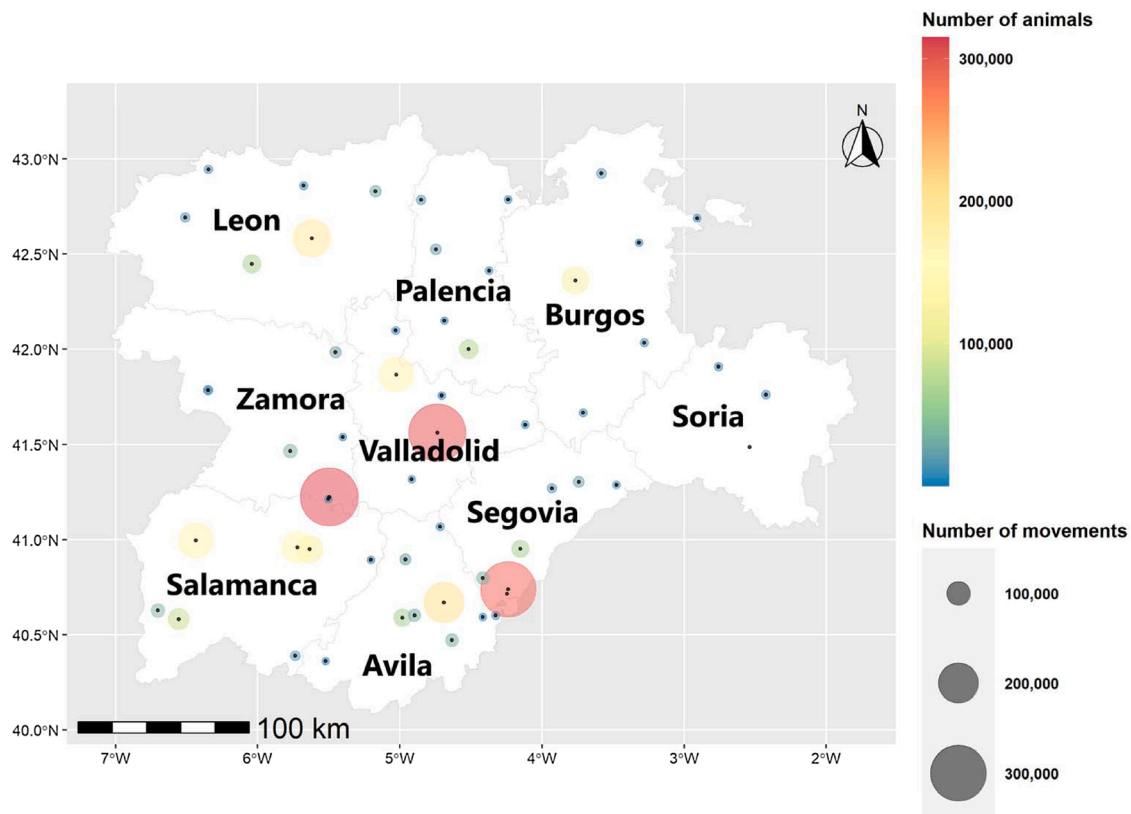


Fig. 2. Number of animals and movements to Castilla y Leon abattoirs from farms located within the region.

Table 2

Number of animals culled in Castilla y Leon (CyL) abattoirs with source farms located in the region during 2010-2017. Cattle population slaughtered in abattoirs in Castilla y Leon is split into those culled in authorized (and based on their bTB ante-mortem tests results) and non-authorized abattoirs. Number of abattoirs where each subpopulation was slaughtered is denoted in brackets.

		Number of animals	%
Origin farm and destination abattoir in CyL		2,842,150	100
bTB ante-mortem tests results	Authorized abattoirs (19)	2,496,044	87.8
	Positive	29,382	1.0
	Negative	1,645,125	57.9
	Not subjected to testing	821,537	28.9
	Non-authorized abattoirs (35)	346,106	12.2

1,481,954 animals for routine slaughter coming from herds not subjected to compulsory ante-mortem testing based on the exceptions mentioned above.

A total of 5,538 animals with bTB-like lesions were detected in 28/54 abattoirs throughout the study period, of which 98.7% (n = 5,468) were detected in the 19 authorized abattoirs. The remaining 70 lesioned animals were detected in 9/35 non-authorized abattoirs. These nine abattoirs that reported bTB-like lesions did not submit any lesion to perform bacteriological culture. A median of 619 (IQR = 568–740) suspect lesions were detected per year, with 2010 and 2011 accounting for 37.4% of the total over the study period. Globally, a significant strong positive correlation ($\rho = 0.85, p < 0.001$) between the number of slaughtered and lesioned animals in an abattoir was observed. The majority (85%, n = 4,710/5,538) of the lesioned animals were bTB reactors (16% of all bTB reactors culled during the study period) and originated from 4,359 farms. Most of them were beef cattle (n = 3,873), came from herds in Salamanca (n = 2,270), and were crossbred (n = 2,486) (Table 3). Lesioned bTB reactors were positive to SIT (61.6%, n =

2,902) and IFN- (49.3%, n = 2,321) tests in similar proportions (Table 3 and Table S1).

Out of the subpopulation of animals for routine slaughter (animals with negative results or not subjected to the ante-mortem tests), 828/2,812,768 (0.03%) animals coming from 415 farms had visible lesions detected at the slaughterhouse (Table 3 and Table S1).

Out of the 5,538 animals with bTB-like lesions, 5,430 (98%) were subjected to bacteriology, and 5,316 (97.9% of the submitted lesions) yielded *M. bovis* positive cultures. For the remaining 114 samples either no isolation was achieved (1.5%, n = 82/5,430) or a different mycobacterial species was recovered (0.6%, n = 32/5,430), though no information on the specific non-tuberculous mycobacterial species retrieved was available. When stratifying the laboratory confirmation results by subpopulation, a higher proportion of lesions confirmed through bacteriology was observed in the case of bTB reactors (98.9%, 4,658/4,710) compared with the animals for routine slaughter (91.4%, 658/720) (Table S1).

