



Assessing the variability in transmission of bovine tuberculosis within Spanish cattle herds

G. Ciaravino^{a,*}, A. García-Saenz^{a,b}, S. Cabras^{c,d}, A. Allepuz^{a,e}, J. Casal^{a,e}, I. García-Bocanegra^f, A. De Koeijer^g, S. Gubbins^h, J.L. Sáezⁱ, D. Cano-Terriza^f, S. Napp^e

^a Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain

^b ISGlobal (Barcelona Institute for Global Health - Epidemiology of Cancer), Campus MAR, Barcelona Biomedical Research Park (PRBB), 08003 Barcelona, Spain

^c Department of Statistics, Universidad Carlos III de Madrid, 28903 Getafe, Madrid, Spain

^d Department of Mathematics and Informatics, Università degli Studi di Cagliari, 09124 Cagliari, Italy

^e Centre de Recerca en Sanitat Animal (CRESA) – Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

^f Departamento de Sanidad Animal, Facultad de Veterinaria, UCO, Campus Universitarios de Rabanales, 14014 Córdoba, Spain

^g Central Veterinary Institute (CVI), Wageningen UR, Lelystad, The Netherlands

^h Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey GU24 0NF, UK

ⁱ Subdirección General de Sanidad e Higiene Animal y Trazabilidad, Dirección General de la Producción Agraria, Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, Madrid, Spain

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ABSTRACT

In Spain, despite years of efforts to eradicate bovine tuberculosis (bTB), the disease is still endemic, with some areas of high prevalence. In this context, the surveillance and control plans may need to be re-evaluated, and understanding the dynamics of bTB spread within Spanish herds may help to develop new strategies for reducing the time for detection of infected herds and for the elimination of bTB from the herds already infected. Here, we developed a compartmental stochastic model to simulate bTB within-herd transmission, fed it with epidemiological data from 22 herds (obtained from a previous work) and carried out parameter inference using Approximate Bayesian Computing methods. We also estimated the “Within-herd transmission potential Number” (R_h), i.e. the average number of secondary cases generated by a single animal infected introduced into a totally susceptible herd, considering different scenarios depending on the frequency of controls. The median global values obtained for the transmission parameters were: for the transmission coefficient (β), 0.014 newly infected animals per infectious individual per day (i.e. 5.2 per year), for the rate at which infected individuals become infectious (α), 0.01 per day (equivalent to a latent period of 97 days), and for the rate at which infected individuals become reactive to the skin test (α_1), 0.08 per day (equivalent to a period of 12 days for an infected animal to become reactive). However, the results also evidenced a great variability in the estimates of those parameters (in particular β and α) among the 22 herds. Considering a 6-month interval between tests, the mean R_h was 0.23, increasing to 0.82 with an interval of 1 year, and to 2.01 and 3.47 with testing intervals of 2 and 4 years, respectively.

1. Introduction

Bovine Tuberculosis (bTB) is defined as a chronic infectious disease of cattle (including all *Bos* species, and *Bubalus bubalis*) and bison (*Bison bison*) caused by any of the disease-causing mycobacterial species within the *Mycobacterium tuberculosis*-complex (Anon., 2013a). Cattle are mainly affected by *Mycobacterium bovis* and *Mycobacterium caprae*, which can also affect other domestic and wild animals as well as humans (Anon., 2013b; De la Rúa-Domenech et al., 2006; Aranaz et al.,

2003). Due to its zoonotic nature and the high economic impact on livestock production, the objective within EU countries is the elimination of bTB through the implementation of eradication programs (Reviriego Gordejo and Vermeersch, 2006).

In Spain, it was not until 1993 that most dairy and beef herds were included within the bTB national eradication program (Anon., 2010). According to the programme, all cattle herds are routinely screened by the single intradermal tuberculin test (SITT), testing all animals above 6 weeks of age. Private veterinarians, accredited to provide government

* Corresponding author.

E-mail address: giovanna.ciaravino@uab.cat (G. Ciaravino).

services, are in charge of performing the tests, which are usually carried out annually, although the frequency may be increased depending on the prevalence in the area. Positive cattle (reactors) are slaughtered and subjected to *post-mortem* examination at the slaughterhouses. Positivity is confirmed by culture of the mycobacteria. Other measures include passive surveillance for bTB lesions at the slaughterhouses. Thanks to the application of the national eradication program in cattle, bTB herd prevalence in Spain decreased from 5.90% in 1993 to 1.80% by the end of 2004 (Anon., 2015a). Afterwards, the bTB prevalence remained quite stable for over one decade (1.72% in 2014), despite the implementation of further measures such as the introduction of compulsory pre-movement tests in 2006 or the establishment of a surveillance plan for wildlife reservoirs in 2009. In 2015 there was a major setback, as bTB prevalence increased to 2.81%, similar to the levels Spain had in 2001 (Anon., 2015b). Within the country the situation is also quite heterogeneous with some regions free of bTB (e.g. the Canary Islands) or with very low prevalence (mainly the north of Spain), and others with very high prevalence, mainly central and southern Spain (e.g. herd prevalence in Andalusia in 2015 was 17.2%) (Allepuz et al., 2011; García-Saenz et al., 2014; Anon., 2015b).

Those results demonstrate the need to re-evaluate the measures currently in place if eradication is to be achieved. Understanding the dynamics of bTB spread within Spanish herds would be helpful for the design of new surveillance and control strategies that would reduce the time needed for both the detection of infected herds and the elimination of the disease from the infected herds.

Dynamic modelling of bTB has been widely applied because studying bTB spread in infected herds is hindered by the long incubation periods; and therefore models offer the opportunity to assess bTB transmission in a more cost-effective way (Brooks-Pollock et al., 2014; Conlan et al., 2012; Pérez et al., 2002). Different mathematical models have been used to describe the dynamics of bTB infection in the herd, with the purpose of estimating bTB within-herd transmission rates and evaluating the effectiveness of surveillance and control strategies (Barlow et al., 1997; Pérez et al., 2002; Álvarez et al., 2012a; Bekara et al., 2014; Brooks-Pollock et al., 2014; O'Hare et al., 2014). As a result, the bTB transmission parameters estimated are quite variable, which may be partially explained by the intrinsic variability in the transmission process, but also on factors such as the modelling approach used, the assumptions made, or the type and quality of the data used to feed models. Transmission dynamics is also influenced by the herd production type or the management practices, and therefore it is essential that parameters are obtained using data from herds that are representative of the bTB context in Spain.

In the present work, we first estimated the variability in the parameters related to bTB transmission in Spanish herds. Then, we used those parameters to simulate the average number of secondary cases caused by a single infected animal introduced into a herd, calling this “quantity” the “Within-herd transmission potential Number” (R_h).

2. Materials and methods

2.1. Selection of herds for parameter inference

In Spain, when a newly infected herd is confirmed by bacteriological culture, a veterinary officer carries out an epidemiological questionnaire, and the data is recorded in a database called BRUTUB, which is maintained by the Spanish Ministry of Agriculture, Fisheries, Food and Environment (Anon., 2010). In a previous work, Guta et al. (2014) developed a methodology to determine the most likely source of infection of bTB affected herds. Briefly: seven possible origins of infection were considered: i) residual infection; ii) purchase of cattle; iii) sharing of pastures; iv) neighbours; v) contact with domestic goats; vi) interaction with wildlife reservoirs and vii) contact with humans. Decision trees were developed for each of the different sources of infection, and a group of bTB experts assigned the probabilities for the

possible events on those decision trees. By feeding the data from a given farm (contained in the BRUTUB questionnaire) to the decision trees, the probabilities of the farm being infected by each of the seven possible sources were quantified.

