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Article

Evaluation of Surveillance for Documentation of Freedom from Bovine Tuberculosis

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Abstract: The objective was to study how surveillance for bovine tuberculosis (bTB) could be made more resource-effective in a bTB free country. A stochastic scenario tree model was developed to: (1) evaluate the sensitivity (CSe) of four surveillance system components (SSC) (*i.e.*, meat inspection of slaughtered domestic cattle, farmed deer and pigs, and tuberculin testing of adult export cattle) given that bTB would enter one of these components, (2) estimate the probability of freedom (PFree) from bTB over time, and (3) evaluate how future alternative programmes based on visual meat inspection would affect the confidence in freedom from bTB at the very low animal-level design prevalence 0.0002% and a low probabilities of introduction (1%). All, except the export cattle component reached a PFree above 96% within five years. The PFree was slightly reduced if surveillance was changed to visual inspection, e.g., PFree was reduced from 96.5% to 94.3% in the cattle component, and from 98.5% to 97.7% in the pig component after 24 years. In conclusion, visual meat inspection of pigs and cattle will only reduce the confidence in freedom from bTB slightly. However, with negligible probability of introduction (0.1%) the PFree could be maintained well above 99% in the cattle, pigs and deer components, which highlights the importance of rigid testing and quarantine procedures in trade of livestock.

Keywords: bovine tuberculosis; livestock; meat inspection; risk-based; freedom

1. Introduction

Documenting freedom from disease is an essential requirement for international trade of live animals and animal products. In accordance with the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) of the World Trade Organization (WTO), science-based evidence is needed to determine the appropriate country sanitary level for international trade [1]. Meat inspection is one way of collecting such evidence. However, meat inspection is up for discussion in several parts of the world. Currently, the European Food Safety Authority (EFSA) is evaluating which public health hazards (biological and chemical) to be covered by meat inspection for several livestock species. Moreover, EFSA suggests using indicators (also called epidemiological criteria) for these hazards in food and animals, when meat inspection procedures do not adequately address these risks. An example of such an indicator could be the housing system [2].

When meat inspection was founded more than 100 years ago, bovine tuberculosis (bTB) was endemic in most countries. Since then, many countries have managed to eradicate this infection. One example is Denmark, which has been declared free from bTB since 1980 by the European Union (EU) (Decision 2004/320/EC). The requirements for a country to be given the bTB free status according to the OIE and EU are that bTB has been found in less than 0.2% and 0.1% of the herds, respectively [3,4], hence allowing the presence of a limited number of bTB cases in the country. A single case of bTB was diagnosed in Denmark in 1988, when one cow with clinical signs was identified at meat inspection. The case was subsequently confirmed by laboratory examination, but no further animals were found bTB positive. The infection has not been observed in cattle since then despite extensive surveillance activities. Furthermore, bTB has never been found in Danish free-ranging wildlife, making the risk of introduction to livestock herds through contact with wildlife negligible [5]. In 1994, the last out of 16 cases of bTB was found in farmed deer in Denmark.

The Danish surveillance programme for bTB consists of four independent components (*i.e.*, slaughtered domestic cattle, pigs and deer as well as tuberculin testing of adult export cattle), each with their own sampling and diagnostic processes. In slaughter cattle, pigs and deer surveillance relies on *post mortem* examination by palpation and/or incision of lymph nodes. For cattle these cover the retropharyngeal, mandibular, parotid, tracheobronchial, mediastinal and mammary lymph nodes, as well as palpation of lungs. In addition, most adult cattle for export are tuberculin tested because of export requirements [5]. For pigs, incisions into the mandibular lymph nodes as well as palpations of the intestinal lymph nodes are required. The current EU meat inspection Regulation (EC) No 854/2004 [6] requires that every single bovine carcass is examined for bTB as described above. This is time-consuming, costly and not necessarily very effective for the detection of infected animals in countries with a very low prevalence of disease.

The overall objective was therefore to evaluate the surveillance system for a country that has been declared free from bTB for many years to elucidate how and where surveillance could be made more effective, using Denmark as a study case. The specific aims of this study were:

(1) to evaluate the sensitivity of each of the surveillance system components given that bTB would enter one of these components, but not necessarily spread between these due to limited contact and risk of transmission between the four components;
(2) to estimate the probability of freedom from bTB in Denmark over time, and

(3) to evaluate how future alternative programmes based on visual meat inspection and risk-based meat inspection would affect the probabilities of freedom from bTB compared to the current meat inspection system, at the very low animal-level design prevalence 0.0002% and a low probability of introduction of 1% and 0.1%.

The results are relevant for livestock industries and veterinary authorities in countries aiming at improving and modernising the meat inspection procedures, while lowering the resources spent on surveillance programme for bTB. Furthermore, the results can support international organisations such as EFSA and OIE in the decision-making processes concerning modernisation of meat inspection, and hereby ensure free trade on safe conditions.

2. Results and Discussion

2.1. Results

The mean surveillance system component (SSC) sensitivity (CSe) of the individual SSCs in the current Danish surveillance programme for bTB and the alternative scenarios, together with their 95% credibility interval, are shown in Table 1. Under the current surveillance system for bTB, the pig SSC had the highest CSe (68%), mainly due to the large average annual number of units processed (n = 20,022,790), compared to the domestic cattle (32%), deer (43%) and export cattle (7%) SSCs. The CSe were generally higher under the current meat inspection system compared to the alternative surveillance system scenarios.

