Received: 7 May 2021 | Revised: 6 June 2021 | Accepted: 9 June 2021

DOI: 10.1111/tbed.14186

Transboundary and Emerging Diseases

WILEY

ORIGINAL ARTICLE

The effectiveness of bovine tuberculosis surveillance in Dutch badgers

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Abstract

Countries survey wildlife for bovine tuberculosis (bTB) to ensure case detection or to ascertain a high probability of freedom from bTB in wildlife. The Eurasian badger (*Meles meles*) is a potential bTB reservoir host. Between 2008 and 2019, 282 badgers were examined post-mortem in the context of general wildlife disease and targeted bTB surveillance programmes in the Netherlands, and no bTB cases were detected. However, it was unclear how effective this surveillance effort was to demonstrate freedom from *Mycobacterium bovis* infection in the badger population of ±6000 or to detect cases if present.

Therefore, surveillance effectiveness was assessed using scenario tree modelling. For lack of standards for wildlife, the models were run against three assumed levels of disease in the population called design prevalence P^*: 0.1%, 0.5%, and 3%. A small risk of introduction (0.015/year) was applied, because the Netherlands are officially free from bTB in cattle, with rare import of bTB-infected cattle and no bTB-infected wildlife reported along the Belgian and German borders with the Netherlands. Surveillance more readily picks up bTB presence in badgers when case detection sensitivity tends towards 100% and demonstrates freedom best when the probability of freedom tends towards 100%.

For P^* 0.1%, 0.5% and 3%, respectively, maximum case detection sensitivity during 2008–2019 was 8%, 35% and 94% and the probability of freedom in 2019 was 46%, 67%, and 95%. At $P^* = 3\%$, performing targeted surveillance on 300 badgers in a year would make it extremely unlikely to miss a case (case detection sensitivity > 99.9%); and if no cases are detected, the adjusted probability of freedom would then reach nearly 98.5%. Stakeholders should be made aware that at $P^* = 3\%$, one case detected implies around 3% infected badgers. Additional surveillance system components to assess bTB in wildlife and its economics are to be explored further.

KEYWORDS
badgers, bovine tuberculosis, freedom of disease, scenario tree modelling, surveillance

1 | INTRODUCTION

Member countries of the World Organisation for Animal Health (OIE) are encouraged to implement efficient wildlife disease surveillance (Stephen et al., 2018). For diseases known to be present in wildlife in a country, the main purposes for surveillance in wildlife species will be to detect cases of the disease and to measure the level of disease in wildlife. However, when a disease is assumed not currently or normally present in the country (e.g. exotic diseases, emerging diseases), the two main purposes of surveillance in wildlife species will be to demonstrate freedom of disease in wildlife and to ensure early detection of cases in wildlife (Cameron, 2012; OIE, 2018). A probabilistic approach is used to demonstrate freedom of disease, because only part of the population can be sampled (Cameron, 2012; OIE, 2018).

The OIE specifies that the implemented wildlife disease surveillance system must be sensitive, specific and timely to be effective. It defines the sensitivity of a surveillance system for early detection as 'the probability that the system would find disease in the population if the population is infected at (or above) a specified level (design prevalence)' (OIE, 2018). The implemented surveillance system can be composed of multiple components, which can be differentiated, amongst others, into general and targeted disease surveillance system components. A general (scanning) disease surveillance system component is oriented towards detecting multiple diseases, whilst a targeted disease surveillance system component focuses on obtaining information on a specific disease (OIE, 2018). Scenario tree modelling is a tool that allows to determine the sensitivity of different surveillance system components and integrate this information with sample size to calculate the overall sensitivity of the performed surveillance for early detection of a disease not normally present in wildlife. Concurrently, if no cases are found, the model allows to calculate the probability of freedom of disease in wildlife, considering the prior probability of freedom and the probability of introduction (FAO, 2014; Martin et al., 2007).

Bovine tuberculosis (bTB) is one of the diseases for which surveillance in wildlife is relevant. bTB affects cattle and many other mammalian hosts and is caused by bacteria from the *Mycobacterium tuberculosis* complex, principally the zoonotic bacterium *M. bovis* (OIE, 2019; Palmer, 2013; Pesciaroli et al., 2014). Globally, bTB significantly impacts on human and animal health, and it affects agriculture and trade economically (WHO, 2017). Countries that have successfully controlled bTB in livestock can obtain the officially tuberculosis free (OTF) status. The presence of bTB in wildlife is an additional complication in the control of the disease in farmed animals (Fitzgerald & Kaneene, 2012; Marais et al., 2019).

Wildlife can be infected with *M. bovis* even in countries that have successfully controlled bTB in livestock and obtained the OTF status. In the Netherlands, a country that obtained the OTF status in 1999, there is currently no indication that bTB is present in wildlife. However, bTB is occasionally introduced by cattle imported into the country (Spierenburg et al., 2014), and if there is direct or indirect contact between such infected cattle and wildlife, the disease may spread from cattle to wildlife. Commingling with infected cattle is a risk factor for transmission to susceptible wildlife (Miller & Sweeney, 2013). In the Netherlands, the risk of commingling with wildlife is greater in dairy farming than in the veal fattening industry. This makes it fortunate that bTB is more rarely introduced into dairy cattle herds than into veal calf herds (four dairy herds versus 18 veal calf herds and one suckling cattle herd during 1999–2013) (de Vos et al., 2015). Imports into dairy cattle herds do sometimes result in infected secondary herds (de Vos et al., 2015). In addition to the risk of introduction of bTB into Dutch wildlife through infected livestock, there is the risk of bTB being introduced by infected translocated wildlife or infected wildlife crossing borders (Maas et al., 2016). The risk of introduction via infected wildlife may increase, because the geographical range of bTB in wildlife is expanding in Europe (Yon et al., 2019). Translocated wildlife always presents some risk, even when animals are tested, because tests used to diagnose bTB either ante-mortem or post-mortem have shortcomings (Corner et al., 2011; Maas et al., 2013). The risk of introduction via infected wildlife naturally crossing the German or Belgian borders with the Netherlands is currently low, because there is no indication that bTB is present in wildlife near the Dutch border in neighbouring countries. Belgium obtained the OTF status in 2003, and a targeted surveillance project for bTB in wildlife conducted in 2014–2017 found no evidence for bTB-infected wildlife (Linden et al., 2018; Welby et al., 2012). Germany obtained the OTF status in 1996, and even though bTB is occasionally detected in farms in north-western Germany (Menge et al., 2017), no publications were found showing recent evidence for bTB in wildlife in the direct border region with the Netherlands.