For the inference of bTB transmission parameters, we selected only infected herds in which we had some certainty that the introduction of bTB into the herd had occurred through purchase of animals, by adapting the methodology developed by Guta et al. (2014). More specifically, from the herds recorded in the BRUTUB database between 2010 and 2013:

- First, we selected herds that met the criteria in relation to introduction through purchase of animals, that is: i) cattle had been purchased between the last negative control and the detection of infection in the herd of destination; ii) at least one of the purchased animals reacted positive to the SITT at the time of detection; iii) the herd of origin of cattle was subsequently confirmed as bTB infected; iv) and the same spoligotype was isolated in both herds or the same spoligotype was isolated during the previous year in the municipality of the herd of origin of introduced cattle.
- Then, from the herds selected, we excluded those that did not meet the criteria of exclusivity in relation to the introduction of bTB only through purchase of animals. It means we further excluded all herds in which the introduction of the disease through any of the other sources was possible. In order to do that, we defined some other “key events” as exclusion criteria. For example, herds with evidence of the presence of some reactor 3 years prior to the last negative control were excluded because of potential residual infection; and herds that reported some sort of contact with wildlife reservoir species were excluded because of potential infection from wildlife.

Besides, any herd with missing data that did not allow ruling out any of the possible origins was also excluded for parameter inference.

2.2. Herd data for parameter inference

On those selected herds, data available included:

- Date of purchase of animals from the herd subsequently found to be infected, i.e. the likely date of introduction of bTB into the herd.
- Date of bTB detection in the herd.

We assumed that the difference between both dates represented the time available for the spread of bTB.

- Number of animals in the herd on the date of bTB detection.

We assumed a constant population size between infection of the herd and detection.

- Number of positives on the date of bTB detection.
- Number of positives among the purchased animals. As it is estimated at the time of detection, not at the time of purchase, it actually represents the maximum number of infected animals introduced into the herd (i.e. the number of occult animals introduced is modelled as a *Uniform* distribution between 1 and the number of positives among the purchased animals).

The difference between the number of infected among the purchased animals and the total infected animals in the herd on the date of bTB detection represented the spread of the infection within the herd since the introduction of bTB.

2.3. Development of the bTB spread model

Bovine tuberculosis within-herd transmission was simulated using a

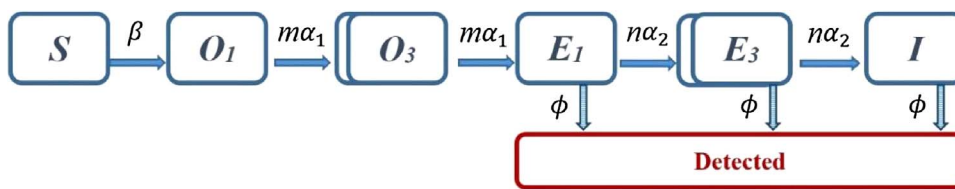


Fig. 1. Flow diagram of the compartmental stochastic SO^mE^nI (Susceptible, Occult, Exposed and Infectious) model with Erlang-distributed occult and exposed sojourn times (where $m = n = 3$), representing the dynamics of the bTB spread within the herd. Animals susceptible to *M. bovis* (S) become occult (O), infected but not detectable by SITT neither infectious, through the contact with shedding cattle at a rate β , the transmission coefficient. Occult

cattle become exposed (E), not infectious yet but detectable by SITT, at a rate α_1 . Exposed animals become infectious and detectable by SITT (I) at a rate α_2 . Exposed (E) and Infectious (I) cattle can be detected as bTB positive based on the SITT sensitivity (ϕ).

compartmental stochastic SOEI (Susceptible, Occult, Exposed and Infectious) model (Conlan et al., 2012; Barlow et al., 1997) (Fig. 1). In this model, occult animals (O) represented animals that were infected, but were not yet detectable by SITT and were not infectious either. Exposed animals (E) represented animals that were infected and were detectable by SITT, but were not infectious yet. Finally, infectious animals (I) represented animals that were infected, were detectable by SITT and were also infectious.

A homogeneous-mixing model with frequency-dependent (i.e. true mass-action) transmission was assumed as described in previous studies (Bekara et al., 2014; Smith et al., 2013; Álvarez et al., 2012a; Fischer et al., 2005; Pérez et al., 2002). Although herd size is known to be correlated with the persistence of the bTB (Brooks-Pollock et al., 2014) and several authors opted for density-dependent models (O'Hare et al., 2014; Barlow et al., 1997; Kao et al., 1997), recent comparison of models have demonstrated a higher predictive ability for the frequency-dependent models (Álvarez et al., 2014; Smith et al., 2013). In contrast to wildlife or human populations, in cattle holdings there is an upper limit to the number of contacts that animals may have, so it is unlike that an increase in the size of the herd would lead to an increase in animal interactions (Sánchez and Hudgens, 2015; Vynnycky and White, 2010).

Although the simplest compartmental models implicitly assumes that the sojourn time in any of the states is exponentially distributed, from a biological point of view, in some situations, the use of more flexible non-exponential residence-time distributions for latent and infectious periods may represent a reasonable alternative (Streftaris and Gibson, 2004; Wearing et al., 2005; Feng et al., 2007; Huppert and Katriel, 2013). In our study, we assumed that the occult and exposed sojourn states followed the Erlang distribution, a subset of the gamma probability density function, with integer-valued shape parameter (Ibe, 2009). The Erlang distribution, due to its properties, offers a computationally tractable way to incorporate gamma-like distributed sojourn times into a compartmental model (Lloyd, 2001; Bame et al., 2008; Yan and Feng, 2010). While this modification does not affect the development of the epidemic as such, it leads to a more flexible and reasonable representation of the occult and exposed sojourn times (Barlow et al., 1997; Lloyd, 2001; Streftaris and Gibson, 2004; Wearing et al., 2005; Feng et al., 2007; Huppert and Katriel, 2013). The Erlang distributed occult and exposed periods were introduced into the compartmental model by using a “box-car” approach, to take advantage of the so-called “linear chain trick” (Wearing et al., 2005; Feng et al., 2007; Lloyd, 2009). The O and E compartments were subdivided into m and n sequential sub-compartments, respectively. We assumed 3 sub-compartments for each state ($m = n = 3$), dubbing the model as SO^mE^nI (Fig. 1).

To ensure that the overall average times spent in the occult and exposed classes were still $1/\alpha_1$ and $1/\alpha_2$, respectively, we constructed the original single compartments as the sum of the respective sub-compartments and the transition rates between successive occult and exposed sub-compartments were defined as $m^*\alpha_1$ and $n^*\alpha_2$, respectively (Fig. 1).

Infection dynamics were modelled in continuous time (with days as units), using the Gillespie's direct algorithm (Vynnycky and White, 2010; Keeling and Rohani, 2008). At each time step transitions between compartments of the SO^mE^nI model occurred according to the following

differential equations:

$$\frac{dS}{dt} = -\frac{\beta S(t)I(t)}{N(t)}$$

$$N(t) = S(t) + O(t) + E(t) + I(t)$$

$$\frac{dO_1}{dt} = \frac{\beta S(t)I(t)}{N(t)} - m\alpha_1 O_1(t)$$

$$\frac{dO_m}{dt} = m\alpha_1 O_{m-1}(t) - m\alpha_1 O_m(t)$$

$$\frac{dE_1}{dt} = m\alpha_1 O_m(t) - n\alpha_2 E_1(t)$$

$$\frac{dE_n}{dt} = n\alpha_2 E_{n-1}(t) - n\alpha_2 E_n(t)$$

$$\frac{dI}{dt} = n\alpha_2 E_n(t)$$

where m and n represented the different sub-compartments within the occult and exposed stages, respectively. The transmission coefficient (β) is defined as the average number of individuals that are newly infected from an infectious individual per unit of time (De Jong, 1995). The parameter α_1 is defined as the rate at which infected non-detectable and non-shedding cattle (O) become reactive to the SITT (E). Thus $1/\alpha_1$, known as *occult period*, is the average time between the infection of the animal and the moment in which that animal is able to develop a (cell-mediated) immune response detectable by SITT. The parameter α_2 is defined as the rate at which infected detectable but non-shedding cattle (E) become infectious (I). The value of α_2 is obtained as:

$$\alpha_2 = \frac{1}{\left(\frac{1}{\alpha} - \frac{1}{\alpha_1}\right)}$$

where α is the rate at which infected individuals become infectious, and $1/\alpha$ is the *latent period*, i.e. the average time between infection of a cow and the moment when that animal becomes infectious.