Figure 1 illustrates the temporal change in the posterior mean probability of freedom from bTB in Denmark, for the period between 1995 and 2012, under the current surveillance system. A design prevalence of 0.0002% was assumed for pigs and domestic cattle. For deer and export cattle the design prevalence was one infected animal out of the number of units processed during a one-year period. The probability of introduction of bTB to the SSCs was set to 0.1% and 1%, respectively, each year in the two illustrated scenarios. With a negligible probability of introduction of 0.1% the pig SCC provided the highest posterior mean probability of freedom at 99.8% (CI: 99.8%–99.9%), compared to 99.7% (CI: 99.6%–99.7%), 99.8% (CI: 99.7%–99.8%) and 97.1% (CI: 96.1%–98.1%) for the domestic cattle, deer and export cattle SSCs, respectively, by year 2012. The posterior mean probability of freedom for the domestic cattle SSC, reached a value above 99% after the end of the sixth year of surveillance (*i.e.*, 2001) and the maximum value was reached after the fourteenth year of surveillance (*i.e.*, year 2009), compared to the second year (*i.e.*, 1997) and fourth year (*i.e.*, 1999) for the pig SSC, and the fifth year (*i.e.*, 2000) and thirteenth year (*i.e.*, 2008) for the deer SSC, while for the export cattle SSC a value above 99% was never reached (Figure 1). In other words, basing the surveillance only on export cattle would not provide a good confidence in freedom from bTB compared to the other SSCs, and therefore the benefit of this surveillance activity would be inferior, if it was going to be used as the only surveillance activity. Basing the surveillance on slaughter pigs and/or cattle was found to increase the confidence in freedom from bTB markedly over time, given that the infection would enter one of these components and spread to the level of the design prevalence.

With a low probability of introduction of 1%, the pig SCC again provided the highest posterior mean probability of freedom at 98.5% (CI: 98.0%–98.8%), compared to 96.9% (CI: 96.4%–97.2%), 97.6%

(CI: 96.7%–98%) and 87.4% (CI: 85.3%–89.5%) for the domestic cattle, deer and export cattle SSCs, respectively, by year 2012. The posterior mean probability of freedom never reached values above 98.5%.

Table 1. Results of simulations with 10,000 iterations for the sensitivities (CSe), with design prevalence and units processed for each of four surveillance system components (SSC) (*i.e.*, all slaughtered animals subjected to invasive meat inspection as well as cattle tested prior to export) in the current Danish surveillance programme for bovine tuberculosis and for four alternative meat inspection surveillance programmes involving more visual meat inspection.

Surveillance programme/SSC	Design prevalence	Units processed	CSe (95% CI)
Current surveillance system (Invasive in all slaughtered animals)			
Domestic cattle	0.000002	498,364 [a]	0.32 (0.28–0.36)
Pig	0.000002	20,022,790	0.68 (0.52–0.80)
Deer [b]	0.000125	8,000	0.43 (0.31–0.53)
Export cattle [b]	0.00038	2,634	0.07 (0.05–0.09)
Alternative scenario 1 (100% visual inspection cattle)			
Domestic cattle	0.000002	498,364	0.18 (0.15–0.20)
Alternative scenario 2 (100% visual inspection pigs)			
Pig	0.000002	20,022,790	0.43 (0.30–0.54)

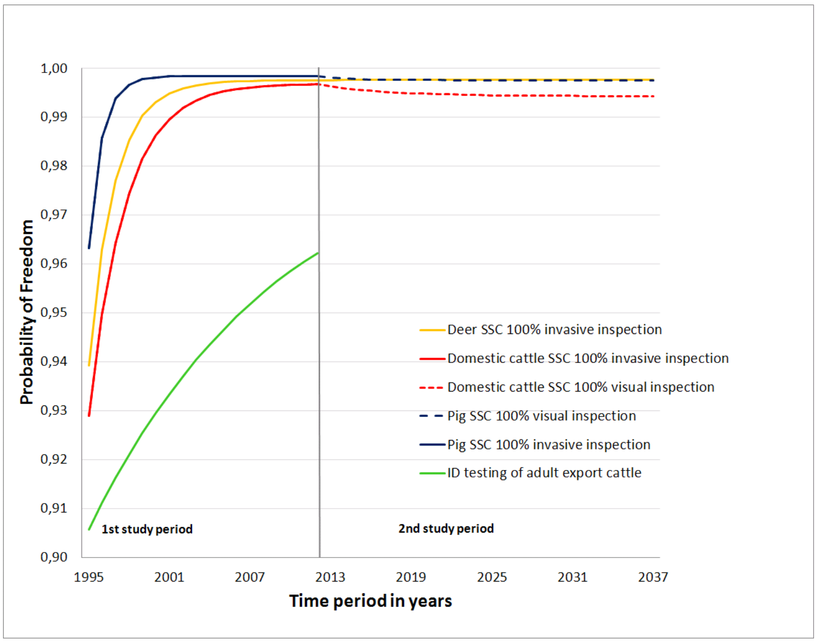
[a] Average number of cattle slaughtered between January 2004 to June 2012 in Denmark;
[b] Alternative surveillance programmes were not investigated for these SSCs.