One of the wildlife species susceptible to bTB is the Eurasian badger (*Meles meles*) (Corner et al., 2012). *M. bovis*-infected badger populations have been reported in several countries in Europe, including the United Kingdom, Ireland, Spain, and France (Balseiro et al., 2011; Corner et. al., 2011; Courcier et al., 2018; Rivière et al., 2015). The species can act as a bTB reservoir (Gortázar et al. 2012), and the risk of spill-back from badgers to cattle has been documented (Donnelly and Nouvellet 2013). In the United Kingdom, the cattle-badger bTB problem has led to sustained economic losses and social tensions (Bennett, 2017). The issue has received media attention abroad, including in the Netherlands, where badger populations made a come-back from around 1200 to 1500 individuals (383 occupied km^2) in the 1980s, to around 3200 to 3700 (948 occupied km^2) in 2001, and currently an estimated 6000 badgers with widespread occurrence in suitable habitat throughout the country (Van Moll, 2005; Zoogdiervereniging, 2020). The Eurasian badger is a protected species in the Netherlands since 1947, and there are compensation schemes for the damage it inflicts. In the past century, the predominant badger framing shifted from 'Cause of damage' to 'Victim of road-traffic accidents (RTA) and habitat loss', and overall badger framings were found to be less polarized in the Netherlands than in the United Kingdom (Runhaar et al., 2015). However, more controversy could arise if bTB were to be detected in Dutch badgers (Runhaar et al., 2015).

The surveillance system for bTB in badgers in the Netherlands includes components with general and targeted disease focus. General wildlife disease surveillance is carried out since 2008 by pathological investigation of wildlife found dead, put out of suffering or more rarely and not applicable to badgers, hunted. In addition, surveillance for bTB

in badgers was targeted for a certain period (December 2012 to April 2014). No bTB cases were detected among the badgers examined, providing no evidence for bTB in badgers. However, the sensitivity of these surveillance activities to detect badger bTB cases or to demonstrate freedom of bTB was unknown.

This study therefore estimated the sensitivity of the performed surveillance activities for detecting bTB-infected badgers during 2008–2019 and the probability of freedom from bTB infection in badgers in the Netherlands at the end of 2019, using scenario tree modelling. In addition, the effect of investigating a greater but still realistic number of badgers in targeted surveillance was explored prospectively. The goal is to design surveillance that ensures ready case detection if bTB has emerged in the badger population at a level of disease corresponding to a predefined low prevalence, and at the same time ascertain a high probability of freedom from bTB in the badger population if the disease is not detected in the badgers under investigation. Both high probability of freedom prior to detection and timely detection are relevant for tailoring disease mitigation measures in the event of a point introduction into previously uninfected populations. The implications of the results for future surveillance activities and the stakeholders involved are discussed.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

No ethical approval was required because all original data were obtained from animals found dead.

2.2 | The surveillance performed for bTB in badgers during 2008–2019

In total, 290 badgers found dead were examined post-mortem for bTB infection between December 2008 and December 2019 in the Netherlands. Of these, 186 were submitted in the context of general wildlife disease surveillance. The remaining 104 were investigated as part of targeted surveillance for bTB (December 2012 to April 2014). Eight badgers (six from general surveillance and two from targeted surveillance) were too autolytic or too incomplete for proper examination and excluded from the study.

The collection of dead badgers relies on voluntary submissions by badger conservation volunteers, foresters and others. In the general wildlife disease surveillance component, the focus is on investigating carcasses from extraordinary mortality events. In badgers, this implies that suspect disease cases are examined with priority over suspect road traffic victims, because road traffic accidents are a common cause of death in badgers (di Giulio et al., 2009; Dekker et al., 2010). In contrast, in the targeted bTB surveillance component, any badger carcass suitable for post-mortem examination is investigated.

In the general surveillance component, post-mortem examination is performed using a standard protocol including histological examination of the main organs and visible lesions. In brief, the carcass is kept at 4°C until pathological examination, normally within 48 h from initial notification. After weighing, the animal is examined externally. Sex, age category (young or adult) and condition (body fat and muscle mass) are determined. Then external orifices and integument are inspected, and any abnormal exudate or lesion is recorded, including wounds, fractures or abscesses. After opening the abdominal and thoracic cavity, any abnormal finding observed in situ is recorded and bloody fluid sampled for storage at −80°C. Impression smears (Hemacolor quick stain, HemacolorR, Merk, D61 Darmstadt, Germany) are made of lung, liver, spleen and rectum content, and of any lesion suggestive of bTB in gross pathology. Ziehl-Neelsen (ZN) staining of the smears for acid-fast bacilli is performed if lesions consistent with bTB were seen, as described for badgers elsewhere (Corner *et al*., 2011). After removing the internal organs from the carcass, tissues are sampled and examined. The gross examination includes multiple incisions in lungs and mandibular, retropharyngeal and bronchial lymph nodes. Standard samples taken for histology and storage at −80°C until diagnostic testing include any tissue with lesions as well as lung, bronchial lymph node, heart, liver, spleen, kidney, brain, stomach, intestines, gonads and uterus. Tissues sampled for histology are fixed in 4% buffered formalin, embedded in paraffin, cut at 4 μm, and stained with haematoxylin and eosin (H&E staining). If a lesion suggestive of bTB in microscopy, duplicate slides are made and ZN stained. In the general surveillance component, the diagnostic procedure is conditional: lesions suggestive of bTB are examined using ZN staining for characteristic acid-fast bacilli. PCR tests (Pinsky & Banaei, 2008) and bacterial culture are performed only if acid-fast bacilli are found.

In the targeted surveillance for bTB in badgers, PCR tests and bacterial culture are performed systematically on any tissue with lesions suggestive of bTB and on multiple samples of bTB predilection tissues without visible lesions (head, thoracic and abdominal lymph node pool samples). Post-mortem examination is performed using the same standard protocol as detailed previously, with in addition the collection of these predilection tissues. The samples are stored at −80°C until PCR tests and bacterial culture are performed.

No evidence for the disease was found in any of the 282 specimens. The data of 180 badgers examined in the general surveillance component and the 102 badgers examined through the targeted surveillance were used in the retrospective models (Figure 1; Online Appendix 1).