The only way of measuring the progress of the infection within the farm is through the detection of infected animals by means of the *in-vivo* diagnostic tests (mainly SITT). As tests are not perfect, some infected animals may be missed. In fact, in the case of the SITT, there is a great deal of uncertainty about the true sensitivity of this test applied in the field (Álvarez et al., 2012b). In this study, we defined a short *occult period*, in which animals were not reactive to the cervical SITT, and then the same sensitivity (ϕ) was assumed for both exposed and infectious individuals. Consequently, the number of animals detected in the herd at any point in time can be estimated as:

$$Detected_t \sim binomial((E_t + I_t), \phi)$$

We assumed a test sensitivity (ϕ) of 94%, the median value for the SIT (cervical) reported in the comprehensive review carried out by the EFSA (EFSA-AHAW, Scientific opinion, 2012).

As purchased animals were assumed to have been subjected to pre-movement tests, the infected animals introduced into the herd were assumed to be in the occult state (O). The within-herd transmission model was built in R version 3.2.1 (R Core Team., 2015).

2.4. Parameter inference

While it is often straightforward to build models that may describe our observations, or even feed some parameters to a model to simulate an artificial data set, it is usually more difficult to estimate the parameter values that could have given rise to a given data set, i.e. carry out parameter inference (Beaumont, 2010). Because of that, some deterministic methods, mainly based on maximum-likelihood estimation, were developed for parameter estimation, but they were constrained by the stochasticity, which is an inherent part of many biological systems (Hartig et al., 2011; Toni et al., 2009). To overcome those limitations further inference methods were developed; among them, the Approximate Bayesian Computing (ABC) (Beaumont, 2010; Tavaré et al., 1997). ABC methods are based on the calculation of summary statistics for a given configuration of the parameters obtained from the stochastic simulation model. Acceptance of that configuration is based on the comparison between observed and simulated data, and that comparison enables us to obtain an approximated posterior distributions of the model parameters (Hartig et al., 2011). The simplest ABC algorithm is the ABC rejection sampler, but it has the disadvantage that the rate of acceptance may be quite low when non-informative prior distributions are used (Toni et al., 2009). Therefore, we used a random walk ABC Markov chain Monte Carlo (MCMC) algorithm (see Toni et al., 2009 for a detailed description; Marjoram et al., 2003) to generate the posterior distributions of the bTB transmission parameters (β , α , α_1 and α_2) within Spanish cattle herds. To build the posterior chains, the algorithm drew candidate samples from a proposal distribution that was normally distributed, centred at the previous state of the chain, and with standard deviations set at 0.003 for β , 0.002 for α , and 0.007 for α_1 .

The study-herds were analysed individually by running MCMC chains with 1,000,000 steps, with the posterior distributions thinned to return 10,000 samples. Therefore, we obtained 22 posterior distributions for each of the parameters estimated. ABC-MCMC simulations were assessed using the “coda” package (Plummer et al., 2006). The estimated posterior distributions of the bTB transmission parameters (β , α , α_1 and α_2) within Spanish cattle herds are summarized with their mean and quantiles, and also displayed graphically as box-and-whiskers plots. For each of the transmission parameters we also calculated a global median value (i.e., aggregated value), obtained by binding together the posteriors distributions inferred from the 22 selected Spanish cattle herds, after determining that each of the individual posterior distributions were satisfactory. Algorithms were implemented within the R environment version 3.2.1 (R Core Team., 2015).

2.4.1. Definition of prior distributions

The uncertainty of β , α , α_1 and α_2 parameters was accounted for by the use of prior distributions. Prior distributions for the different parameters, and the sources from which they were derived, are described in Table 1.

Table 1

Prior distributions for the bTB within-herd transmission model parameters, their values and the sources from which those values were derived. **Pert distribution*: a special version of the beta distribution defined by the minimum, most likely and maximum values (Vose, 2008).

Parameter	Description	Distribution	Inputs of the distribution	Source
β	Transmission coefficient	<i>uniform(min, max)</i>	Minimum = 0.0003 days ⁻¹ Maximum = 0.0276 days ⁻¹	Bekara et al. (2014) Bekara et al. (2014)
α	Rate at which infected individuals become infectious.	<i>uniform(min, max)</i>	Minimum = 0.0009 days ⁻¹ Maximum = 0.0164 days ⁻¹	Bekara et al. (2014) Bekara et al. (2014)
α_1	Rate at which infected individuals become reactive to SITT.	<i>pert*(min, most likely, max)</i>	Minimum = 1/63 days Most likely = 1/uniform(21,42) days Maximum = 1/7 days	Bekara et al. (2014) De la Rúa-Domenech et al. (2006) De la Rúa-Domenech et al. (2006) and OIE Terrestrial Manual (2012) De la Rúa-Domenech et al. (2006)

2.4.2. Optimization of the sampling algorithm

A potential disadvantage of the ABC-MCMC algorithm is that when there is a high degree of uncertainty in relation to the prior distributions, the candidate parameters sampled from those priors may be potentially very far from the posterior distribution, and the ABC-MCMC may result in low acceptance rates (Toni et al., 2009). In order to avoid that problem and optimize the sampling, we developed an algorithm that, before the initiation of the Markov chains, drew samples from the prior distributions, simulated the spread within a given herd, calculated the summary measure for that simulation and compared it with summary measure observed for that herd. Samples were drawn until the difference of those summary measures was within the tolerance limit (set at 0.1), in which case, the values sampled from the priors were accepted, and used as the values that initiated the Markov chains. That enabled us to avoid samples from priors that are too distant from posterior values.

2.4.3. Choice of the summary measure (SM)

The most obvious approach for comparing the bTB within-herd spread observed in the herds with the values simulated using the within-herd spread model, would be to use the difference in the absolute number of infected animals. However, while a difference of a few infected animals may be considered as acceptable in a large herd, the same difference may not be acceptable in a small herd. On the other hand, if we used prevalence to account for the size of the herd, while a relatively small difference in prevalence may be considered as acceptable in a small herd, the same difference may not be acceptable in a large herd (as it would represent a huge difference in the number of infected animals). Because of that, we chose a combination of absolute number of infected animals and prevalence (i.e. number of infected animals times prevalence) as the summary measure. The tolerance limit of SM was set at 0.1, which corresponds to a difference (between observed and simulated values) of 0 infected animals for herds with less than 10 animals; 1 infected animal for herds between 11 and 39 animals; 2 infected animals for herds between 40 and 90 animals; 3 infected animals for herds between 91 and 159 animals, and so on.