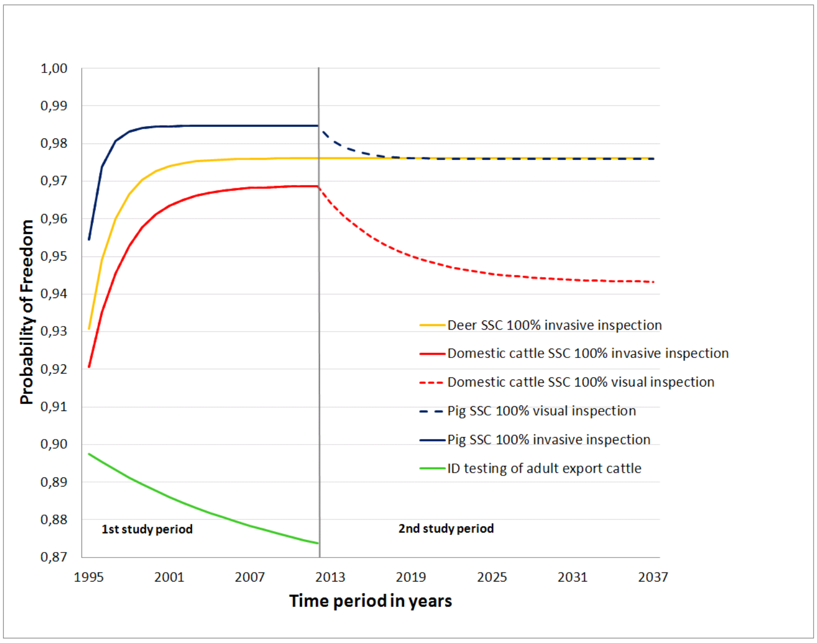
Figure 1 also shows the results of the temporal discounting evaluating the posterior mean probability of freedom from bTB in Denmark for the two alternative surveillance programmes assuming a change in the meat inspection procedure starting in the year 2013. Out of the alternative scenarios at the end of the second study period (*i.e.*, 2013–2037), Scenario 2 where all pigs would be visually inspected had higher posterior mean probability of freedom at 99.8% (CI: 99.7%–99.8%), compared to 99.4% (CI: 99.3%–99.5%) for Scenario 1 where 100% of the domestic cattle would be visually inspected. These results show that even with lower CSe for the alternative surveillance programme scenarios (Table 1) for the domestic cattle and pig SSCs, the posterior mean probability of freedom would be maintained well above 99% (Figure 1, top graph) if a negligible probability of introduction can be obtained. However, more realistically the probability of introduction is 1% and as illustrated in the bottom graph in Figure 1 this leads to posterior mean probabilities of freedom below 99% for the SSCs under the current surveillance programme and the alternative surveillance programmes. Moving to 100% visual inspection in cattle resulted in a posterior mean probability of freedom of 94.3% (CI: 93.5%–95.1%) by year 2037, and 100% visual inspection in pigs reached 97.6% (CI: 96.6%–98.1%).

The results of the scenario analyses showed that if the proportion of granulomas detected in the alternative surveillance system, based on visual inspection only, was set to 40% or 60% rather than the 50% used in the original model, no noteworthy changes were observed in the results.

If in the pig SCC the population of adults (2% of all slaughtered pigs) was inspected using the current meat inspection procedures and the rest of the pig population was only visually inspected, no noteworthy changes were observed in the results. Nor did it change the results much if the submission of detected lesions for bacteriological culture was set to 100% instead of 10%, as in the current scenario.

Figure 1. Temporal discounting, estimating the probability of freedom from bovine tuberculosis in Denmark, for the first study period (1995–2012) under the current surveillance system for the four surveillance system components and for the second study period (2013–2037) under the alternative surveillance programmes: Scenario 1 where all the domestic cattle would be subjected to visual meat inspection and Scenario 2 where all pigs would be subjected to visual meat inspection. Export cattle were tested using intradermal tuberculin test (ID). The top graph shows the results from the scenario with 0.1% and the bottom graph the scenario with 1% probability of introduction.





If the design prevalence was changed to 0.01% corresponding to the EU requirement of a herd-level design prevalence of 0.1% multiplied with an assumed animal-level prevalence within infected herds of 10%, the posterior mean probability of freedom would reach a maximum level of 99.9% at the end of the first year of surveillance and would be maintained that high throughout the two study periods.

If start probabilities of freedom in 1995 were set to 0.5 (corresponding to an uninformed prior) instead of 0.9, the posterior mean probability of freedom from bTB would reach values above 99% after the end of the fifth, tenth and thirteenth year of surveillance for the pig, deer and domestic cattle SSC. If changed to 0.7 instead of 0.9, the posterior mean probability of freedom from bTB would reach values above 99% after the end of the fourth, eighth and tenth year of surveillance for the pig, deer and domestic cattle SSC.