2.3 | The two scenario trees and their input data

The sensitivity of each surveillance system component to detect bTB depends on the probability of the badgers being infected with bTB and the probability of the infected badgers being detected. Not all badgers have the same risk of being infected, and the diagnostic procedure affects the probability of detection. In the scenario tree method, the population is divided into subpopulations based on risk factors, infection status and detection probabilities (FAO, 2014; Martin et al., 2007). This is graphically represented by a scenario tree. This study comprises two surveillance components and therefore two scenario trees (Figure 2).

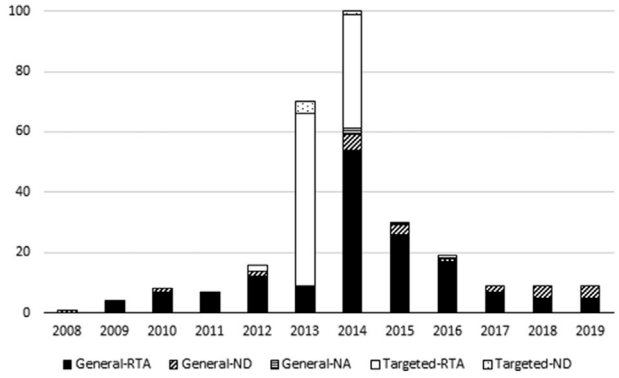

FIGURE 1 Number of badgers investigated per surveillance system component and cause of death category per year (total = 282). Abbreviations: General, general surveillance system component; NA, not available; ND, natural death; RTA, road traffic accident; Targeted, targeted surveillance system component

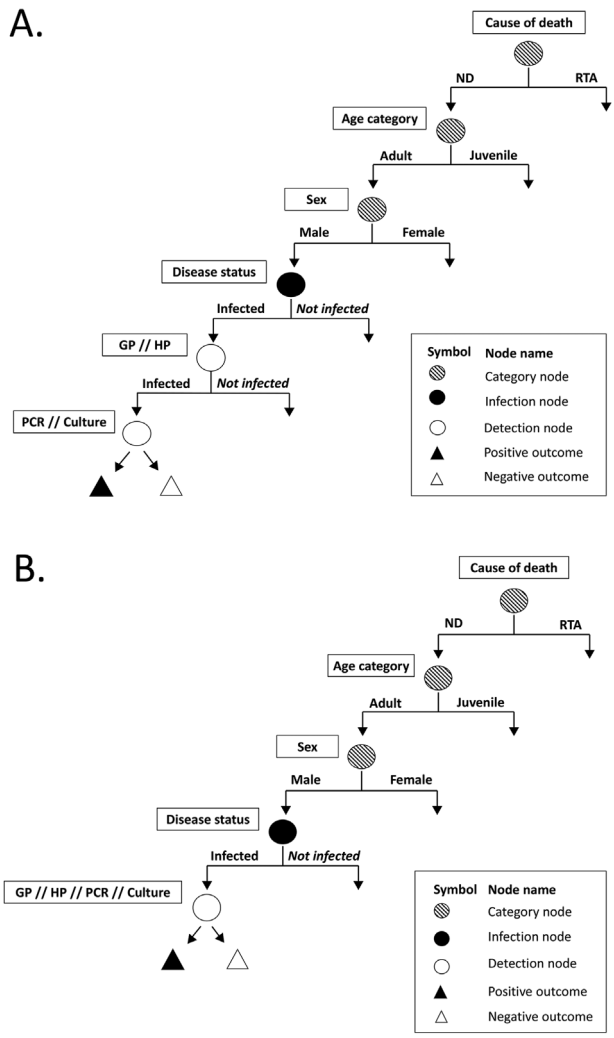

FIGURE 2 Scenario trees illustrating the two different surveillance components: (a) general wildlife disease surveillance; (b) targeted bTB surveillance in badgers. Abbreviations: GP, gross pathology; HP, histopathology; ND, natural death; PCR, PCR test; RTA, road traffic accident

Risk factors are included in the scenario tree model through risk category nodes. At each consecutive node, the population branches into subpopulations that have different risks of infection. To define the risk category nodes in the bTB badger scenario trees, a literature review was carried out using the search engines Web of Science and Pub Med, using the keywords 'bovine tuberculosis', '*Mycobacterium bovis*', 'badger', 'wildlife', 'epidemiology', 'pathology', 'surveillance' and 'scenario tree' (Online Appendix 2). This led to the identification of three risk category nodes, identical for both surveillance system components (Figure 2a, b; Table 1; Online Appendix 2). The first risk node '*cause of death*' grouped the sample population into 'natural death' (ND) and 'road traffic accident' (RTA). Some countries could have a third sub-group, 'culled badgers', which is not considered here because the Eurasian badger is a protected species in the Netherlands. The models consider that when bTB is present in the population this disease occurs more frequently in ND than in RTA badgers (Table 1; Online Appendix 2). The second risk category node '*age category*', sub-grouped the population into 'adult' (AD) and 'juvenile' (JUV; ≤ 1 year old), with a higher risk of bTB in the first (Table 1; Online Appendix 2). The third risk category node '*gender*' sub-grouped the population into 'male (M)' and 'female (F)', with a higher risk of bTB in the first (Table 1; Online Appendix 2). The relative risk and population proportions used in the calculation of the adjusted risk at each risk category node branch in each scenario were derived from the literature (Table 1; Online Appendix 2), whereas the sample proportions used for the branches of the risk category nodes were derived from the 282 badgers examined post-mortem in the Netherlands in 2008–2019 (Table 1; Online Appendix 1). Consistent with the submission criteria, ND cases were more frequent among the badgers examined in general surveillance (ND 13%, RTA 87%) than in the targeted surveillance (ND 5%, RTA 95%). This difference was significant ($p < 0.05$), and therefore the observed ND–RTA proportions were each applied in the corresponding models. The proportions of AD and JUV, as well as the proportions of M and F, did not differ significantly between general and targeted surveillance datasets, so that the proportions obtained from the whole dataset (AD 96%, JUV 4%; M 50%, F 50%) were used in both surveillance system component models.

Infection status is included in the model through the infection node. The sensitivity of surveillance is measured against a standard, an assumed level of disease in the population named design prevalence P^*, incorporated in the infection node as probability of being infected. The models were run with three different assumed levels of disease, or design prevalence (P^*) values, given the lack of an agreed international standard for the design prevalence of bTB surveillance systems in wildlife (Rivière et al., 2015). The lowest P^* (0.1%) was the design prevalence used in cattle to reach the OTF status at individual animal level. The highest P^* (3%) was the design prevalence used in a study on bTB in wildlife in France (Rivière et al., 2015). The third was an intermediate, P^* (0.5%).