3.3. Detection and confirmation risks

Throughout the 8-year period, the overall probability of detection among positive animals to the ante-mortem tests was 1,603 per 10,000 animals, with a large heterogeneity between abattoirs (median = 1,358 per 10,000 animals, range 515–3,142). Among the animals for routine slaughter, the overall probability of detection was lower, 2.9 per 10,000 animals, and also varied largely between abattoirs (median = 0.37 per 10,000 animals, range 0–17.9). Crude detection probabilities in authorized versus non-authorized abattoirs were also very different, with median probabilities of 14.3 (IQR = 10.5–34.4) per 10,000 animals and 0 (IQR = 0–0.2) per 10,000 animals, respectively, when considering all culled animals, and 2.5 (IQR = 1.6–4.5, authorized abattoirs) per 10,000 animals versus 0 (0–0.2) per 10,000 animals in non-authorized abattoirs when considering only the non-reactor subpopulation ($p < 0.001$, Mann-Whitney test). In the authorized abattoirs, detection probabilities

Table 3
Animal and herd level characteristics of cattle slaughtered and bTB lesioned/non-lesioned animals detected in Castilla y Leon abattoirs during 2010-2017.

		Total number of culled animals				Reactors n = 29,382				Non-reactors n = 2,812,768					
Variable	Exposure level	Total	%	Total	%	Lesioned	%	Non-lesioned	%	Total	%	Lesioned	%	Non-lesioned	%
	Beef	746,027	26.2	23,055	78.5	3,873	82.2	19,182	77.7	722,972	25.7	557	67.3	722,415	25.7
	Fattening	1,794,079	63.1	310	1	11	0.2	299	1.2	1,793,769	63.8	34	4.1	1,793,735	63.8
Unit type	Dairy/mixed	176,536	6.2	3,366	11.5	166	3.5	3,200	13	173,170	6.2	169	20.4	173,001	6.1
	Bullfighting	22,636	0.8	950	3.2	336	7.1	614	2.5	21,686	0.8	23	2.8	21,663	0.8
	Others	102,872	3.6	1,701	5.8	324	6.9	1,377	5.6	101,171	3.6	45	5.4	101,126	3.6
	Avila	401,740	14.1	4,718	16.1	1,207	25.6	3,511	14.2	397,022	14.1	204	24.6	396,818	14.1
	Burgos	105,130	3.7	1,969	6.7	193	4.1	1,776	7.2	103,161	3.7	64	7.7	103,097	3.7
Origin province	Leon	308,463	10.9	3,063	10.4	314	6.7	2,749	11.1	305,400	10.9	81	9.8	305,319	10.9
	Palencia	118,480	4.2	1,500	5.1	189	4	1,311	5.3	116,980	4.2	37	4.5	116,943	4.2
	Salamanca	863,789	30.4	11,621	39.6	2,270	48.2	9,351	37.9	852,168	30.3	251	30.3	851,917	30.3
	Segovia	504,413	17.7	2,127	7.2	152	3.2	1,975	8	502,286	17.8	61	7.4	502,225	17.9
	Soria	21,920	0.8	884	3	104	2.2	780	3.2	21,036	0.7	11	1.3	21,025	0.7
	Valladolid	277,621	9.8	1,279	4.3	51	1.1	1,228	5	276,342	9.8	63	7.6	276,279	9.8
	Zamora	240,594	8.5	2,221	7.6	230	4.9	1,991	8.1	238,373	8.5	56	6.8	238,317	8.5
	Crossbreed	1,912,325	67.3	15,961	54.3	2,486	52.8	13,475	54.6	1,896,364	67.4	379	45.8	1,895,985	67.4
	Holstein Friesian	320,244	11.3	3,023	10.3	92	2	2,931	11.9	317,221	11.3	159	19.2	317,062	11.3
	Bullfighting	48,913	1.7	1,059	3.6	425	9	634	2.6	47,854	1.7	42	5.1	47,812	1.7
Breed	Others	560,668	19.7	9,339	31.8	1,707	36.2	7,632	30.9	551,329	19.6	248	29.9	551,081	19.6
	Negative	1,193,630	42	16,539	56.3	1,807	38.4	14,732	59.7	1,177,091	41.8	430	51.9	1,176,661	41.8
	Positive	12,826	0.5	12,826	43.7	2,902	61.6	9,924	40.2	0	0	0	0	0	0
SIT	No information	1,635,694	57.6	17	0	1	0	16	0.1	1,635,677	58.2	398	48.1	1,635,279	58.2
	Negative	1,115,923	39.3	8,425	28.7	1,755	37.3	6,670	27	1,107,498	39.4	659	79.6	1,106,839	39.4
	Positive	17,551	0.6	17,551	59.7	2,321	49.3	15,230	61.7	0	0	0	0	0	0
IFN- γ	No information	1,708,676	60.1	3,406	11.6	634	13.5	2,772	11.2	1,705,270	60.6	169	20.4	1,705,101	60.6

were significantly higher in the subpopulation of bTB reactors (median = 1,358 per 10,000 animals, IQR = 1,048–1,853) than among negative animals (median = 2.4 per 10,000 animals, IQR = 1.5–4.5, $p < 0.001$, Mann-Whitney test). No significant differences on detection probabilities were found between authorized or non-authorized abattoirs depending on the province of the abattoir ($p = 0.235$ and $p = 0.236$, respectively, Kruskal-Wallis test).

The likelihood ratio tests indicated that the models including both farm and abattoir random effects were better than models including only the farm ($p < 0.001$), and are the ones described below. In the univariable analyses, the variables herd size, unit type, breed, age, and season and year of slaughter were potentially associated with the probability of lesion detection in the reactor subpopulation, whereas the effect of the year of slaughter on the probability of laboratory confirmation could not be evaluated due to lack of model convergence (Tables 4 and 6). In contrast, for the animals for routine slaughter, age and year of slaughter were associated with the risk of detection while season of slaughter was the only significant variable associated with the risk of confirmation, and thus no multivariable analyses were performed ($p > 0.2$, Tables 5 and 7). The inclusion of the variables province, unit type and breed, all highly homogenous for animals coming from the same herd, along with the herd-level random effect, led to unstable models (in both subpopulations) with very large errors due to redundancy in their information. Results of the univariable and multivariable models are shown in Tables 4–7. Therefore, final models fitted for the risk of detection using the reactor subpopulation considered age, year, and slaughter season, whereas for the non-reactors, models included only

age and year of slaughter. The model without the interaction term of age and season of slaughter was preferred over the model one including the interaction term based on Akaike’s information criteria (AIC) scores in the subpopulations of reactors and non-reactors. There were no additional significant interaction terms. The risk of lesion detection increased with the age in both the bTB reactor and non-reactor subpopulations. In the former, animals of 12–24 months (OR = 1.31, 95% CI 1.1–1.6) and older than 2 years had higher odds (OR = 1.77, 95% CI 1.5–2) compared with animals ≤ 1 year (Table 4). In the animals for routine slaughter a much higher risk was observed in animals older than 2 years (OR_{>2} = 11.09, 95% CI 8.4–14.6), but no significant differences between 12–24 month animals and those less than one year old were detected ($p = 0.063$) (Table 5). Reactor animals culled between 2011 and 2017 were at lower risk of being detected with bTB-like lesions than those slaughtered during 2010, whereas non-reactor cattle culled during 2011–2014 had lower odds compared with animals culled in 2010 (Tables 4 and 5).