The most influential input parameters identified in the tornado plots for the current surveillance system and posterior probability of freedom outputs were the proportion of granulomas submitted for laboratory investigation in the pig SSC and the probability of granulomas present in young animals from the domestic cattle and pig SSC. In the case of the alternative surveillance system CSes and posterior probabilities of freedom outputs, the most influential input parameters were the proportion of granulomas present in the adult animals of the domestic cattle and pig SSC, and the proportion of granulomas submitted from the domestic cattle SSC.

2.2. Discussion

The present study has shown that a country officially recognized as being free from bTB for many years can retain a high confidence in being free, even if meat inspection procedures are changed to visually based procedures, but only if the probability of introduction is kept negligible. This underpins the importance of effective import testing and quarantine procedures when import of cattle into bTB free countries cannot be avoided. The prediction of freedom from bTB in Denmark was made using the SSCs independently, because of the lack of contact and hence, low risk of transmission of bTB between cattle, pigs and deer representing the different surveillance components. We believe that the introduction of bTB into one of the components is unlikely to lead to a spread to the other components; therefore the results are presented for each component separately. The benefit of not palpating and making incisions is that the inspector can have more time to focus on other food safety hazards (*e.g.,* faecal contamination), which might constitute a non-negligible food safety issue.

The presented results indicate that the current surveillance programme - requiring palpation and/or incisions of relevant lymph nodes of cattle, pigs and deer at meat inspection - has the highest CSe of detecting at least one slaughter animal with bTB at the design prevalence used in this study (*i.e.*, 32% for domestic cattle and 68% for pigs). This was compared to the alternative surveillance programmes based on more visual meat inspection, which all had CSe <50% (Table 1), if bTB was present at a design prevalence corresponding to 40 infected pigs in the pig SSC, and one infected animal in each of the cattle and deer SSCs. This national animal-level design prevalence is much lower than the OIE and EU requirements for a country to obtain the bTB free status. If we had used the design prevalences suggested by the OIE or EU then we would have expected to have around 300 herds with at least one positive animal before one bTB infected animal would be detected. This was considered an unacceptably high number of infected herds for a country that has been truly free for many years. Other studies have used a higher animal-level design prevalence than we did [7,8], but we find that it is appropriate to use such a low design prevalence for a slowly spreading disease compared to a fast spreading disease, as also stated by [9], in order to evaluate the ability of the system to detect the infection early, should it enter the country. For the deer SSC, the design prevalence used was higher than 0.0002% because we needed to be able to detect at least one infected deer. Therefore, the CSe appears to be higher for deer than for the domestic cattle component, but the two CSes are not directly comparable due to the different assumptions about underlying design prevalence. Still, the CSe in both SSCs indicate the probability of detecting one infected animal that goes to slaughter in each SSC. This is the lowest prevalence an infected country can have. Therefore, the used method provides very conservative estimates of the CSes and confidence in freedom. In many cases more than one animal would be infected in the SSCs, if bTB had been introduced.

Assuming a probability of 0.9 that Denmark was free of bTB at the beginning of 1995 and an annual probability of introduction of disease into the country of 0.1% and 1%, the posterior mean probability of freedom increased rapidly, except in export cattle under the 1% scenario. The sensitivity analyses showed that the posterior mean probability of freedom would be affected, if start probabilities of freedom in 1995 of 0.5 (corresponding to an uninformed prior) or 0.7 were assumed instead of 0.9. The posterior mean probability of freedom from bTB would reach values above 99% much later in time, which underpins the importance of striving to keep the bTB-free status continuously. The results indicated that it took around five years to obtain the highest obtainable confidence in freedom after bTB had been eradicated with the level of surveillance used in Denmark.

The probability of introduction of bTB into Denmark was set to 1% due to the fact that Denmark mainly imports a low number of animals and mostly from bTB free countries. Testing is performed before cattle are moved in from countries that are not officially free of bTB according to OIE criteria. The scenario with negligible probability of introduction (0.1%) was evaluated to investigate the effect of improving import biosecurity procedures such as import testing and quarantine procedures on the confidence of freedom over time. The number of import cattle was reduced from 1235 in 2009 to 170 in 2011, and a large proportion of the imported animals came from the bTB free countries Sweden and Germany. Therefore, the assumption about the probability of introduction of 1% is believed to be a valid reasonable estimate for current Danish practices, but lowering this probability through improved testing and quarantine practices would be beneficial for the level of confidence in freedom.

For the second study period, assuming a change in the current meat inspection procedures to visual inspection with a negligible probability of introduction of 0.1%, the posterior mean probability of freedom from bTB decreased slightly for the domestic cattle alternative scenario (Scenario 1) by the end of 2013 from 99.7% to 99.6%. Furthermore, the pig alternative scenario (Scenario 2) had the highest posterior mean probability of freedom by the end of year 2037 at 99.8% (Figure 1). However, we also found that if the design prevalence was changed to 0.01% corresponding to the EU requirements, the posterior mean probability of freedom would be maintained above 99.8%, at least until 2037 in all SSCs, except for the adult export cattle in which it will be maintained above 98.4%. This provides further confidence in freedom for Denmark, even if visual inspection is introduced in both the pig and cattle components. In the EU, visual inspection is going to become standard practice in pigs from June 2014 onwards, according to the EU Commission Standing Committee on the food chain and animal health (SCFCAH), whereas visual inspection of cattle is less likely to be introduced in the near future.