Finally, detection probability is included in the model through the detection nodes. The sensitivity of the diagnostic procedures performed during surveillance will influence the probability of detection of cases. The diagnostic procedures differ for the general (Figure 2a)

TABLE 1 Deterministic model input data obtained from the data from the literature review (italics) and based on characteristics of the badgers surveyed for bTB during 2008–2019 in the Netherlands (regular font)

Level	Parameter	Value (s)	*Source (details of literature in Online Appendix 4)*
Risk node 'Cause of death'	*Population proportions ND-RTA*	*22% ND, 78% RTA*	*Cheeseman et al. (1989); Clifton-Hadley et al. (1993); Gallagher et al. (1979); Rogers et al. (1997) (bTB present).*
	Relative risk (RR) of bTB+ ND/RTA	*3.3*	*Balseiro et al. (2011); Barron et al. (2018); Clifton-Hadley et al. (1993); Courcier et al. (2018); Gallagher et al. (1979); Goodchild et al. (2012); Rogers et al. (1997)*
	Adjusted risk (AR) bTB+ ND	2.203	Calculation:(1/ ((3.3*0.22) + 0.78)) *3
	Adjusted risk (AR) bTB+ RTA	0.661	Calculation: 1/ ((3.3*0.22) + 0.78)
	Sample proportions ND-RTA (General SSC)	13% ND, 87% RTA	Dutch surveillance data (bTB absent)
	Sample proportions ND-RTA (Targeted SSC)	5% ND, 95% RTA	Dutch surveillance data (bTB absent)
Risk node 'Age category'	*Population proportions AD-JUV*	*73% AD, 27% JUV*	*Rogers et al. (1997)* (bTB present)
	Relative risk of bTB+ AD/JUV	*1.5*	*Barron et al. (2018); Gallagher and Clifton-Hadley (2000) citing Gallagher (1998) and Nolan (1991); Jenkins et al. (2008); Murphy et al. (2010); Woodroffe et al. (2009)*
	Adjusted risk (AR) bTB+ AD	1.099	Calculation: (1/ ((1.5*0.73) + 0.27)) *1.5
	Adjusted risk (AR) bTB+ JUV	0.733	Calculation: 1/ ((1.5*0.73) + 0.27)
	Sample proportions AD-JUV	96% AD, 4% JUV	Dutch surveillance data (bTB absent)
Risk node 'Sex'	*Population proportions M-F*	*40% M, 60% F*	*Rogers et al. (1997) (bTB present)*
	Relative risk (RR) of bTB+ M/F	*1.4*	*Barron et al. (2018); Cheeseman et al. (1989); Clifton-Hadley et al. (1993); Gallagher et al. (1979); Murphy et al. (2010); Réveillaud et al. (2018); Wilesmith et al. (1986)*
	Adjusted risk (AR) bTB+ M	1.207	Calculation: 1/ ((1.4*0.4) + 0.6) *1.4
	Adjusted risk (AR) bTB+ F	0.862	Calculation:1/ ((1.4*0.4) + 0.6)
	Sample proportions M-F	50% M, 50% F	Dutch surveillance data (bTB absent)
Design prevalence *P**	*P* value Cattle OTF*	*0.1%*	*OIE*
	*P** value intermediate	0.5%	This study
	P value Wildlife France*	*3.0%*	*Rivière et al. (2015)*
Sensitivity	*Sensitivity Gross Pathology (GP) (parameter underlying //-test calculations)*	*50%*	*Balseiro et al. (2011); Corner et al. (2012); Gallagher et al. (1979); Murphy et al. (2010); Payne et al. (2013); Pritchard et al. (1986); Réveillaud et al. (2018); Woodroffe et al. (2009)*
	Sensitivity Histopathology (HP) (parameter underlying //-test calculations)	*80%*	*Courcoul et al. (2014)*
	Sensitivity PCR-test (PCR) (parameter underlying //-test calculations)	*75%*	*Courcoul et al. (2014); Hénault et al. (2006)*
	Sensitivity Culture (Culture) (parameter underlying //-test calculations)	*66%*	*Courcoul et al. (2014)*
	Sensitivity GP//HP	0.9	Calculated: 1 – ((1-SeGP) * (1-SeHP))
	Sensitivity PCR//Culture	0.915	Calculated: 1 – ((1-SePCR) * (1-SeCulture))
	Sensitivity GP//HP & if suspect PCR//Culture (General SSC)	0.824	Calculated: (SeGP//HP * SePCR//Culture)
	Sensitivity GP//HP//PCR//Culture (Targeted SSC)	0.992	Calculated: 1- ((1-Se GP)* (1-SeHP) * (1-SePCR)* (1-SeCulture)

Abbreviations: AD, adult; bTB, bovine tuberculosis; F, female; JUV, juvenile; M, male; ND, natural death; OTF, officially tuberculosis free; RTA, road traffic accident; Se, sensitivity; SSC, surveillance system component.

and targeted (Figure 2b) surveillance components. To define the sensitivities of these diagnostic procedures to detect bTB in badgers, the key words used for the literature search in addition to the previous were 'Diagnosis', 'Sensitivity', 'PCR', 'Post-mortem', and 'Bacterial culture' (Online Appendix 2). The combined sensitivity considers that in the targeted surveillance component all four diagnostic tests are done in parallel, while in the general surveillance component it is the result of a serial procedure, consisting of gross pathology (GP) and histopathology (HP) performed in parallel, followed only in suspect and probable cases by PCR and culture performed in parallel (Table 1; Online Appendix 2). The models assume no false positives, or 100% specificity of the detection methods. This assumption can be made for bTB because any sample positive in culture will also be PCR-tested for *M. bovis* confirmation (Pinsky & Banaei, 2008). Positive culture samples may also be spoligotyped for epidemiology purposes (Kamerbeek et al.,1997).

2.4 | Sensitivity of the surveillance system components at animal level

Using the scenario tree model input data described in Section 2.3., the component sensitivity at animal level (Cse_u) was estimated for each value of P^*. First, deterministic scenario tree models were constructed per component in Microsoft Excel (Office 365) (Online Appendix 3; sheets 'General' and 'Targeted'). Probabilities were multiplied down each limb of the tree: the effective probability of infection (EPI) was calculated by multiplying P^* by the adjusted risks of the category node branches, and then EPI was multiplied in turn by the sample risk category proportions and diagnostic test sensitivity of the limb. The Cse_u was estimated by adding the results of the limbs that gave a positive outcome (i.e. disease is detected).