The odds of lesion detection were higher among reactor animals slaughtered in July to September (1.29, 95% CI 1.2–1.4) and April to June (1.23, 95% CI 1.1–1.4) compared with animals culled in October to December, whereas the probability of bTB-like lesion detection was the same among the animals for routine slaughter irrespective of the season of slaughter ($p = 0.514$, Tables 4 and 5). The overall median predicted probability of lesion detection in the reactor subpopulation was 0.09 (IQR = 0.05–0.21, max. = 0.89), and was highest in animals older than 2 years and culled in July to September (Fig. 3). Much lower predicted values were obtained in the animals for routine slaughter

Table 4

Results from univariable and multivariable logistic regression models using the detection of bTB-like lesions (yes/no) as the outcome variable for the reactor subpopulation.

Variable	Exposure level	Culled animals	Lesioned animals	Adjusted OR for detection (95 % CI)	P-value ^a	P-value ^b	Adjusted OR for detection (95 % CI)	P-value ^a	P-value ^b
				Univariable			Multivariable ^c		
Population	Reactors	29,382	4,710						
	Beef	23,055	3,873	NA ^d	–	<0.001			
	Fattening	310	11	0.24 (0.1–0.5)	<0.001				
Unit type	Dairy/mixed	3,366	166	0.32 (0.2–0.4)	<0.001				
	Bullfighting	950	336	2.12 (1.4–3.1)	<0.001				
	Others	1,701	324	1.18 (0.9–1.5)	0.162				
	Crossbreed	15,961	2,486	3.71 (2.7–5.1)	<0.001				
	Holstein								
Breed	Freisian	3,023	92	NA	–	<0.001			
	Bullfighting	1,059	425	9.87 (6.4–15.1)	<0.001				
	Others	9,339	1,707	4.81 (3.5–6.6)	<0.001				
Herd size ^e	1Q (0–42)	2,827	533	1.57 (1.3–1.9)	<0.001				
	2Q (43–77)	5,015	992	1.49 (1.2–1.8)	<0.001				
	3Q (78–133)	6,831	1,130	1.21 (1–1.5)	0.041				
	4Q (>133)	12,882	1,730	NA	–	0.032			
	2010	3,971	871	NA	–	<0.001	NA	–	<0.001
Year of slaughter	2011	4,083	881	0.21 (0.2–0.3)	<0.001		0.74 (0.6–0.9)	<0.001	
	2012	2,611	519	0.73 (0.6–0.8)	<0.001		0.62 (0.5–0.7)	<0.001	
	2013	3,561	477	0.63 (0.5–0.7)	<0.001		0.38 (0.3–0.5)	<0.001	
	2014	4,008	439	0.38 (0.3–0.5)	<0.001		0.31 (0.3–0.4)	<0.001	
	2015	3,857	486	0.31 (0.3–0.4)	<0.001		0.42 (0.3–0.5)	<0.001	
	2016	4,674	530	0.38 (0.3–0.5)	<0.001		0.37 (0.3–0.4)	<0.001	
	2017	2,617	507	0.34 (0.3–0.4)	<0.001		0.58 (0.5–0.7)	<0.001	
	≤ 1	2,984	336	NA	–	<0.001	NA	–	<0.001
Age (years)	1–2	3,394	445	1.33 (1.1–1.6)	0.001		1.31 (1.1–1.6)	0.002	
	>2	23,004	3,929	1.83 (1.6–2.1)	<0.001		1.77 (1.5–2)	<0.001	
	January–March	6,330	813	0.82 (0.7–0.9)	0.001		0.92 (0.8–1)	0.182	
Season	April–June	9,230	1,496	1.18 (1.1–1.3)	0.002		1.23 (1.1–1.4)	<0.001	
	July–September	6,387	1,168	1.25 (1.1–1.4)	<0.001		1.29 (1.2–1.4)	<0.001	
	October–December	7,435	1,233	NA	–	<0.001	NA	–	<0.001

^a Wald test.

^b Likelihood ratio test.

^c Generalized linear multivariable mixed model results with 29,382 observations and random intercept terms for 19 abattoirs and 4,359 farms.

^d Reference category.

^e Generalized linear univariable mixed model results with 27,555 observations and random intercept terms for 19 abattoirs and 3,895 farms for which herd size was available.

Table 5

Results from univariable and multivariable logistic regression models using the detection of bTB-like lesions (yes/no) as the outcome variable for the non-reactor subpopulation.