Overall, the level of confidence in freedom from bTB remained close to the same regardless of how large a proportion of the cattle or pigs would be subjected to visual inspection, *i.e.*, if visual inspection was only used in the 98% of the pig SSC consisting of young slaughter pigs, or 80% rather than 100% of the adult cattle. The confidence in freedom remained high, mainly due to the high probability of freedom at the starting point (90%) and low probability of introduction, but also due to a high number of inspected animals in the surveillance components. It is important to notice that visual inspection performs much better in a population with a long history of surveillance and negligible probability of introduction, compared to a population higher probability of introduction and lower prior probability of freedom from bTB.

A posterior mean probability of freedom reaching levels above 96%, under the current and alternative surveillance programme scenarios, suggests that there is a low probability of undetected bTB cases in the country. However, in the Danish case the probability of freedom is calculated for a country where there have been no bTB cases in cattle since 1995 (more than 18 years), and therefore, the design prevalence was set much lower than the international standards. This provides a higher confidence in the level of freedom, even with a change in the meat inspection procedures, compared to countries that still have a few bTB cases in the country.

Bovine tuberculosis is a notifiable disease [10,11] due to its zoonotic nature and the economic implications for livestock and international trade [12], and it is considered an unwanted infection in most countries. Each year, suspect cases of tuberculosis are found primarily in cattle and pigs in Denmark. A publicly accessible database lists the suspects and show how these are dealt with [13]. In 2009, 15 suspicions in pigs were raised by abattoirs, which had detected granulomatous lesions in lymph nodes in a number of slaughtered pigs from nine herds. The epidemiological investigation revealed that most of the farms had used non-heat-treated bedding (peat), originating from countries not free from bovine tuberculosis, for the piglets. Because of the risk of bovine tuberculosis, the farms were placed under official veterinary supervision with movement restrictions. However, laboratory analyses revealed *Mycobacterium avium* in all cases and therefore the restrictions on the farms were lifted [5]. Subsequently, the Danish agricultural industry banned the use of non-heat-treated peat at pig farms, unless specific approval is obtained - as described in the Danish Product Standard [14].

The main routes for transmission of bTB to humans are the consumption of unpasteurized milk and milk products, and direct contact through aerosols or skin cuts [10,11]. In most developed countries, bTB in humans has been eradicated [15]. Pasteurisation of milk has resulted in a substantial decline in the incidence of human cases infected with *M. bovis*. In Denmark, between zero and two human cases of bTB were identified annually between 2007 and 2011. All cases consisted either of a reactivation of childhood infection in elderly people or immigrants infected in their home countries [16]. This suggests that human infection with *M. bovis* has become an occupational hazard in developed countries. This points to a benefit in using human cases of tuberculosis in the countryside for the tracing-back of infected premises in a country believed to be free from bTB.

3. Methods

The stochastic scenario tree methodology described by Martin *et al*. (2007) [17] was used to estimate the sensitivity of each SSC and the country-level probability of freedom from bTB provided by each SSC. Historical data is used in this methodology to determine the confidence in freedom from an infection, therefore the bTB free status in our study is not based on a single year of data. Others have used this approach [18–20]. This model is based on the assumption that all final results of the surveillance system are negative [17], therefore referring to true freedom from infection as in the case of Denmark. This is different from the status defined by the OIE and EU, where bTB might be found in 0.2% and 0.1% of the herds, respectively, allowing the occurrence of bTB cases in the country [3,4].

The four components of the Danish surveillance programme for bTB were assessed separately to evaluate each their sensitivities, because of limited contact and hence low risk of transmission of bTB between the different surveillance components. The sensitivity of a SSC specifies the probability that at least one bTB test positive animal will be detected given that bTB has been introduced into the SSC and spread to the level of a predefined design prevalence (see below). The four SSCs of the current Danish surveillance programme for bTB are slaughtered domestic cattle, farmed deer and pigs as well as testing of adult cattle for export. Hence, there is no overlap between these components. All slaughtered domestic cattle, farmed deer and pigs are examined at meat inspection for clinical signs or lesions indicative of bTB, and suspect findings during meat inspection are tested by bacterial culture for confirmation. Adult cattle for export are tested with an intradermal tuberculin test (ID). If there is a suspect case of bTB, the herd is put under veterinary official supervision and all or a large proportion of the animals in the herd are tested with ID [5].

Due to very few imports of livestock into Denmark and negligible risk of infection from wildlife (*i.e.*, the two most obvious herd-level risk factors of introduction of bTB into livestock herds), we did not include herd-level risk factors in the model. The sensitivity of the component (CSe) was defined as the probability of detecting at least one bTB infected animal at a design prevalence of 0.0002% corresponding to a herd-level design prevalence of 0.2% multiplied with an animal-level design prevalence of 0.1% when inspecting or testing all units processed in the domestic cattle and pig SSCs. These two prevalence levels were stated in the OIE guidelines for a country to qualify as free from bTB [3]. However, for the deer and export cattle this design prevalence would result in less than one infected animal in those populations, therefore the design prevalence for these two SSCs was estimated as the 1/N, where N is the total number of deer and tested export cattle in the SCC in 2011, respectively. The diagnostic test sensitivity and number of units processed were also entered in the stochastic scenario tree model. The structure and contents of the scenario tree model is described in the sections below. The values for the key parameters entered in the stochastic model were estimated using data from on-going surveillance for bTB and information from the literature (Table 2).