Subsequently, the same model but with stochastic simulation was constructed in R (R Core Team, 2013; Online Appendix 4, Part 1). Taking into consideration the input data confidence intervals found in other studies (Online Appendix 2), this study arbitrarily attributed intervals of 10% of the relative risk on each side of the relative risk factors, intervals of 5% on each side of the population and sample proportions, and intervals of 10% on each side of the diagnostic procedure sensitivity values except for GP sensitivity which had an interval of 30% on each side. A total of 15,000 iterations with pert distribution for the input values was performed to obtain the Cse_u confidence interval.

2.5 | Sensitivity at country level and current probability of freedom of disease

After the calculation of the component sensitivity at animal level, a retrospective analysis was conducted to estimate the sensitivity of the surveillance at country level using the three different values for design prevalence, in Microsoft Excel (Online Appendix 3, deterministic model, Sheet 'Retrospective and Prospective') and in R (Online Appendix 4, stochastic model, Part 2). The sensitivity of the surveillance at country level (surveillance system sensitivity SSSe) estimates the probability that the surveillance system would be able to detect at least one positive case, if the population is infected at 0.1%, 0.5% or 3% (P^*). In other words, SSSe is the 'case detection sensitivity'. The retrospective analysis used the badger surveillance data from 2008 to 2019 and applied 'year' as the surveillance time period. It considered the relative contributions of the two surveillance system components (the 'General' and 'Targeted') per year and a constant probability of introduction of 0.015 (1 introduction in 65 years; Online Appendix 5).

The probability of freedom can be calculated from this as the negative predictive value of the diagnostic process. At the start, because there was no bTB surveillance in badgers before 2008, it is assumed that the probability of freedom is as great as the probability of being infected (50%). This uninformed prior is also used in other studies (Calvo-Artavia et al., 2013). The final posterior adjusted probability of freedom obtained, under the assumed parameters and with the performed surveillance in 2008–2019, is considered the probability of freedom of bTB in badgers in the Netherlands on 31 December 2019.

The values obtained for 'case detection sensitivity' and the 'posterior probability of freedom' were used to assess the effectiveness of the surveillance. No standards for design prevalence and probability of freedom have been agreed upon internationally against which to measure the effectiveness of the surveillance effort for bTB in badgers or in other wildlife species. However, clearly, if bTB was emerging in the badger population, surveillance effort ought to be sufficient to detect at least one case while the prevalence is still fairly low at a high probability (well above 99% and close to 100%); and if inversely if bTB is not present in badgers in a country, surveillance effort should demonstrate a high probability of freedom, close to 100%, adjusted only for the probability of introduction during the year.

2.6 | Prospective surveillance projections

Surveillance for bTB in dead badgers is limited by the number that can be realistically obtained for investigation. Four badger field coordinators or national experts were asked to estimate the highest number of dead badgers that Dutch badger conservation networks could reasonably be expected to deliver in a year. They converged to an estimate of 300, based on field experience or reasoned as the (upper limit of) proportion of the badger population (6000 badgers) that dies annually in RTAs (15–20%) (di Giulio et al., 2009; Dekker et al., 2010) divided by four (estimating 25% suitable for post-mortem examination and deliverable). This number corresponds to 5% of the estimated badger population in the Netherlands.

The scenario tree models were then used to determine, for each of the three P^* values, the case detection sensitivity and probability of freedom of bTB that could be performed when targeted surveillance was performed continuously on 300 badgers for a decade. The same constant probability of introduction was considered as in the retrospective analysis (0.015 probability of introduction). In view of cost reduction and assuming slow spread of bTB, this analysis was repeated for cycles of targeted surveillance on 300 badgers for 1 year, followed by 2 years of general surveillance on 20 badgers (Online

TABLE 2 Component sensitivity at unit level of the two surveillance system components for three different design prevalence levels

Design prevalence P^*	Cse_u general SSC	Cse_u targeted SSC
0.001 (0.1%)	0.000794 [0.000718, 0.000875]	0.000821 [0.000751, 0.000894]
0.005 (0.5%)	0.003971 [0.003594, 0.004369]	0.004102 [0.003753, 0.004463]
0.03 (3%)	0.023840 [0. 021479, 0.026228]	0.024641 [0.022531, 0.026810]

Abbreviations: Cse_u, component sensitivity at animal level; SSC, surveillance system component.

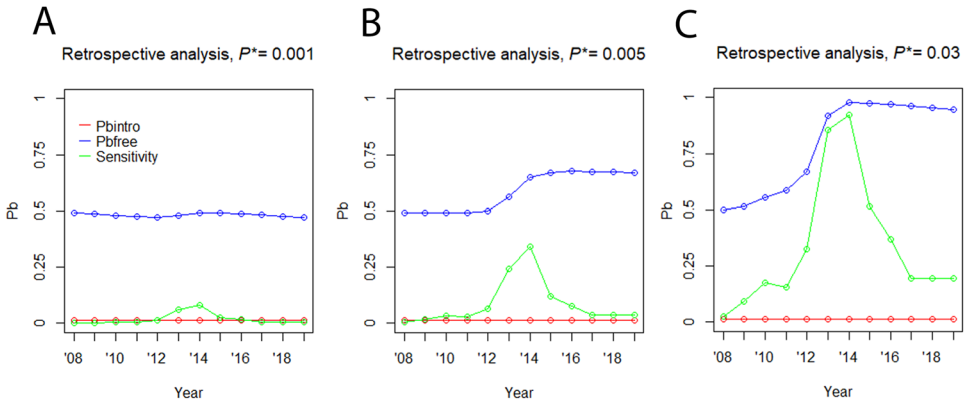

FIGURE 3 Retrospective analysis, 2008–2019. Plots of the sensitivity of the surveillance for bTB in badgers at country level and the probability of freedom of disease, considering the three different levels of design prevalence, assuming a low constant risk of disease introduction (0.015 introduction/year) and no information at the start of surveillance activities in 2008 (50% probability of freedom). Abbreviations: P^* , design prevalence; Pb , probability; Pbintro , probability of introduction; Pbfree , probability of freedom; sensitivity, surveillance system sensitivity at country level, that is case detection sensitivity

Appendix 3, Microsoft Excel deterministic model, Sheets 'Retrospective and Prospective_300cont' and 'Retrospective and Prospective_300inter'; Online Appendix 4, R stochastic model, Part 2).