Variable	Exposure level	Culled animals	Lesioned animals	Adjusted OR for detection (95 % CI) Univariable ^c	P-value ^a	P-value ^b	Adjusted OR for detection (95 % CI) Multivariable	P-value ^a	P-value ^b
Population	Non-reactors	2,812,768	828						
	Beef	722,972	557	NA ^d	–	<0.001			
	Fattening	1,793,769	34	0.05 (0–<0.1)	<0.001				
Unit type	Dairy/mixed	173,170	169	0.61 (0.4–0.9)	0.019				
	Bullfighting	21,686	23	1.38 (0.6–2.9)	0.411				
	Others	101,171	45	0.79 (0.4–1.4)	0.402				
	Crossbreed	1,896,364	379	0.91 (0.6–1.3)	0.631				
Breed	Holstein	317,221	159	NA	–	<0.001			
	Freisian	47,854	42	1.91 (1–3.6)	0.047				
	Others	551,329	248	1.62 (1.1–2.4)	0.018				
	1Q (0–19)	185,617	52	1.27 (0.7–2.2)	0.393				
Herd size ^e	2Q (20–48)	138,039	105	1.61 (1–2.5)	0.032				
	3Q (49–93)	283,117	167	1.35 (0.9–1.9)	0.107				
	4Q (>93)	724,041	455	NA	–	1			
	2010	366,496	168	NA	–	<0.001	NA	–	<0.001
	2011	371,812	153	0.85 (0.6–1.1)	0.231		0.89 (0.7–1.2)	0.396	
	2012	370,902	91	0.68 (0.5–0.9)	0.014		0.65 (0.5–0.9)	0.007	
Year of slaughter	2013	340,520	59	0.42 (0.3–0.6)	<0.001		0.44 (0.3–0.6)	<0.001	
	2014	322,313	32	0.27 (0.2–0.4)	<0.001		0.27 (0.2–0.4)	<0.001	
	2015	351,810	142	1.07 (0.8–1.4)	0.629		1.21 (0.9–1.6)	0.193	
	2016	346,353	112	1.05 (0.8–1.4)	0.764		1.12 (0.8–1.5)	0.453	
	2017	342,562	71	0.79 (0.6–1.1)	0.176		0.83 (0.6–1.2)	0.282	
	≤1	836,395	67	NA	–	<0.001	NA	–	<0.001
Age (years)	1–2	1,511,777	72	0.72 (0.5–1)	0.069		0.72 (0.5–1)	0.063	
	>2	464,596	689	10.95 (8.3–14.4)	<0.001		11.09 (8.4–14.6)	<0.001	
	January-March	642,048	180	0.97 (0.8–1.2)	0.831				
	April-June	701,145	223	1.12 (0.9–1.4)	0.291				
Season	July-September	762,569	245	1.35 (1.1–1.7)	0.006				
	October-December	707,006	180	NA	–	0.514			

^a Wald test.

^b Likelihood ratio test.

^c Generalized linear univariable mixed model results with 2,812,768 observations and random intercept terms for 54 abattoirs and 21,028 farms.

^d Reference category.

^e Generalized linear univariable mixed model results with 1,330,814 observations and random intercept terms for 54 abattoirs and 15,376 farms for which herd size was available.

(median = <0.001, IQR = <0.001–<0.001, max. 0.63). Further results of the predicted probabilities of lesion detection per unit type, breed, year of slaughter, age of the animal, season of slaughter and province of origin for the reactor and non-reactor subpopulations are included in Figure S2.

When considering the probability of laboratory confirmation in samples collected from the bTB reactor subpopulation, age was again significantly associated with the risk of being confirmed; culled animals between 1 and 2 years and animals older than 2 years were at higher (OR_{1–2} = 4.71 95 % CI 1.2–18.4; OR_{>2} = 3.02 95% CI 1.4–6.5, respectively) risk of being bTB-confirmed than the animals ≤1 year (Table 6). Only season of slaughter was significant when the analysis was performed in the animals for routine slaughter. Median predicted probabilities of laboratory confirmation for the reactors and the animals for routine slaughter were 0.99 and 0.96, respectively.

Normality of the residuals was checked with quantile-quantile (qq) plots and the KS statistic (Figure S3). No deviations from the expected distributions were observed for the detection and laboratory confirmation risks models in the reactor to the ante-mortem tests subpopulation (KS test, *p* > 0.05, Figure S3).

Comparison of the abattoir random effects revealed several slaughterhouses with a higher probability of detection for both subpopulations of animals, and no significant differences depending on the province. Comparison of the farm-level random effects revealed that coefficients from bullfighting herds (and herds in which bullfighting was the predominant breed) were significantly higher compared with the other three categories in the reactor subpopulation (*p* < 0.05, Kruskal-Wallis

test with Bonferroni corrections, Fig. 4). A similar trend was observed when the estimates from the model for the risk of laboratory confirmation was assessed, although differences were less evident (Figure S4).

In the case of the animals for routine slaughter, significant (*p* < 0.001, Kruskal-Wallis test with Bonferroni corrections) differences in farm random effects estimates in the models for lesion detection were also observed depending on the unit type/predominant breed of the farm (Figure S5), while no differences were observed in the model for laboratory confirmation (*p* > 0.05, Kruskal-Wallis test, Figure S6). Significant (*p* < 0.001, Kruskal-Wallis test with Bonferroni corrections) differences in the herd-level random effects depending on the province of origin of the farm in both subpopulations were also observed (Figure S7).

The abattoir detection and confirmation probabilities adjusted by the covariates included in the model for bTB reactors ranged from 603 to 3,070 per 10,000 animals (median = 1,367 per 10,000 animals, IQR = 1,058–1,843) and from 97.3 to 99.3 over 100 lesioned animals (median = 98.9 per 100 animals, IQR = 98.3–99.1), respectively (Table S2). There was a very high positive correlation ($\rho = 0.99, p < 0.001$) between the crude and adjusted detection risks per abattoir, suggesting that the adjustment for the effects of the confounding variables had little effect on the ranking of the slaughterhouses according to their detection risk. A moderate correlation ($\rho = 0.69, p < 0.001$) was seen between the crude and adjusted probabilities of laboratory confirmation (Table S2).

In the animals for routine slaughter, the adjusted abattoir detection probabilities ranged between 0.2 and 16.1 per 10,000 animals, with a

Table 6

Results from univariable and multivariable logistic regression models using the laboratory confirmation of bTB-like lesions (yes/no) as the outcome variable for the reactor subpopulation.

Variable	Exposure level	Lesioned animals	Confirmed animals	Adjusted OR for confirmation (95 % CI) Univariable	P-value ^a	P-value ^b	Adjusted OR for confirmation (95 % CI) Multivariable ^c	P-value ^a	P-value ^b
Population	Reactors	4,710	4,658						
	Beef	3,873	3,841 ^d	NA ^e	–	0.093			
	Fattening	11							
Unit type	Dairy/mixed	166	165	1.69 (0.2–13.8)	0.624				
	Bullfighting	336	334	1.66 (0.3–8.8)	0.549				
	Others	324	318	0.51 (0.2–1.4)	0.19				
	Crossbreed	2,486	2,449	0.74 (0.1–5.9)	0.78				
Breed	Holstein	92	91	NA	–	0.011			
	Freisian								
	Bullfighting	425	424	3.96 (0.2–73.8)	0.356				
	Others	1,707	1,694	1.51 (0.2–12.7)	0.706				
Herd size ^f	1Q (0–42)	533	525	0.78 (0.3–2)	0.614				
	2Q (43–77)	992	986	1.96 (0.7–5.4)	0.197				
	3Q (78–133)	1,130	1,120	1.27 (0.5–3)	0.592				
	4Q (>133)	1,730	1,708	NA	–	0.232			
	≤1	336	326	NA	–	0.007	NA	–	0.009
Age (years)	1–2	445	442	4.81 (1.2–18.6)	0.023		4.71 (1.2–18.4)	0.026	
	>2	3,929	3,890	3.08 (1.4–6.6)	0.004		3.02 (1.4–6.5)	0.005	
	January-March	813	797	0.57 (0.3–1.3)	0.059		0.58 (0.3–1.3)	0.197	
	April-June	1,496	1,482	1.12 (0.5–2.5)	0.713		1.1 (0.5–2.4)	0.878	
Season	July-September	1,168	1,158	NA	–		1.3 (0.5–3.2)	0.567	
	October-December	1,233	1,221	1.31 (0.5–3.2)	0.551	0.201	NA	–	0.239

^a Wald test.