3.1. Scenario Tree

Four scenario trees were constructed, one for each of the SSCs in the Danish bTB surveillance programme. The trees consisted of nodes dividing the population into risk groups. Within each of the risk groups the units had the same probability of detection, given the presence of disease (Figure 2). The probabilities of a positive outcome were estimated for each limb of the scenario tree by multiplication of the population proportions in the risk groups and the probabilities of infection and detection in each branch of the tree. The probabilities of detection resulting in a positive outcome (*i.e.*, an infected unit) were then combined to obtain the CSe. The scenario for the deer component differs from the cattle and pig components at the detection node section, because detailed information on lesions present, detected and submitted was not readily available, as it was for the other two components. Therefore, only one combined node for detection was used at this step in the deer SSC.

Figure 2. Conceptual scenario tree developed to evaluate the performance of the four surveillance system components (SSCs) for detection of bovine tuberculosis (bTB) at meat inspection in Denmark. $Prop_A$ and $Prop_Y$ are proportions of animals in the adult and young risk groups, respectively, and *RR* is the respective relative risk. EPI_Y is the effective probability of infection in the young animal group. *Prop_T*, *Prop_D*, *Prop_L* and *Prop_S* are the proportions of animals for each detection node according to the current invasive meat inspection procedures for bTB and its corresponding SSC.

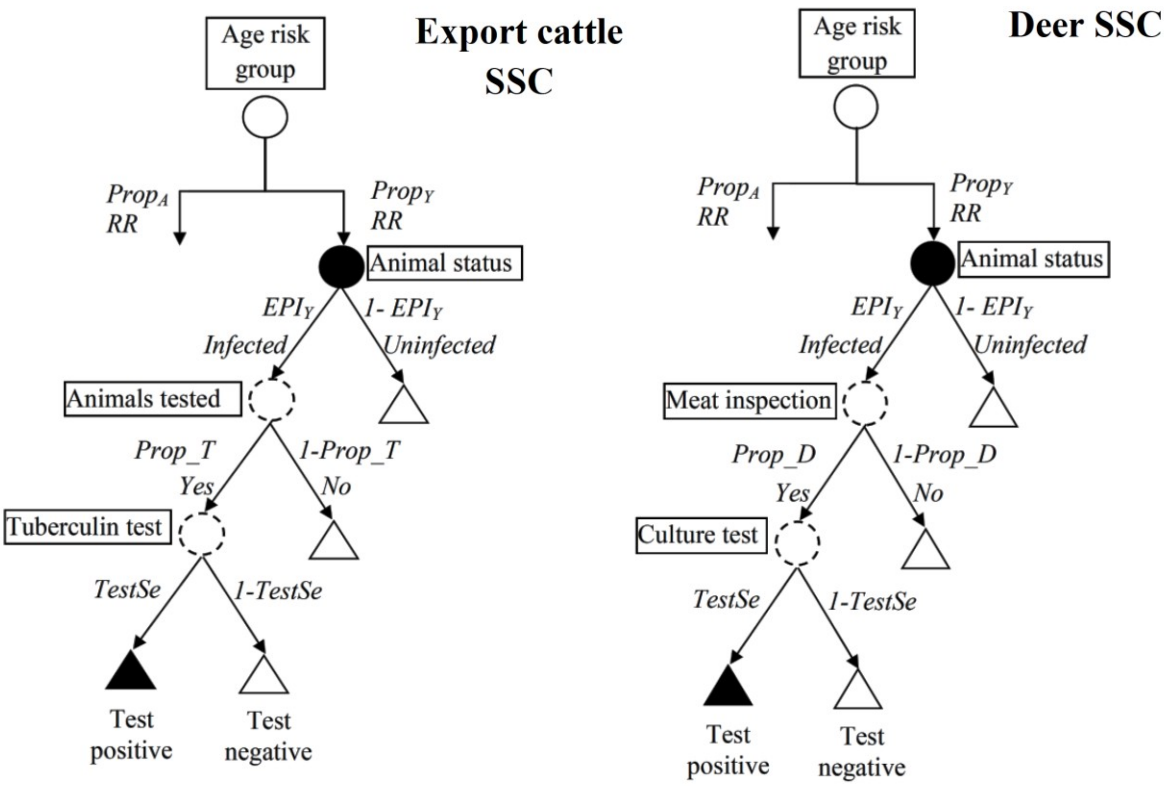
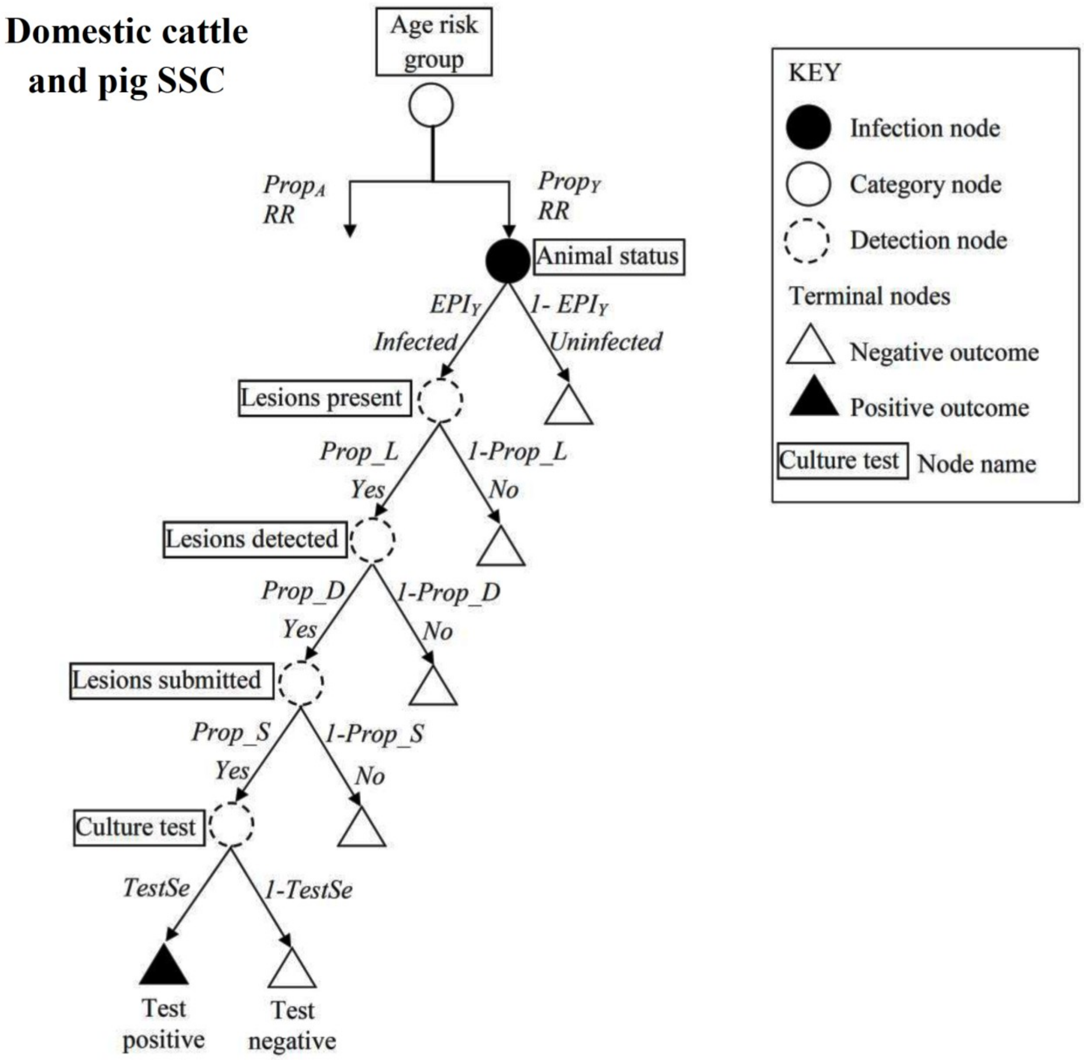