3 | RESULTS

3.1 | Sensitivity of the surveillance system components at animal level

Targeted surveillance had a higher diagnostic sensitivity than the general surveillance (99% vs. 82%). However, in the general surveillance more specimens with a higher risk for bTB were examined (13% ND vs. 5% ND). The net effect of these countering features is that the Cse_u of the targeted surveillance system component was barely greater than that of the general surveillance system component (Table 2; ratio Cse_uTargeted SSC/Cse_u General SSC = 1.034). The Cse_u values obtained for each component are highly dependent on the design prevalence applied (Table 2), as to be expected (see Section 2.4).

3.2 | Surveillance sensitivity at country level and current probability of freedom of disease

The number of badgers sampled in a year impacted the annual surveillance sensitivity at country level (case detection sensitivity) and the posterior probability of freedom of disease. Regardless of the design prevalence, the lowest case detection sensitivity occurred in 2008 when only one badger was investigated, and the highest in 2014 when most badgers (n = 100) were examined. Also, the probability of freedom was boosted in the period 2012–2014, when more badgers were investigated per year (Figure 3).

The design prevalence greatly affected the effectiveness of surveillance during the period 2008–2019 (Figure 3). Considering first the 0.1% design prevalence, which corresponds to the level of disease in cattle accepted for a country to maintain the OTF status, the estimated sensitivity of the bTB badger surveillance varied among years between < 1% and 8% (Figure 3a). This indicates that, if 0.1% of the Dutch badger population was infected (corresponding to six infected out of 6000 badgers), the probability of detecting at least one of these badger bTB cases with the performed surveillance never exceeded 8%, that is, was very low throughout 2008–2019. In addition, at P^* = 0.1%, the probability of freedom of bTB in badgers declined from 50% in 2008 to 46% in 2019 (Figure 3a), because of the poor case detection sensitivity and the very small but continuous risk of disease introduction. Thus, the surveillance effort performed since 2008 did not make progress in demonstrating freedom of disease when measured against an assumed prevalence of 0.1%.

Considering the 0.5% design prevalence, the posterior probability of freedom from bTB did increase from the initial 50% in 2008 to 67% in 2019, but the probability of detecting a bTB case if 0.5% of the badger population were infected (corresponding to 30 infected out of 6000 badgers) was mostly very low and never exceeded 35% (Figure 3b).

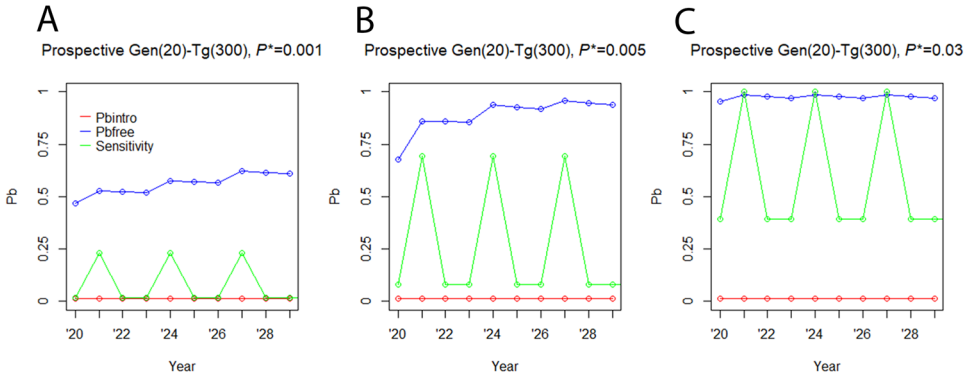

FIGURE 4 Prospective analysis, 2020–2029. Plots of the sensitivity of the surveillance for bTB in badgers at country level and the probability of freedom of disease, considering the three different levels of design prevalence, and an annual probability of bTB introduction into badgers of 0.015 (1 in 70 years), following a 3-year cycle composed of 1 year of targeted surveillance on 300 badgers followed by 2 years of general surveillance. Abbreviations: Gen, general surveillance; P^*, design prevalence; Pbintro, probability of introduction; Pbfree, probability of freedom; sensitivity, surveillance system sensitivity at country level, that is case detection sensitivity; Tg, targeted surveillance

Finally, considering the 3% design prevalence applied for bTB in wildlife in a French study (Rivière et al., 2015), surveillance sensitivity at country level had a median value of 20%, varying among years between 2% (2008) and 94% (2014) and the posterior probability of freedom from bTB in badgers in 2019 was 95% (Figure 3c). This implies that if the population were infected at 3% (corresponding to 180 infected out of 6000 badgers), the probability was quite high in 2014 (94%) that one of these 180 badger bTB cases would have been detected, and that, having not detected any cases since 2008, the surveillance effort performed is sufficient for claiming a relatively high (95% in 2019) probability of freedom of disease.

3.3 | Prospective surveillance projections

At $P^* = 0.1\%$, if targeted surveillance were performed continuously on 300 badgers a year from 2020 onwards, case detection sensitivity would be only 22%, and if no cases were detected, the probability of freedom in 2029 would raise to 85%. Under the cyclic surveillance conditions (1 year of targeted surveillance on 300 badgers alternated with 2 years of general surveillance on 20 badgers), case detection sensitivity would be either 22% or 2%, and the probability of freedom after 10 years around 59–60% (Figure 4a).

At $P^* = 0.5\%$, under conditions of sustained investigation of 300 badgers/year, the probability of freedom would reach nearly 98% in 2029 if no cases were detected, but the case detection sensitivity would be only 71%. Under the cyclic surveillance conditions, case detection sensitivity would be either 71% or 8%, and the probability of freedom after 10 years 94–96% (Figure 4b). A case detection sensitivity of 71% at $P^* = 0.5\%$ indicates that, if the disease is present, there is only 71% probability of detecting at least one case that year if 30 of 6000 badgers were infected. In other words, even in years of intensive surveillance there is still a 29% chance that a case is not detected when considering this level of bTB prevalence in the population.

Finally, at $P^* = 3\%$, under conditions of sustained investigation of 300 badgers/year, case detection sensitivity would be nearly 100% (99.943%), and if no cases were detected, the probability of freedom would maximize at nearly 98.5% (>98.499%) within 3 years; under the cyclic surveillance conditions, case detection sensitivity would be either nearly 100% or 38%, and the probability of freedom continuously above 97%, reaching nearly 98.5% (>98.498%) every third year (Figure 4c). A case detection sensitivity >99.9% at $P^* = 3\%$ indicates that, if the disease is present, that it is extremely likely at least one case would be detected if 180 of 6000 badgers were infected.