^b Likelihood ratio test.

^c Generalized linear multivariable mixed model results with 4,710 observations and random intercept terms for 19 abattoirs and 1,323 farms.

^d “Beef” (3,830 confirmed animals) and “Fattening” (11 confirmed animals) were merged as no fattening animal was unconfirmed.

^e Reference category.

^f Generalized linear univariable mixed model results with 4,385 observations and random intercept terms for 19 abattoirs and 1,214 farms for which herd size was available.

Table 7

Results from univariable and multivariable logistic regression models using the laboratory confirmation of bTB-like lesions (yes/no) as the outcome variable for the non-reactor subpopulation.

Variable	Exposure level	Lesioned animals	Confirmed animals	Adjusted OR for confirmation (95 % CI) Univariable ^c	P-value ^a	P-value ^b
Population	Non-reactors	828	658			
	Beef	557	442	NA ^d	–	0.006
	Fattening	34	21	0.53 (0.1–3)	0.473	
Unit type	Dairy/mixed	169	139	0.37 (0.1–1)	0.049	
	Bullfighting	23	18	1.42 (0.1–18.2)	0.785	
	Others	45	38	1.68 (0.2–11.9)	0.603	
	Crossbreed	379	293	2.39 (0.9–6.5)	0.088	
Breed	Holstein Freisian	159	128	NA	–	<0.001
	Bullfighting	42	237 ^e	5.81 (1.7–19.6)	0.004	
	Others	248				
Herd size ^f	1Q (0–19)	47	41	0.71 (0.2–3.2)	0.653	
	2Q (20–48)	87	81	1.34 (0.3–5.1)	0.669	
	3Q (49–93)	141	130	1.25 (0.4–3.6)	0.679	
	4Q (>93)	403	365	NA	–	0.275
	≤1	67	54	NA	–	0.257
Age (years)	1–2	72	58	0.47 (0.1–2.7)	0.402	
	>2	689	546	0.66 (0.2–2.8)	0.574	
	January-March	180	131	0.15 (0.0–0.6)	0.004	
	April-June	223	187	0.77 (0.2–2.8)	0.691	
Season	July-September	245	191	0.34 (0.1–1.1)	0.071	
	October-December	180	149	NA	–	<0.001

^a Wald test.

^b Likelihood ratio test.

^c Generalized linear univariable mixed model results with 720 observations and random intercept terms for 19 abattoirs and 355 farms.

^d Reference category.

^e “Bullfighting” (36 confirmed animals) and “others” (201 confirmed animals) were merged as no bullfighting animal was unconfirmed.

^f Generalized linear univariable mixed model results with 678 observations and random intercept terms for 19 abattoirs and 325 farms for which herd size was available.