2.5. Estimation of the average number of secondary cases (within-herd transmission potential number, R_0)

The basic reproduction ratio (R_0) is the most extensively used parameter in epidemic theory and it is an essential tool for understanding the behaviour of infectious diseases. It is defined as the average number of secondary cases produced when a single infected individual is introduced into a fully susceptible population (Anderson and May, 1991). If $R_0 > 1$ then the disease tends to persist within that population, while if $R_0 < 1$ the disease tends to die out, and this threshold behaviour makes R_0 the most useful measure of the transmission potential of a pathogen within a population (Heffernan et al., 2005). It also allows evaluating which control measures would be most effective in reducing R_0 below one and therefore eliminating the disease

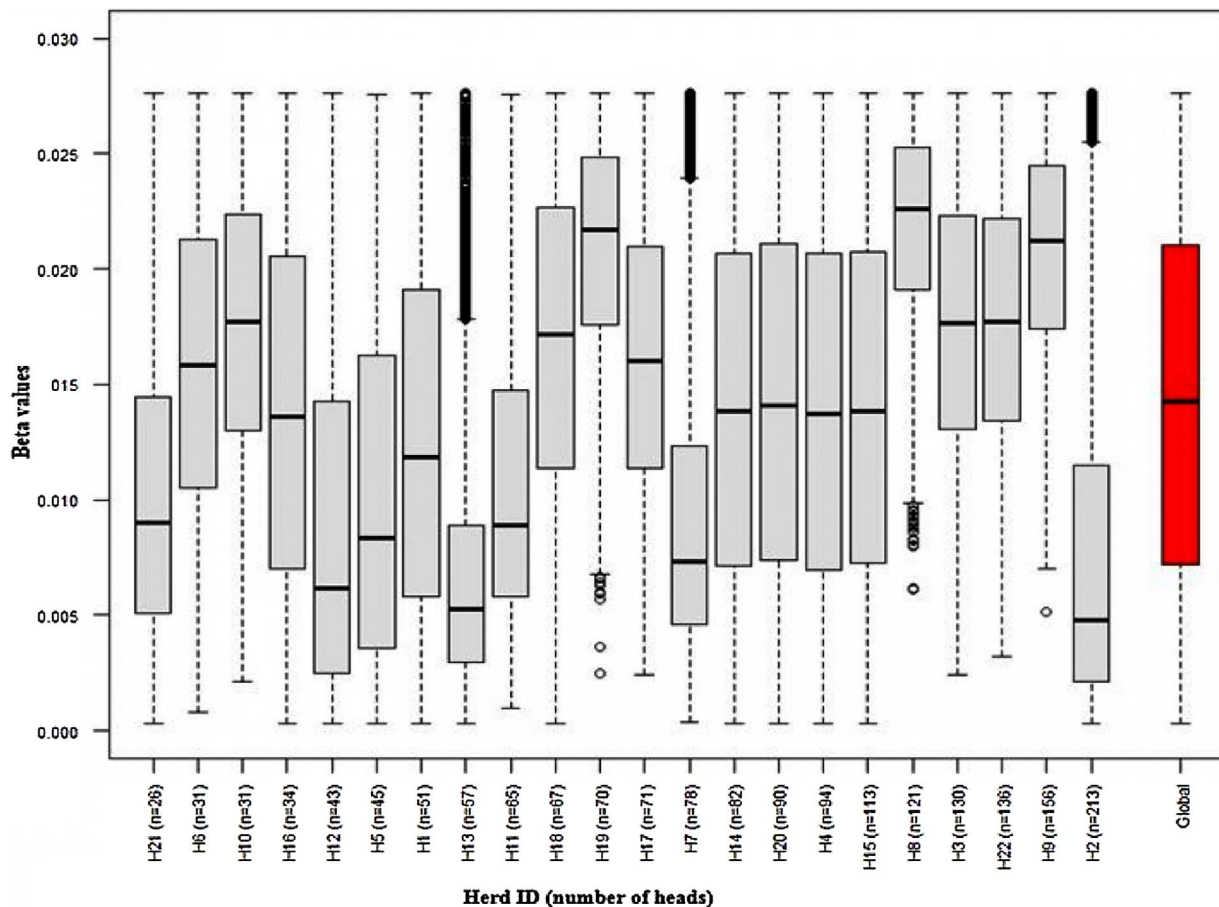


Fig. 2. Box and whisker plots summarizing the posterior distribution of the β parameter. The horizontal line inside the box represents the median value (Q50%), and the limits of the box are the lower (Q25%), and upper quartiles (Q75%). The upper and lower whiskers (the two lines extending vertically from the box) represent respectively the highest datum still within the 1.5 interquartile range (IQR) of the upper quartile and the lowest datum still within the 1.5 IQR of the lower quartile. Values higher than the upper whisker and lower than the lower whiskers are considered “outliers” and plotted as individual points. In grey: the 22 posterior distributions of the bTB transmission coefficient (β) obtained for the individual herds. The x-axis indicates the herd’s ID number, and for each herd, the corresponding herds’ size (cattle heads) is indicated in brackets; herds are ordered by its size. In red: the global β value, calculated binding together the posteriors distributions inferred from the 22 selected Spanish cattle herds.

from that population (Heffernan et al., 2005; Diekmann et al., 2010).

In our study, we used an intuitive epidemiological approach to quantify the number of secondary cases produced by the introduction of an infected animal into a totally susceptible herd, and we called this quantity the “Within-herd transmission potential Number” (R_h). In order to do that, we used the compartmental transmission model described in Section 3 to simulate bTB spread after the introduction into the herds of a single infected animal. Given that in Spain cattle are subjected to pre-movement tests, the introduced infected animal was assumed to be in the occult stage (O). By tracking down the number of new infections generated, we obtained an estimate of R_h . As once infectious, animals are considered to remain in that state for life, the number of secondary infections generated will depend on the time available for disease spread. We assumed that bTB spread within the herd until the disease was discovered by routine SITT testing. Therefore, bTB spread, and ultimately R_h , depend on the frequency of those controls.

We simulated bTB transmission within the herds considering different times for the disease to spread freely within the herd, which is equivalent to the assumption that the disease was indeed detected after those periods. The periods chosen for the simulations were related to the frequency of testing considered within the Spanish eradication program. In Spain the spatial distribution of bTB is highly heterogeneous (Allepuz et al., 2011; García-Saenz et al., 2014), and therefore, the frequency of routine testing was adapted to account for that. In general, herds are subject to one whole herd test per year. However,

within regions where the herd prevalence is below 1% (low prevalence regions), the provinces where the herd prevalence has remained below 1% for two consecutive years may reduce the frequency to one testing every two years. In contrast, within regions where the herd prevalence is above 1% (high prevalence regions), the counties where the herd prevalence is above 3% need to increase the frequency of controls to two per year. Therefore, the spread of the disease was then simulated in absence of control interventions, for fixed time periods of 90, 180, 365 and 730 days. Where for example a time period of 90 days represents the average time bTB would have to spread when routine testing are carried out twice a year.

For each of the 22 selected herds, we simulated the number of secondary infections generated by the introduction of a single occult animal using the compartmental transmission model from Section 3 with the values of the posterior distributions of bTB transmission parameters (β , α , α_1 and α_2) inferred for that herd. For each herd and each time-spread period, the model was run for 1000 iterations. For each time-spread period, the global values of R_h were obtained by combining the estimates from the 22 study-herds. We also estimated the proportion of simulations in which R_h was zero (i.e. no bTB transmission) and the proportion of simulations in which R_h was equal or higher than one (i.e. bTB transmission) for the different time-spread periods. To gain a deeper knowledge of the mechanisms of transmission, within simulations in which R_h was zero, we quantified the cases in which the infected animal, a) remained as occult, b) became exposed, or c) reached the infectious state. And within simulations in which R_h was

equal or higher than one, we calculated the proportion of cases in which a) the transmission occurred but secondarily-infected cattle did not have enough time to become infectious; and b) the transmission occurred and at least one of the secondarily-infected cattle became infectious.

3. Results

3.1. Herds selected for parameter inference

Of the 1869 bTB-infected herds recorded in the BRUTUB system between 2010 and 2013, only 22 met the inclusion and exclusion criteria (i.e. infection likely to have been caused by the purchase of an infected animal and not by other causes). The majority of holdings were located in South-Central Spain, including 13 herds in Andalusia, six in Extremadura and two in Castile La Mancha, while there was only one herd from the North of Spain, Navarre region. All the selected herds were extensive beef herds, with sizes ranging between 26 and 213 cattle heads, although the majority were small to medium size beef herds (only 27% had more than 100 cows).