4 | DISCUSSION

To assess the effectiveness of bTB surveillance among Dutch badgers, the sensitivity of surveillance to detect cases and to demonstrate freedom from disease was estimated retrospectively and prospectively using scenario tree modelling. The retrospective analysis showed that sensitivity of the surveillance system performed in 2008–2019 was insufficient, even considering the highest design prevalence $P^* = 3\%$. There were many years in which there was a high probability that 3% infection in the badger population would have gone undetected, and case detection sensitivity was never well above 99%. However, given that no cases were found, the effort did result for $P^* = 3\%$ in a probability of freedom of 95% in 2019, which is a step forward from the uninformed prior of 50% in 2008. Thus, at $P^* = 3\%$, a much higher probability of freedom can be used as prior for the next decade of surveillance. The prospective analysis showed that the investigation of a greater but still retrievable number of dead badgers in a year (300 badgers, i.e. 5% of the badger population) could ensure with an extremely high probability (>99.9%) that cases are detected that year if present at $P^* = 3\%$, in addition to maximizing the probability of freedom at $P^* = 3\%$ when no cases are detected. However, such surveillance sensitivity could be reached only when applying the design prevalence used in a study on bTB in wildlife in France ($P^* = 3\%$) (Rivière et al., 2015), not when

applying the design prevalence used in cattle to reach the OTF status at individual animal level ($P^* = 0.1\%$), or $P^* = 0.5\%$.

Investigating 300 badgers in a year will make it almost impossible to not detect at least one case at $P^* = 3\%$, and multiple successive years of apparent absence under such surveillance conditions will be a very strong argument for a badger population free of bTB. If a case is then suddenly detected, it is most probable that the disease has been introduced into a previously bTB free badger population. For the moment, the risk of point introduction by bTB-infected cattle or wildlife appears to be greater than diffuse introduction across borders in the Netherlands (see Section 1), and probability of introduction is considered low, provided the country remains watchful around importations (Calvo-Artavia et al., 2013). Stakeholders with fear of bTB in badgers therefore need to accept that, if a case is detected under such conditions, the disease probably does not occur widespread throughout the badger population, and control measures could be implemented locally rather than country-wide when compartmentalization is feasible. Inversely, given the design prevalence $P^* = 3\%$, stakeholders in favour of badgers need to be made aware in advance that 3% of the badger population can be infected at the time of case detection (i.e. 180 infected badgers / 6,000 badgers), that is that disease control measures will have to fit at least this level of disease in the population. If the Dutch badger population is indeed currently free of bTB, and a point introduction were to occur, it is possible that the other infected badgers may be within range of the detected case. Badgers are social animals that live in small to large groups (2–27 animals), and in high-density settings, when they move it is generally only to move one or two social groups away (Rogers et al., 1998). In undisturbed high-density badger population, it has been shown that the risk of an individual becoming infected with bTB is greatest within an infected group (Vicente et al, 2007). However, in low-density settings the distance that badgers move may be greater and given that around 180 badgers could be infected at the time of first detection, the geographical area will already be rather substantial. Raising awareness among stakeholders about the surveillance conditions and keeping them annually informed of surveillance results should limit the controversy that is otherwise likely to impede control measures to prevent further spread in the event of detection of a case.

To enhance case detection sensitivity and thereby the probability of freedom, in the prospective analysis the sample size was increased (Figure 5; Section 2.5). Sample size significantly contributes to increasing the case detection sensitivity. The number of samples should be practically obtainable and economically sustainable. The first point was covered in this study, but the economics are to be explored further. Annual investigation of 300 badgers ensures the timeliest detection of cases at $P^* = 3\%$, but the alternative cycle of targeted surveillance on 300 badgers for 1 year followed by 2 years of general surveillance on 20 badgers reduces the number of badgers per 3 years by 62% (340 instead of 900). Economic analyses of surveillance alternatives should involve the stakeholders (Peyre et al., 2019).

In addition to sample size, two other parameters influence the case detection sensitivity: the diagnostic procedure and the proportion of high-risk animals in the sample (Figure 5; see Sections 2.3 and 2.4). Concerning the diagnostic procedure, the four possible diagnostic methods on dead animals are GP, HP (with ZN-staining), PCR test and culture; immunohistochemistry is to the best of our knowledge not used for routine diagnostic purposes. GP is not a sensitive diagnostic procedure in badgers because, in contrast to many other reservoir hosts, infected badgers often have lesions that are too small to be detected in GP (Corner et al., 2011). This is probably because badgers are readily infected by bTB, but resistant to disease and therefore lengthy latent infection often precedes generalized disease (Corner et al., 2011). Such latent infections are more likely to be detected in HP (with ZN staining), which should be performed on multiple lung tissue sections and lymph nodes from thorax and head (Crawshaw et al., 2008; Corner et al., 2011). The sensitivity of culture is affected by the number of tissues examined, contamination, storage conditions, incubation period and the pooling of tissues (Balseiro et al, 2011; Corner et al., 2011). Assuming all four tests are optimized, the greatest diagnostic test sensitivity is achieved when all four diagnostic methods are performed in parallel, as implemented in the targeted surveillance component (Figure 2b; Table 1).

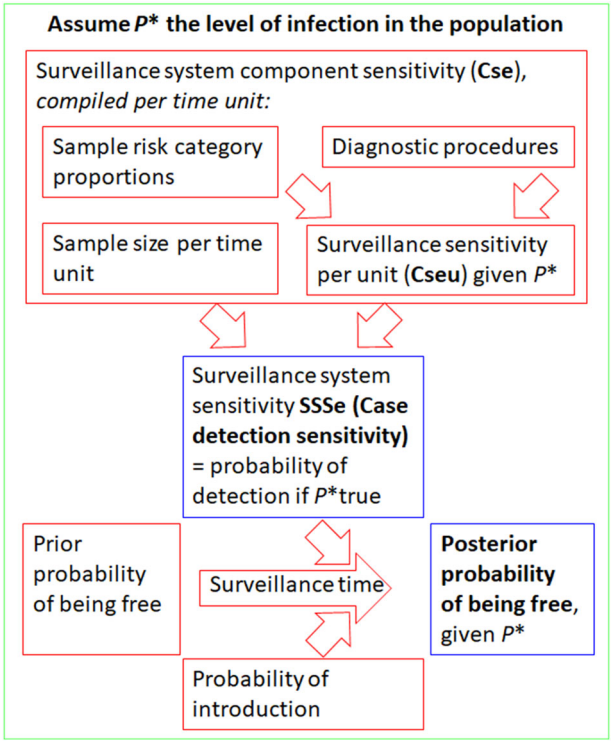

FIGURE 5 The effectiveness of the bTB badger surveillance system, measured through the surveillance sensitivity output parameters (boxes with blue outline), which depend on P^* (green outline) and the input parameters (red outline). Abbreviation: P^* = design prevalence