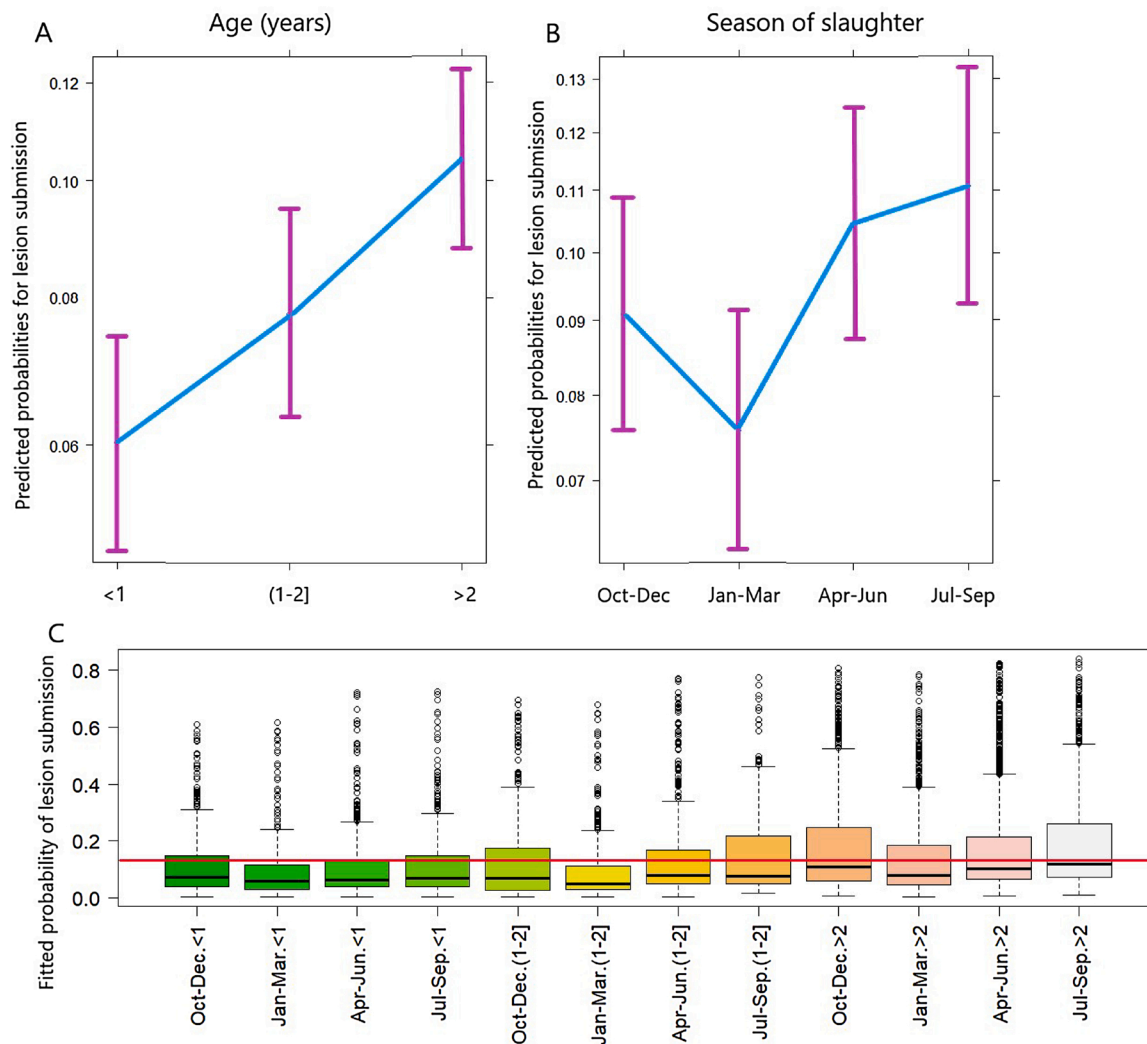


Fig. 3. Predicted probabilities of lesion detection per age (A), season of slaughter (B), and combined (C) for the positive to the ante-mortem tests subpopulation. Middle bars in the boxes indicate median values, and the boxes indicate the interquartile range. Whiskers denote the 95th quantiles and dots represent outliers.

median of 0.85 per 10,000 animals (IQR = 0.5–2.2, Table S3). The probability of laboratory confirmation in the 19 factories that detected (and submitted) bTB lesions among the animals for routine slaughter varied from 82.58 and 96.88 per 100 animals (median 92.5 per 100 animals, Table S3). There was again a very high positive correlation between the crude and adjusted detection ($\rho = 0.98, p < 0.001$) and laboratory confirmation ($\rho = 0.95, p < 0.001$) probabilities per abattoir in the subpopulation of animals for routine slaughter, which again indicated that the confounding factors included in the models did not affect the ranking of the slaughterhouses according to their detection and confirmation risks in the non-reactor subpopulation (Table S3).

4. Discussion

In this study the performance of passive surveillance in detecting and subsequently confirming lesions due to MTBC infection in cattle slaughtered in Castilla y Leon during 2010–2017 was evaluated. Although other studies have been conducted on the herd- and animal-level factors that hamper bTB control in Spain, these have mostly focused on active surveillance (i.e., ante-mortem testing).

Because of data availability, analyses were limited to animals originating from farms in Castilla y Leon. Given the expected difference in the probability of having bTB-like lesions in reactor animals (to the SIT and/or IFN- γ assay), multivariable analyses were performed separately in

each animal subpopulation (reactors vs. non-reactors/animals for routine slaughter). Despite these low proportions of non-reactors in which bTB infection was detected in the post-mortem, the risk posed by these false negative/undetected in the ante-mortem tests cattle should not be underrated as substantial program costs are associated with them.

Overall, the univariable and multivariable analyses showed that there was a significant variation in the probability of lesion detection and laboratory confirmation by abattoir and farm of origin and depending on age and/or season and year of slaughter depending on the subpopulation analyzed. A strong effect of increasing age in the risk of detection of bTB-like lesions was observed in both subpopulations, with lesions in older (>1 year in reactors, >2 in non-reactor cattle) animals more likely to be detected than in younger animals (<1 year). This increased risk associated with the age of the animal has been previously observed in Ireland, Northern Ireland, and Great Britain (Frankena et al., 2007; Pascual-Linaza et al., 2017; McKinley et al., 2018). The chronic nature of the disease together with the complexities linked to its early detection could explain this increased probability of lesion detection (and in fact of bTB infection) as the age of the animal increases. However, the effect of age on the probability of laboratory confirmation was only significant in the reactor subpopulation, suggesting that once well-developed gross lesions are detected in a bTB exposed and undetected testing animal the likelihood of being confirmed as *M. bovis* is equal irrespective of the age of the animal. Additionally, lesions were

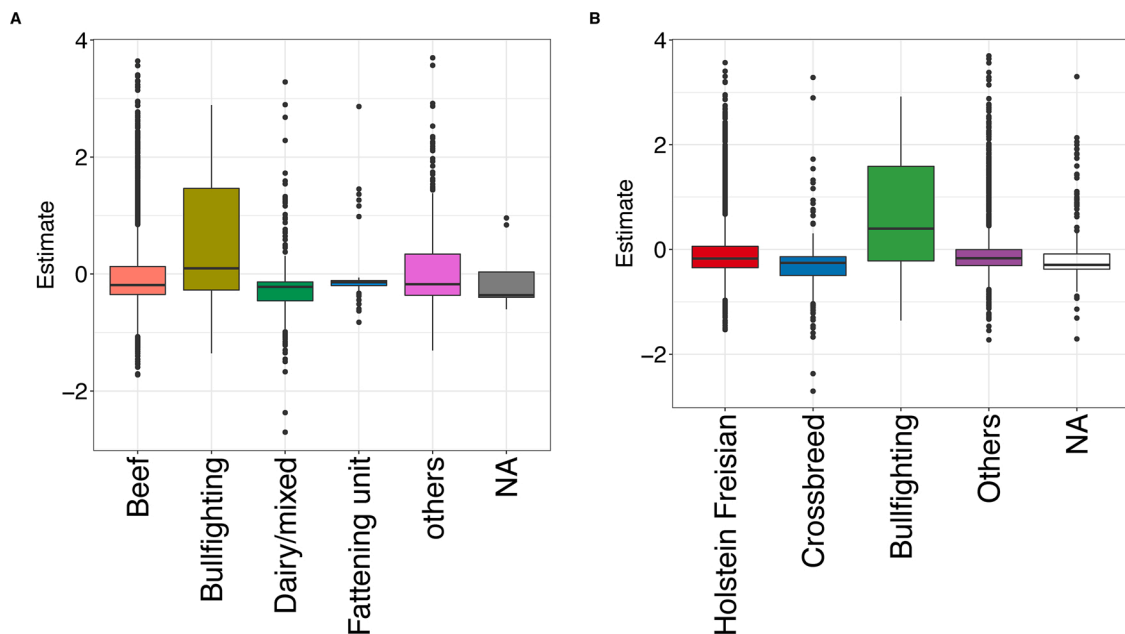


Fig. 4. Random effect estimates of lesion detection per unit type (A) and breed (B) for the reactors to ante-mortem tests subpopulation. NA represents farms with no predominant unit type or breed. Middle bars in the boxes indicate median values, and the boxes indicate the interquartile range. Dots represent outliers.