3.2. bTB spread model and parameter inference

The median global value for the transmission coefficient (β) was 0.014 newly infected animals per infectious individual per day (percentiles 5 and 95 of 0.002 and 0.026, respectively) (Fig. 2, Table 2), equivalent to a median of 5.2 newly infected animals per infectious individual per year (percentiles 5 and 95 of 0.69 and 9.49, respectively). The individual median β values inferred from the 22 herds (Fig. 2) ranged between 0.005 and 0.023 (corresponding to a range of 1.8 and 8.3 newly infected animals per infectious individual per year, respectively). Further details on the estimated β posterior distributions obtained for the 22 study-herds are given in the Supplementary material (Table S1).

The median global value for α_1 (i.e. the rate at which infected non-detectable and non-shedding cattle (O) become reactive to the SITT (E)) was 0.081 per day (percentiles 5 and 95 of 0.022 and 0.137, respectively) (Table 2). Thus, the median estimate of the occult stage (i.e. the time between the infection of an animal and when it becomes detectable by SITT), $\frac{1}{\alpha_1}$, was 12 days (percentiles 5 and 95 of 7.3 and 45.5 days, respectively). Median estimates of the individual occult stage obtained from the 22 selected herds ranged between 11 and 13 days (see Supplementary material (Table S2) for the summary of the posterior α_1 distributions for each of the 22 study-herds).

The median global value for α_2 (i.e. the rate at which infected cattle reactive to the SITT but not infectious (E) yet, become infectious (I)) was 0.012 per day (percentiles 5 and 95 of 0.002 and 0.026, respectively) (Table 2). Therefore, the median estimate of the exposed stage (i.e. the time between when an infected animal becomes detectable by SITT and when that animal becomes infectious), $\frac{1}{\alpha_2}$, was 82 days (percentiles 5 and 95 of 39 and 500, respectively). Median estimates of the exposed stage obtained for each of the 22 selected herds ranged between 59 and 263 days. Further details on the posterior α_2 distributions obtained for the 22 herds are given in the Supplementary material

Table 2

Mean and quantiles obtained for the global value of the bTB transmission parameters (β , α_1 and α_2).

bTB Transmission parameter	Mean	Quantiles				
		5%	25%	50%	75%	95%
β	0.014	0.002	0.007	0.014	0.021	0.026
α	0.010	0.002	0.006	0.010	0.014	0.016
α_1	0.080	0.022	0.049	0.081	0.112	0.137
α_2	0.014	0.002	0.007	0.012	0.017	0.026

(Table S3).

The median global value for α (i.e. the rate at which infected non-detectable and non-shedding cattle (O) become infectious (I)) was 0.010 per day (percentiles 5 and 95 of 0.002 and 0.016, respectively) (Fig. 3, Table 2). Therefore, the median estimate for the latent period (i.e. the time between the infection of an animal and when it becomes infectious), $\frac{1}{\alpha}$, was 97 days (with percentiles 5 and 95 of 62 and 500, respectively). The median value for α inferred from the individual herds ranged between 0.004 and 0.014 (corresponding to 72 and 250 days, respectively) (see Fig. 3 and Supplementary material (Table S4)).

3.3. Within-herd transmission potential number for Spanish herds

Summary statistics of the distributions obtained for the global Within-herd transmission potential number (R_h) at times of 90, 180, 365 and 730 days are shown in Fig. 4. Our results indicate that when bTB was allowed spread for 90 days, the global mean value of R_h was 0.23 (percentiles 2.5 and 97.5 of 0 and 2, respectively), which increased to 0.82 (percentiles 2.5 and 97.5 of 0 and 3, respectively) when the time for spread was 180 days. The mean R_h value rose to 2.01 (percentiles 2.5 and 97.5 of 0 and 6, respectively) when the spread period was 365 days and to 3.47 (percentiles 5 and 95 of 0 and 8, respectively) when the period was 730 days. Further details on the R_h estimates obtained for each of the 22 study-herds are given in the Supplementary material (Table S5).

We also estimated the proportion of simulations in which R_h was equal to zero, equal to one, between two and four, between five and nine and equal or higher than 10 (Fig. 5), using the same times for disease spread as previously described. For disease-spread periods of 90 days, there was an 81.5% probability that R_h was equal to 0, while the probability of R_h being equal to one was 14.8%, and only in 3.7% of simulations R_h was higher than 1. For disease-spread periods of 180 days, the probability of R_h being equal to zero decreased to 49.4%, while the probability of R_h being equal to 1 was 28.5%, and in 22.1% of simulations R_h was higher than 1. When bTB was allowed spread for 365 days there was a 21.8% probability that R_h was equal to zero, a 22.1% probability that R_h was equal to one, there was a 47.5% probability for R_h being between 2 and 4, and in 8.6% of simulation R_h was higher than 4. Finally, for disease-spread periods of 730 days, the probability that R_h was equal to zero dropped to 8.1%, the probability of R_h being equal to 1 was 11.4% and there was a 50.1% probability for R_h being between 2 and 4. In 29.9% of the simulations R_h was between five and nine, and in 0.41% equal or higher than 10.

Considering 90 days for disease-spread, in 49.7% of simulations the infected animal introduced did not have enough time to become infectious, while in 15.8% of cases bTB transmission occurred, but the secondary cases did not have enough time to become infectious (Table 3). For disease-spread periods of 180 days, in 27.6% of cases the animal introduced was able to become infectious, but failed to transmit the disease; and in 25% of simulations the transmission occurred but the secondarily-infected cattle had not enough time to become infectious (Table 3). For disease-spread periods of 365 and 730 days, the probabilities that at least one of the secondarily new infected cattle became infectious were 64.6% and 86.0%, respectively (Table 3).

4. Discussion

In Spain, even though the bTB eradication program has been implemented at the national level for almost 25 years, the Officially Tuberculosis-Free (OTF) Status is far from being achieved. Given the situation, new strategies for improving the detection of infected herds and then to help to eliminate bTB from those herds, are needed, and for that, knowledge of the dynamics of bTB spread within Spanish herds is essential. However, the long time-scales associated with the disease, the lack of clinical symptoms in infected animals, the ambiguity of the mechanisms of transmission or the effect of varying control policies