Badgers with a greater risk for bTB according to the literature are the so-called ND cases compared to those hit by traffic (RTA), adults compared to juveniles, and males compared to females (Table 1; Online Appendix 2). It is probable that badgers found dead in the proximity of known imported bTB cases in (dairy) cattle or wildlife have a higher bTB infection risk than those found further from these sites. However, such

a '*location*' infection risk category node was not included in the models because import case locations are not made public for privacy reasons. The greater risk in ND cases is best explained by the inclusion of deaths associated with bTB disease if present. The greater risk in adults is probably due to bTB being a chronic disease. Only rare studies found a greater bTB risk in juveniles, a discrepancy possibly owing to a difference in the proportion of adult females infected because this impacts on the likelihood of cubs being infected (Gallagher & Clifton-Hadley, 2000). The higher risk of bTB in males is possibly explained by sex-related differences in behaviour and in response to bTB infection: compared to females, males would have a wider ranging activity, greater territorial aggression, more bite wound-associated progressive bTB infections and androgenic suppression of immune response (Gallagher, 1979; Graham et al., 2013; Tomlinson et al., 2013). The majority of the badgers obtained are adults, therefore to improve the case detection sensitivity, the focus would need to be on raising the proportion of ND (as attempted in the later years, 2018 and 2019; Figure 1) and the proportion of males. This assumes that obtainable carcasses are sufficient to allow for being selective.

Potentially, improvement of the surveillance system for bTB in badgers could result from additional surveillance system components that do not rely on badgers found dead, such as environmental monitoring of *M. bovis* in badger faeces from latrines or badger sett soil (King et al. 2015; Sweeney et al., 2007) or trapping and testing of live animals (King et al. 2015; Rivière et al., 2015). However, these are not implemented to date. Also, because bTB is a multi-host pathogen, it could make sense for the surveillance system to consider multiple susceptible wildlife species rather than only badgers, as it is done in France with the Sylvatub system (Rivière et al., 2015). As part of the hunted large game food chain, all hunted deer species (*Cervus elaphus*, *Capreolus capreolus*) and wild boar (*Sus scrofa*) are examined for macroscopic lesions by trained hunters. These trained hunters can be encouraged to submit cases with bTB-like lesions. This may not be very sensitive in terms of diagnostic procedure but does have the advantage of the numbers investigated (all hunted) to increase case detection sensitivity (Rivière et al., 2015).

Scenario tree modelling in general is a valuable tool for assessing the effectiveness of surveillance activities for already recognized wildlife diseases that are exotic or emerging in a country. The model can deal with non-representative population samples and can exploit the results of surveillance activities that differ in setup and are irregular in intensity over time, as is often the case in wildlife disease surveillance. However, the model does require input data on the sensitivity of the diagnostic procedures, and preferably also on the risk factors for the disease under investigation and the relative proportions of the risk categories in the host population. Such data may not be available for the infection in the wildlife species examined. Also, it should be fair to assume that the diagnostic procedure is 100% specific. Finally, for international comparison of surveillance effectiveness, standards should be set for the design prevalence and the minimum probability of freedom. In setting these, consideration should be given to the fact that case detection sensitivity at country level may be limited in wildlife by sample availability and quality. In this study, by performing targeted surveillance on 300 dead badgers a year, the highest design prevalence ($P^* = 3\%$) was associated with a case detection sensitivity close to 100%; however, decreasing this design prevalence does not allow a high case detection sensitivity, because the parameters that increase case detection sensitivity (sample size, selection of high-risk animals, sensitivity of diagnostic procedure) cannot be stretched beyond field reality.

To conclude, this study clarified the conditions for effective bTB surveillance in badgers in the Netherlands, where the badger is a protected species comprising around 6000 animals. Plainly $P^* = 3\%$ was the only realistic value to use as design prevalence. Past surveillance efforts raised the probability of freedom in 2019 to 95% at $P^* = 3\%$; however, to optimize the probability of freedom and ensure timely case detection, the investigation of 300 badgers in a year is required. To limit the controversy around badgers, it is important that stakeholders are informed of the results and realize the implications for disease control measures in the event of detection of a case. The economics and the added value of further surveillance system components for bTB surveillance in Dutch badgers or in multiple wildlife species are to be explored further.

ACKNOWLEDGEMENTS

We thank those who contributed to the information in the dataset used. These include the submitters of the badgers, the Dutch Wildlife Health Centre pathologists M. L. Kik and J. IJzer for the post-mortem examinations, N. Buijs-Ennen for the material transfers and R. Ruuls for laboratory assistance at Wageningen Bioveterinary Research. Finally, we thank the badger experts who shared their knowledge with us and in particular Friso and Karin Derikx (Stichting Dassenwerkgroep Noord Brabant). The study received financial support from the Dutch Ministry of Agriculture, Nature and Food Quality; the Dutch Ministry of Health, Welfare and Sport; and the Faculty of Veterinary Medicine, Utrecht University. PCR tests and bacterial culture were performed under the project WOT-01-002-002.02 – Dutch Ministry of Agriculture, Nature and Food Quality.

CONFLICT OF INTEREST

The authors declare they have no competing interests.

ETHICS STATEMENT

Ethical review and approval were not required for the animal study because the animals were found dead and investigated in the context of wildlife disease surveillance programmes.

AUTHOR CONTRIBUTIONS

JMR and GvS conceived the study; MO collected and MO, JMR, AK, MLH and GvS assessed the input data; MM organized the database; MO, JMR, JvdB and GvS were involved in the modelling; MO and JMR wrote the manuscript; All authors contributed to manuscript revision, read and approved the submitted version.

DATA AVAILABILITY STATEMENT

The dataset and details of the statistical methods can be found in the Online Appendixes.

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SUPPORTING INFORMATION

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How to cite this article: Orrico, M., van Schaik, G., Koets, Ad., van den Broek, J., Montizaan, M., La Haye, M., & Rijks, J. M. (2021). The effectiveness of bovine tuberculosis surveillance in Dutch badgers. *Transbound. Emerg. Dis*, 1–13. https://doi.org/10.1111/tbed.14186