more likely to be detected in reactor animals culled between April and September compared with reactors slaughtered between October and December. Observed differences could be related with a seasonal pattern in the eradication campaigns: first routine herd test (more likely to disclose infected animals that would not have been tested for a year and thus could have more advanced lesions) is typically performed in the first half of the year, while follow-up tests (second rounds performed in bTB-positive farms) performed later in the year would more likely lead to the detection of early-stage infections due to the few months elapsed since the previous herd test. Additionally, differences may be also related to the availability of experienced/motivated temporarily hired meat inspectors during the spring-summer holiday period. Animals culled during 2010 were at higher risk of being detected with bTB-like lesions at the abattoir in both subpopulation of animals, but this effect tended to be lower with time. These results were in line with the decreasing trend in the bTB herd prevalence observed in the region during the study period [2.6% in 2010 and 1.6% in 2017, (Anon, 2020c)], and suggested that improved measures (i.e., increased testing pressure in bTB positive farms) applied in the framework of the Spanish eradication program might be successful in controlling the disease.

According to the univariable analyses and the estimates of the farm random effects, the risk of detecting bTB-like lesions among reactors was significantly higher in bullfighting herds and animals of bullfighting breeds compared to beef (and Holstein Friesian cattle), whereas in the animals for routine slaughter was not. This result may be due to the low sample size of this population (<3% of the submitted lesions in the non-reactor subpopulation). In the animals for routine slaughter, there was also an increased odds of detecting suspect lesions (and also confirming those in the laboratory in this case) in bullfighting cattle compared to Holstein Friesian. Bullfighting (and beef) herds, typically managed in extensive systems, are exposed to certain risk factors (i.e., an increased risk of contact with infected wildlife and other cattle, and access to shared pastures) that may influence the effectiveness of control programs, and our results suggest that when bTB-infected bullfighting animals are detected through ante-mortem tests the disease is in a more advanced state compared with other breeds, what could be related with lack of performance of the tests at earliest stages of disease and/or a faster progression of the infection. The performance of the SIT test may be impaired when performed in bullfighting animals due to the

temperament of these animals and a possible breed-specific effect (Keck et al., 2018). Although the IFN- γ assay can help to increase test performance in infected herds, it was not applied on the negative bullfighting animals in which lesions were found. This result confirms the potential usefulness of the IFN- γ as an ancillary test to decrease the number of false negative animals, as observed previously in these animals in Castilla y Leon (Alvarez et al., 2014). Overall, the odds of finding lesions were significantly lower in dairy herds/Holstein Friesian cattle, although no differences were observed when considering the risk of laboratory confirmation. These results are in agreement with previous research suggesting that reactors from dairy herds are less likely to have gross pathology characteristic of *M. bovis* infection than other types exposed to bTB (Downs et al., 2016; Byrne et al., 2017).

Unsurprisingly, the highest proportion of animals with bTB-like lesions disclosed in Castilla y Leon abattoirs (and the overall slaughtered cattle population) was linked to farms located in Salamanca and Avila, which also account for the highest number of bTB-positive herds during 2010–2017 (Pozo et al., 2020). In contrast, animals from farms located in Soria represented the lowest percentage (<1%) out of the overall culled population in Castilla y Leon (and thus the smallest number of reactor and lesioned animals), although proportionally, Soria had the highest percentage of reactors over its population slaughtered in abattoirs in the region (4% of reactors vs. 1.2% in Avila and 1.3% in Salamanca) what could also explain in part the higher overall proportion of lesioned animals in this province (0.52% of lesioned animals vs. 0.35% in Avila and 0.29% in Salamanca, Table 3). This strong effect of the region where animals were originated concurs with results obtained elsewhere (McKinley et al., 2018), with animals coming from high-risk areas being at higher risk of being detected than animals from low-risk areas. Nevertheless, when considering only the animals for routine slaughter, the proportion of lesioned animals from Soria was similar (or higher) to the one observed in these high-prevalence provinces (0.05% in Soria vs. 0.05% and 0.029% in Avila and Salamanca, respectively, Figure S7) even if other identified risk factors (such as older or bullfighting cattle) are not frequent there. Results obtained in previous study performed in the area showed a higher risk of bTB persistence in herds located in Soria compared to other high and low prevalence regions in Castilla y Leon (Pozo et al., 2020). These results, pointing at possible differences in test performance of ante-mortem

diagnostics should be further examined at the animal/herd level.

The overall median predicted probability of lesion detection in the population positive to the ante-mortem tests was 9%, a much lower value than the 45% reported by Garcia-Saenz and collaborators in reactors from Catalonia, Spain (Garcia-Saenz et al., 2015). This difference may be related to the variability in testing regime intervals in both regions with distinct herd level prevalences (0.16% in Catalonia vs. 1.4% in Castilla y Leon in 2018) (Anon, 2020b). Herds in Catalonia, a low prevalence region, are less frequently routine tested and there is a more frequent use of the single intradermal comparative cervical tuberculin test (SICCT). Therefore, the minimum time for detection in case of an animal being bTB infected may be longer, which allows for longer times for bTB lesions to develop and thus higher chances of being detected during meat inspection (Napp et al., 2019).

Median crude lesion detection probabilities in Castilla y Leon abattoirs for the reactors and the animals for routine slaughter (1,358 per 10,000 animals and 0.4 per 10,000 animals, respectively) fall outside the range of the mean abattoir estimates reported in Northern Ireland (30 per 10,000 animals, Pascual-Linaza et al., 2017) and Ireland (22–25 per 10,000 animals, Frankena et al., 2007; Olea-Popelka et al., 2012). However, these studies were based on the culled population irrespective of their ante-mortem status. Median crude lesion probability estimated here for animals subjected to routine slaughter was slightly lower than values reported in Castilla y Leon in 2019 (0.5 per 10,000 animals), and than the overall probability reported in Spain [2.7 per 10,000 animals (Anon, 2020a)]. In the USA, where bTB is only endemic in a restricted area of Michigan, detection probabilities reported in 2004 were nevertheless significantly higher [9.3 granuloma submissions per 10,000 cattle (United States Animal Health Association, 2004)]. If only the 28 slaughterhouses in Castilla y Leon in which at least one animal was detected were considered, the detection probability increased to 11.5 per 10,000 animals (IQR = 2–22.2). These results suggest that even in abattoirs where an enhanced bTB surveillance is performed, detection probabilities reach levels similar to those reported in scenarios where bTB cases are anecdotal. Nevertheless, direct comparisons between studies should be drawn with caution as the population of culled animals included in the analyses differed with these studies. A substantial variability in the detection probabilities has been observed in other regions of Spain, with values ranging between 0 per 10,000 animals in the Basque Country and 25.2 per 10,000 animals in Murcia, both low-prevalence areas of the country (Anon, 2020a). In contrast, reported detection probabilities in other high bTB prevalence regions such as Castilla-La Mancha and Extremadura were substantially higher than those observed in Castilla y Leon (0.9 and 12.2 per 10,000 animals, respectively) although prevalences were also higher than that reported in Castilla y Leon in 2019 (Anon, 2020c, a). A thorough evaluation of the slaughterhouse surveillance as the one performed here should be conducted in other high and low prevalence regions to evaluate the overall degree of efficiency of meat inspection and factors associated with the detection of bTB-compatible lesions in Spain.