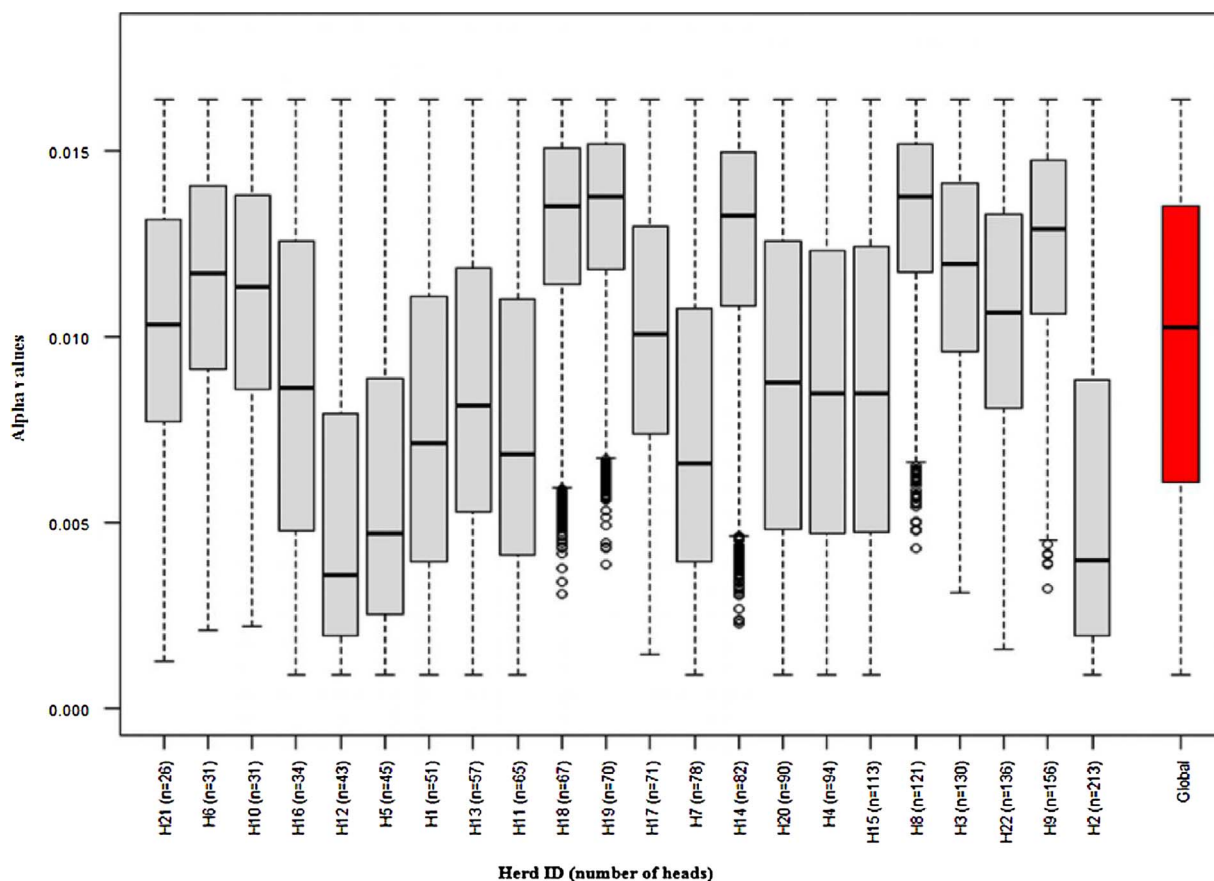


Fig. 3. Box and whisker plots summarizing the posterior distribution of the α parameter. The horizontal line inside the box represents the median value (Q50%), and the limits of the box are the lower (Q25%), and upper quartiles (Q75%). The upper and lower whiskers (the two lines outside the box) represent respectively the highest datum still within the 1.5 interquartile range (IQR) of the upper quartile and the lowest datum still within the 1.5 IQR of the lower quartile. Values higher than the upper whisker and lower than the lower whiskers are considered “outliers” and plotted as individual points. In grey: the 22 posterior distributions of the parameter α obtained for the 22 study-herds. The x-axis indicates the herd’s ID number, and for each herd, the corresponding herds’ size (cattle heads) is indicated in brackets; herds are ordered by its size. In red: the global α value, calculated binding together the posteriors distributions inferred from the 22 selected Spanish cattle herds.

complicate the study of bTB dynamics (Brooks-Pollock et al., 2014). Because of that, mathematical models have been extensively used for improving our knowledge on bTB transmission and developing evidences that can help decision-making (Álvarez et al., 2014). However, there are factors such as the type of model used and the assumptions made, or the type and quality of the data used to feed models, that have a critical impact on the values of the transmission parameters estimated, and therefore the extrapolation of the results from other studies is not recommended (Álvarez et al., 2014; Bekara et al., 2014). Within-herd transmission dynamics is also influenced by the herd production type or the management practices, and that is why it is essential that parameters are obtained using data from herds that are representative of the bTB context in Spain.

The availability and the quality of data is one of the main limitations when trying to estimate bTB transmission parameters. In fact, data obtained under experimental conditions (Neill et al., 1988, 1989; Costello et al., 1998; Dean et al., 2005) may not be representative of the infection dynamics under natural field conditions. Some authors have based their parameter estimations on data obtained from field studies, but with a low number of observations (Fischer et al., 2005; Pérez et al., 2002; Barlow et al., 1997), which may not reflect the whole complexity and variability of bTB spread among different farms. On the other hand, when local (Bekara et al., 2014; Álvarez et al., 2012a) or national-based data sets are used (O’Hare et al., 2014; Conlan et al., 2012; Kao et al., 1997), they are unlikely to contain the level of detail needed for the accurate estimation of transmission parameters. To overcome those difficulties, we took advantage of the information recorded between

2010 and 2013 in the national BRUTUB database by the Spanish Ministry of Agriculture and Fisheries, Food and Environment, that contained very detailed data of the epidemiological investigations carried out by the veterinary officers. Based on the methodology developed by Guta et al. (2014), we applied a very restrictive selection criteria for a) the inclusion of herds where we had clear evidence that bTB had been introduced through the purchase of infected animals, and b) the exclusion of herds that may have been infected by any other origin. By doing so, we ended up with 22 herds for which we had all the data we needed for the inference of the bTB transmission parameters. They were small to medium size extensive beef herds, located mainly in South-Western Spain. Those are indeed the type of herds that represent the majority of bTB-infected herds in Spain, and the location also coincides with the areas of Spain with the highest risk of infection (Allepuz et al., 2011; García-Saenz et al., 2014). Therefore, they may be considered as representative of the herds affected by bTB in Spain.

In relation to the types of models, different approaches have been used to evaluate within-herd transmission, including deterministic models (Barlow et al., 1997), though in small populations stochastic models are preferred (Vynnycky and White, 2010; Keeling and Rohani, 2008). Transmission parameters for bTB have been also calculated using modifications of the Reed-Frost model (Pérez et al., 2002; Álvarez et al., 2012a), but they imply strong assumptions, for example in relation to the duration of the latent and infectious periods. We developed a stochastic continuous-time compartmental model with gamma distributed occult and exposed period ($SO^{\text{occ}}E^{\text{m}}I$), assuming a frequency-dependent transmission, as used in the majority of bTB models, and as

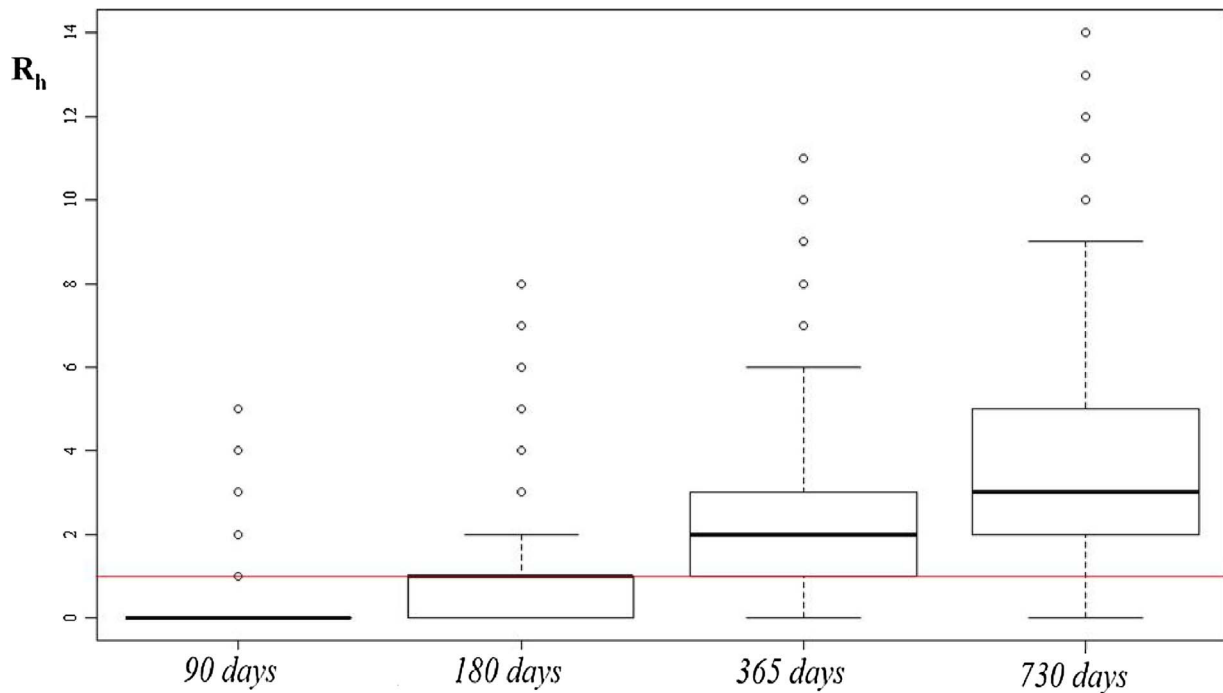


Fig. 4. Box and whisker plots summarizing the R_h estimates at times 90, 180, 365, 730 days (x-axis). For each time, the horizontal line inside the box represents the global median value (Q50%) including all the 22 herds, and the limits of the box are the lower (Q25%) and upper quartiles (Q75%). The upper and lower whiskers (the two lines extending vertically from the box) represent respectively the highest datum still within the 1.5 IQR (interquartile range) of the upper quartile and the lowest datum still within the 1.5 IQR of the lower quartile. Values higher than the upper whisker and lower than the lower whiskers are considered “outliers” and plotted as individual points. The horizontal continuous line (in red), set at the R_h point value of one, indicates that transmission occurred. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recommended by different authors (Álvarez et al., 2014; Smith et al., 2013).