Out of the 54 Castilla y Leon abattoirs, no bTB-like lesions were detected during the study period in 26. Animals slaughtered in these abattoirs mostly originated from fattening farms (67.7%) followed by beef (31.4%) and dairy (3.14%), and were therefore significantly younger than the ones slaughtered in abattoirs where at least one lesioned animal was detected ($p < 0.001$, Mann-Whitney test), in which animals from fattening units constituted 49.1% of all slaughtered animals. No significant differences were found on the abattoir-level random effects depending on the predominant unit type, breed and province of origin of the slaughtered animals in these 26 abattoirs where no bTB-like lesion was detected ($p > 0.05$, Kruskal-Wallis test, data not shown).

After adjustment for age and season of slaughter, detection probabilities in the reactor and non-reactor cattle subpopulations differed significantly between abattoirs (from 603 to 3,070 per 10,000 and from 0.2 to 16.1 per 10,000 animals, respectively). This variation could be due to the differences in the characteristics of the animals slaughtered in

each abattoir not considered in the model, but also to differences in the accuracy/efficiency of the abattoirs in detecting bTB-like lesions as seen in Ireland (Frankena et al., 2007; Olea-Popelka et al., 2012), Northern Ireland (Pascual-Linaza et al., 2017), GB (Shittu et al., 2013), USA (Kaneene et al., 2006), and Catalonia in Spain (Garcia-Saenz et al., 2015). In contrast, confirmation probabilities did not differ substantially among abattoirs in both subpopulations of slaughtered animals (reactors: 97.3–99.3% and non-reactors: 82.6–96.9%) meaning that once bTB-suspect lesions are detected, confirmation as *M. bovis* was not affected by the abattoir in which these were identified. These values were well above other confirmation probabilities reported in GB (67%, Shittu et al., 2013) and Ireland (64–69%, Frankena et al., 2007; Olea-Popelka et al., 2012), what could indicate a higher specificity in lesion detection (that could in turn suggest a lower sensitivity). To reduce this possible limited sensitivity, the establishment of baseline targets of disclosure of bTB-like granulomatous lesions even in the absence of bTB as suggested in the EU (EU - DG SANCO, 2013) and other countries [USA and Canada, with a minimum of 1 granuloma per 2,000 adult cattle slaughtered (USDA-APHIS, 2009, 2015)], is of critical importance, and in Spain was implemented in 2017 after the audit carried out by the European Commission on the progress of the Spanish program to eradicate bTB through the establishment of standards for granuloma submissions (EFSA Panel on Biological Hazards, 2013; Anon, 2020a). Again, the population of culled animals included in our analyses differed from the one analyzed in the previously cited articles, which were based on animals sent for routine slaughter. When the present analysis was performed on the negative cattle subpopulation in which bTB-like lesions were detected and subjected to bacteriological culture ($n = 693$), the laboratory confirmation probability was still higher than 91%, confirming the overall high likelihood of confirming *M. bovis* infection in suspect lesions in Castilla y Leon (data not shown). The apparently low proportion of lesioned but unconfirmed animals described here, along with the relatively low detection probability mentioned before, suggests that a proportion of animals with lesions compatible with bTB may not be effectively detected at the slaughterhouse, a hypothesis that if confirmed would require efforts to improve sensitivity in post-mortem inspection.

Differences in detection and laboratory confirmation probabilities observed between abattoirs could not be explained by differences in age and season of slaughter alone, as ranking of abattoirs by disclosure and confirmation probabilities did not change between crude and adjusted analyses, with correlation estimates >0.7 ($p < 0.001$) in both subpopulations. These results were in agreement with the abovementioned studies conducted in UK and Ireland despite the differences in the predictors included in the models (i.e., previous bTB history and severity of previous outbreaks in the herd, time since last breakdown, testing interval, and number of farms in which the animals lived before slaughter). Other factors not considered such as those related to inspection procedures or operation of abattoirs (line speed, light, thoroughness of the examination, expertise, interest and workload of the meat inspectors) may affect the accuracy of detection of bTB in cattle in Castilla y Leon, as suggested elsewhere (Corner et al., 1990; Shittu et al., 2013; Pascual-Linaza et al., 2017).

5. Conclusions

Abattoir surveillance plays a pivotal role in bTB eradication programs and the assessment of its efficiency and accuracy should be monitored, especially in endemic areas. Results obtained here allow estimating the overall probability of detection of bTB-like lesions (and their confirmation in the laboratory) in reactor and non-reactor animals in ante-mortem tests, what could be used as a baseline for future studies and to fully investigate the differences between slaughterhouses. Moreover, we have demonstrated the existence of large differences in the probabilities of detection of lesions between abattoirs, that could not be attributed solely to the different populations being slaughtered in

each of them. Enhanced surveillance during the post-mortem examination for cattle with certain characteristics assessed here could be applied in the Spanish eradication program framework.

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CRediT authorship contribution statement

Pilar Pozo: Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Nicolas Cespedes Cardenas:** Formal analysis, Methodology, Writing - review & editing. **Javier Bezos:** Supervision, Writing - original draft, Writing - original draft, Writing - review & editing. **Beatriz Romero:** Supervision, Writing - original draft, Writing - review & editing. **Anna Grau:** Data curation, Writing - review & editing. **Jesus Nacar:** Data curation, Writing - review & editing. **Jose Luis Saez:** Conceptualization, Funding acquisition, Writing - review & editing. **Olga Minguez:** Conceptualization, Data curation, Funding acquisition, Writing - review & editing. **Julio Alvarez:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2021.10.5307>.

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