In relation to parameter estimation, to avoid the limitations of deterministic methods, we used an ABC Markov chain Monte Carlo (MCMC) algorithm. As the ABC-MCMC algorithm may result in low acceptance rates when non-informative prior distributions are used, we developed an algorithm that, ensured that the values drawn from the prior distributions for the initiation of the Markov chains were not too distant from posterior values, and that enabled us to improve the computational efficiency.

For the estimation of bTB within-herd transmission parameters, we

considered that spread was only the result of the transmission from one or more infected animals introduced into the herd. Although not implicitly stated, that transmission may include not only direct, but also some sort of indirect transmission. We did not consider any external sources of infection such as wildlife reservoirs or spread from neighbouring herds, which have been included in other models (Kao et al., 1997; Brooks-Pollock et al., 2014; O’Hare et al., 2014). However, in the process of selecting the herds to be included in the study, we did exclude the possibility of infection by other sources such as wildlife reservoirs or infected neighbours.

Considering only cattle-to-cattle transmission, our median estimate

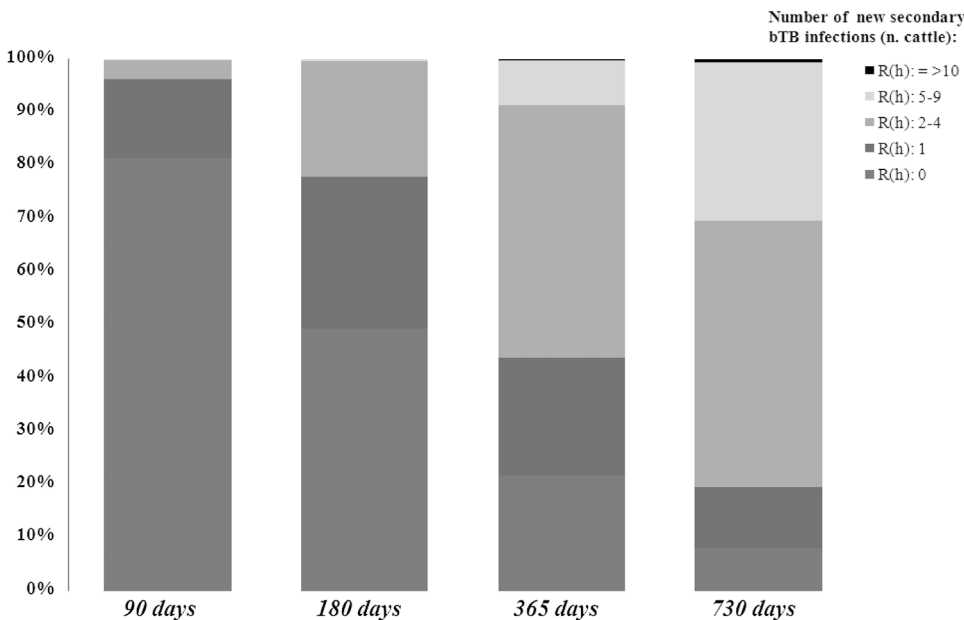


Fig. 5. Range of R_h values considering 90, 180, 365 and 730 days for disease spread (bar graphs from left to right). The average number of secondary cases generated after introducing an occult animal into a totally susceptible herd was categorized in 5 groups: R_h equal to zero, R_h equal to one, R_h ranging between two and four, between five and nine and R_h higher or equal to 10. Categories are indicated with the different gradients of grey (see legend in the figure).

Table 3

Possible events in the case of a) No bTB transmission (infected animal remains as Occult, becomes Exposed, or reaches the Infectious state); and b) bTB transmission (one Infectious animal or more than one Infectious animal).

	90 days (n. 22,000)		180 days (n. 22,000)		365 days (n. 22,000)		730 days (n. 22,000)	
	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)
No transmission, one O animal	77	0.4%	1	0.0%	0	0.0%	0	0.0%
No transmission, one E animal	10866	49.4%	4808	21.9%	1869	8.5%	547	2.5%
No transmission, one I animal	6983	31.7%	6062	27.6%	2936	13.3%	1226	5.6%
NO bTB transmission, Total	17,926	81.5%	10,871	49.4%	4805	21.8%	1773	8.1%
Transmission, one I animal only	3472	15.8%	5551	25.2%	2990	13.6%	1318	6.0%
Transmission, more I animals	602	2.7%	5578	25.4%	14205	64.6%	18909	86.0%
bTB transmission, Total	4074	18.5%	11,129	50.6%	17,195	78.2%	20,227	91.9%

of β for extensively reared beef herds in Spain, was 0.014 newly infected animals per infectious individual per day, equivalent to 5.2 per year. The median transmission coefficient (β) calculated by Álvarez et al. (2012a) for Spanish beef herds was 2.3, lower than our estimate, however when the improvements introduced in the eradication program in 2006 were taken into account, they observed an increase in the values of β for beef to 5.7, much similar to our estimate. Barlow et al. (1997), estimated a β value of 2.6 new infections per infectious animal per year, but the value was for a typical dairy herd in New Zealand (200 cattle heads in a pasture-based system). Similarly, Pérez et al. (2002) obtained a β value of 2.2 for dairy herds managed in pasture in Argentina. Bekara et al. (2014) reported a median β value of 5.16 per year during the stabling period, but only of 0.96 per year during the grazing period. Variations in the transmission coefficient (β) estimated for the different countries may be explained by differences in the model design and assumptions made, but also by differences in management practices.

Moreover, we observed a wide variation in the median estimates of β among the 22 herds included in the study, ranging between 1.8 and 8.3 newly infected animals per infectious cow per year. Although certain variability in the estimations of β is described in the literature, such extreme differences are rarely reported.

Variations in β estimates among herds do not seem to be related to the size of the herd, but may be the result of other factors such as the implementation of different herd management practices (that may help or prevent the transmission of bTB). Discrepancies in β may also be the result of factors related to individual animals. Differences in the infectiousness of the infected animals have been reported: while most individuals seem not to be very infectious, the presence of “super-spreaders” has also been described (Goodchild and Clifton-Hadley, 2001; O’Hare et al., 2014). The level of infectiousness of individual animals may reflect differences in terms of the infective dose of *M. bovis* received or in terms of the immune status of the individuals (Neill et al., 1988; Morrison et al., 2000; Menzies and Neill, 2000; Goodchild and Clifton-Hadley, 2001; Pollock and Neill, 2002). Variations in β estimates may also reflect differences in behavior and/or social ranking of infected cattle (some animals, usually those on the top of the social hierarchy, are more curious and dominant than others, increasing the probability of infection by increasing both number and intensity of contacts) (Menzies and Neill, 2000; Goodchild and Clifton-Hadley, 2001). The β parameter was by far the most influential parameter in bTB transmission within herds, and therefore the study of the factors, either related to the herd management or related to the individual animals, which influence β , deserves further attention.

Previous studies evidence a high degree of uncertainty in relation to the duration of the latent period (i.e. from the infection of an animal until it becomes infectious) (Barlow et al., 1997; Goodchild and Clifton-Hadley, 2001; Conlan et al., 2012). Even though we used weakly informative priors for the duration of the latent period (uniform: 2–36 months), we obtained a median latent period of 97 days with a narrow interquartile range (i.e. 25th and 75th percentiles (IQR), 74 and

164 days, respectively). This result is consistent with those of other models (Barlow et al., 1997; Bekara et al., 2014; O’Hare et al., 2014) and some experimental studies (Neill et al., 1991; Menzies and Neill, 2000), which described the total duration of the latent period ranging between 2 and 9 months. In contrast to other studies reporting latent periods longer than 20 months (Kao et al., 1997; Pérez et al., 2002; Smith et al., 2013), we did not obtain median values above 9 months in any of the herds evaluated. Observed variation in latency may be influenced by the intermittency of shedding, or reflect differences in factors such as the infective dose, the individual host susceptibility or environmental factors (for example housing condition or nutritional status, which may affect the level of stress of animals, which, may in turn, influences immune competence) (Menzies and Neill, 2000; Goodchild and Clifton-Hadley, 2001; Pollock and Neill, 2002).

The *in-vivo* diagnostic tests for bTB are mainly based on the detection of the cellular mediated immune (CMI) response, since it is the predominant mechanism of defence in infected cattle, and antibodies against *M. bovis* are generated only in the more advanced stages of infection (De la Rua-Domenech et al., 2006). However, there is a period between the infection of an animal and the development of a detectable cellular immune response, known as *occult* or *unreactive* period, during which infected animals test negative to the SITT (Vordermeier et al., 2004; De la Rua-Domenech et al., 2006). Even though some models did not consider this occult stage (Pérez et al., 2002; Bekara et al., 2014), we included it, because it influences our capacity to detect bTB-infected animals, and there is a lot of uncertainty about its duration. We estimated a median duration of the occult stage of 12 days (IQR: 9–21 days), with very low variability among the 22 herds studied (median values ranging between 11 and 13 days). Although slightly lower, our median estimate of the duration of the unreactive period remains in line with observations reported from experimental studies, which report a period of 3 weeks (Thom et al., 2006), and with the values estimated by Conlan et al. (2012), which calculated a mean duration of 28 days. Differences observed to values reported by Conlan et al. (2012) may be due to the assumed sensitivity of the test and the choices made on priors distribution of the model parameter.

There are numerous factors that may affect the detection of bTB infection by the tuberculin test (reviewed by De la Rua-Domenech et al., 2006), including factors related to the animal (e.g. concurrent infections, immunosuppression post-partum or nutrition deficiencies) and factors related to the test (e.g. failures of the tuberculin or errors in administration or interpretation).

In the advanced stages of bTB infection (generalisation phase), some animals may spontaneously revert to an anergic state in which they would not react to the diagnostic tests measuring the CMI response (i.e. tuberculin test and γ -Interferon test), although they would potentially be detected by tests that measure the humoral immune response (Domingo et al., 2014; Pollock and Neill, 2002). However, we did not include such a stage in our model because the mechanism of bTB-associated anergy is not well understood and the frequency of this phenomenon is unknown (Pollock and Neill, 2002). Besides, in countries

such as Spain, where eradication programs (with regular test and slaughter) have been applied for many years, anergy tends to be less frequent (García-Saenz et al., 2015).

The great variation in the values of the parameters inferred (mainly β and the parameters related to the latent period) are partially related to the variability that is to be expected in nature, but also to the uncertainty associated to them. The available information on bTB transmission parameters (β , α , α_1 , α_2) is scarce and compromised by the difficulties in their estimation, as well as the heterogeneity of the methods by which they were obtained. Therefore, further research would be essential for increasing the precision of those estimates, and ultimately, help in the decision-making process. In any case, while for some herds β and α estimates were not very informative (evidenced by wide interquartile ranges), for others (i.e., Herds 'ID 19, 8 and 9) their posterior distributions were narrower than the priors, which indicates that data provided additional information and the model allowed us to obtain more accurate estimates of those parameters.

Considering a period between two consecutive tests of 6 months (as in highly prevalent counties), which results in average period for disease spread of around 90 days, the results of our model (given the assumptions) indicate that bTB transmission would not be efficient (mean R_h value of 0.23). In fact, in more than 80% of cases transmission would not occur, and in almost half of the cases, the infected (occult) animal introduced would not even reach the infectious stage. Considering a period between two consecutive tests of 1 year (as for the majority of herds in Spain), which results in average period for disease spread of around 180 days for the spread of bTB, the results of our model indicate that while mean R_h value remains below 1 (0.82), and bTB transmission would occur in approximately half of the cases. Increasing the period between testing to 2 years (as in low-prevalence provinces), which represents an average period for disease spread of around 365 days, would result in mean values R_h clearly above 1 (2.01). In fact, in almost half of the cases R_h would reach values between 2 and 4, and in almost ten percent of cases higher than 4. Even longer periods (testing every 4 years) would result in mean R_h values of 3.47, and bTB transmission would occur in more than 90% of the cases.

Our results indicate that in Spain frequencies of routine SITT testing above below once a year would not be effective to control bTB. Even annual testing would result in bTB being transmitted in half of the cases, which would increase the probability of at least one of the infected animals not being detected and preventing the elimination of bTB from the herd. Clearance of bTB from the herds is often a lengthy process that results in serious economic burden for both the farmers and the Public Administration.

Although our estimates of R_h are not directly comparable with the R_0 estimates reported by other authors due to the differences in the modelling approach and/or the assumptions made, our findings that when the time between controls is short, the mean value of R_h/R_0 remains below 1 coincide with those of other authors. For example, for a period between tests of 6 months, we obtained a mean R_h value of 0.23, while Smith et al. (2013), under the assumption of a test-based culling strategy implemented at 3-month intervals calculated a mean R_0 estimate of 0.02. However, they also estimated that, R_0 would remain lower than 1 if testing was performed more frequently than every 4 years; and estimated a R_0 of only 4.13 without test-based culling 10 years-after the disease introduction (Smith et al., 2013). In contrast, our mean estimate of R_h was 3.5 already with testing every 4 years. On the other hand, Conlan et al. (2012) calculated median R_0 estimates of 1.5 in a herd of 30 cattle and 4.9 in a herd of 400 cattle, considering testing every 5 years; and O'Hare et al. (2014), estimated that the within-herd R_0 in Great Britain ranged between 1.3 and 1.9 for high-risk areas tested annually and between 0.6 and 1.4 for low-risk areas under quadrennial testing. The observed differences may reflect the impact of the testing frequency, herd management practices and pattern of movements according to the size of the herd and the prevalence of the area.

Even though the sensitivity of the SITT is not 100%, and therefore a small proportion of the infected animals introduced into the herd may be actually exposed or infectious, accounting for that would result in the introduction of much more uncertainty in the parameters estimated. Since only bTB-free herds are allowed to move animals, that all herds are subjected to regular controls for detection of infection, and that all purchased animals are subjected to pre-movement tests, which have very high sensitivity for exposed and infectious individuals, the assumption that only occult animals were introduced into the herds seems sensible.

Finding the right balance between the capturing the complexity of the biological processes and the computational feasibility of the model is challenging. In general, model complexity involves a trade-off between simplicity and accuracy of the model: adding complexity improves the realism of a model, but, at the same time, it can pose computational problems and instability, and make the model difficult to understand and analyse (Vynnycky and White, 2010). Here, we developed a method to estimate the variability of the transmission parameters for bTB within-herd spread using field data from the Spanish eradication campaign. The results obtained can be used to improve the strategies for both the detection of bTB in infected herds and the elimination of bTB from affected herds. This methodology could be applied for the estimation of the within-herd transmission parameters of other infectious diseases given that a limited number of inputs are available.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.epidem.2018.01.003>.

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