Figure 2. *Cont.*



3.2. Population and Risk Category Node

Register data were obtained from the Danish Cattle Database (DCD) for the period from January 2004 to June 2012 of all recorded cattle slaughtered in Denmark. The average number of cattle slaughtered per year during this period was calculated (n = 498,364). Data describing the approximated average population of deer (n = 8000) in 2011 and pigs (n = 20,022,790) slaughtered from 2006 to 2011 were obtained from the Danish Agriculture & Food Council. The average number of cattle exported (n = 24,655) per year from 2009 to 2011 was obtained from the Danish Knowledge Centre for Agriculture. However, only 2634 of the adult cattle exported for breeding purposes were tested using the ID test (Annual Report on Zoonoses in Denmark 2011).

The first node in the tree was a risk category node at animal level (*i.e.*, age), which divided all slaughtered cattle into two branches with proportions of young ($Prop_Y$) and adult animals ($Prop_A$), respectively. In general, a young animal was less than or equal to one year old at slaughter and an adult animal was above one year old. However, in the pig SSC, sows and boars were considered as adult animals and finisher pigs were categorized as young animals (Table 2).

Table 2. Input variables used to estimate the probability of freedom from bovine tuberculosis in Denmark represented either as fixed values or distributions, and sources of information.

Input variable		Input value and distribution	Source
Design prevalence (P_A) for domestic cattle and pigs		0.000002	Adjusted from [3]
Design prevalence (P_A) for deer		0.000125	Calculated: 1 animal out of 8000
Design prevalence (P_A) for exported cattle		0.00038	Calculated: 1 animal out of 2634
Proportion of young and adult animals *per* SSC *(PropUG)*			
Domestic cattle	Young	0.3	Danish Cattle Database (DCD)
	Adult	0.7	
Pig	Young	0.98	Danish Agriculture & Food Council
	Adult	0.02	
Deer	Young	0.9	Danish Agriculture & Food Council
	Adult	0.1	
Export cattle	Young	0.77	Danish Knowledge Centre of Agriculture, Cattle
	Adult	0.23	
Relative risk of infection between the young and adult groups *(RR)*		RiskPert (1.5, 2, 3)	[7]
Proportion of lesions present ($Prop_L$)			
	Adult animals	RiskBeta (84, 58)	[21]
	Young animals	50% of adults	
Proportion of lesions detected ($Prop_D$)			
	Current programme	RiskPert (0.9, 0.95, 0.99)	[7]
	Alternative programme	50% of the current	
Proportion of lesions submitted ($Prop_S$)			
	Pig SSC	RiskPert (0.005, 0.1, 0.15)	Expert opinion
	Other three SSC	RiskPert (0.7, 0.8, 0.9)	[7]
Meat inspection Se ($Prop_D$)		RiskPert (0.29, 0.6, 0.86)	[9,21–24]
Bacteriological culture test Se (*TestSe)*		RiskPert (0.92, 0.95, 0.98)	[7]
Intradermal tuberculin test Se (*TestSe*)		RiskPert (0.53, 0.7, 0.96)	[25]
Probability of introduction (*PIntro*)		0.001 and 0.01	Evaluation of import practices

3.3. Population and Risk Category Node

The next node in the scenario tree was the infection node, where the design prevalence (P_A) was used in the calculations to obtain the effective probabilities of infection (*EPI*) within each subpopulation defined by the previous category node. The relative risk (*RR*) of infection between the young and adult groups—irrespective of animal species—was based on [7] and modeled using a pert distribution. Then the relative risks for the adult animals compared to the young animals (Table 2), and the proportion of adult animals was used for calculation of the adjusted risk (AR) for the young animals (AR_Y), and multiplied by the *RR* to obtain the AR of the adult animals (AR_A) (Martin *et al*., 2007). Finally, the ARs were multiplied by P_A to calculate the *EPI* for each risk group (EPI_Y and EPI_A) as shown in Equation 1 and Equation 2, respectively [17].

$$EPI_Y = \left(\frac{1}{RR \times Prop_A + (1 - Prop_A)}\right) \times P_A \quad (1)$$

$$EPI_A = \left(\frac{1}{RR \times Prop_A + (1 - Prop_A)} \times RR\right) \times P_A \quad (2)$$

Where EPI_Y is the effective probability of infection for the group of young animals, EPI_A is the effective probability of infection for the adult animals, RR is the relative risk for the adult animals compared to the young animals, $Prop_A$ is the proportion of all slaughtered cattle (or pigs or deer, respectively) in the group of adult animals and P_A is the design prevalence.

3.4. Detection Nodes

The next nodes correspond to the probability of detecting bTB in each group. For the domestic cattle and pig SSC, the meat inspection was divided into three nodes:

(1) The proportion of lesions present (*i.e.*, granulomas) in the animals. This was set 50% lower for young animals than adult animals, and was modeled using a beta distribution based on an efficiency study of inspection procedures for detection of tuberculous lesions in cattle [21];
(2) The proportion of granulomas detected at meat inspection, modeled using a pert distribution based on [7];
(3) The proportion of granulomas submitted for confirmation by bacterial culture. Based on expert opinion, the proportions were set higher for domestic cattle than for pigs (Table 1), because around 90% of tuberculous lesions found in pigs are detected in the gastro-intestinal tract and according to the current meat inspection legislation, these lesions will not be submitted for confirmatory bacterial culture, as they are in the cattle abattoirs [26]. The last detection node for domestic cattle and pigs SSCs was the probability of testing positive at culture, modeled using a pert distribution (Table 2) [7].

For the export cattle SSC the detection node was the probability of testing positive to the ID test, which was also modeled using a pert distribution, based on a field trial literature review by [25]. For farmed deer SSC, the detection node was the probability of tuberculous lesions detected at meat inspection. The sensitivity of meat inspection for the farmed deer SSC was modeled using a pert distribution based on literature [9,21–24].

3.5. Component and Overall System Sensitivity Calculations

To estimate the overall CSe of each SSC, the component unit sensitivity (CSeU) was first calculated by the sum of the estimated probabilities with a positive outcome at the end of each limb (Equation 3). These probabilities were the result of the product of all branch probabilities and proportions. For the tree of the domestic cattle SSC (Figure 2) this was:

$$CSeU = \sum_{k=1}^{2} PropUG_k \times EPI_k \times Prop_L \times Prop_D \times Prop_S \times TestSe \quad (3)$$

Where $PropUG_k$ is the proportion of animals on the k risk group (*i.e.*, adult or young animals), EPI_k is the effective probability of infection for k risk group, $Prop_L$ is the proportion of lesions present in the animals to be inspected, $Prop_D$ is the proportion of lesions detected at meat inspection, $Prop_S$ is the proportion of lesions submitted for bacteriological culture test and *TestSe* is the sensitivity of the bacteriological culture test.

Once the CSeU was calculated, then the probability that one or more positive units would be detected given that the SSC was infected at the design prevalence (CSe) was estimated for each of the four SSC (Equation 4).

$$CSe = 1 - (1 - CSeU)^n \quad (4)$$

Where *CSeU* is the component unit sensitivity and *n* is the number of units (*i.e.*, animals) processed in the SSC of interest.

3.6. Probability of Freedom

To calculate the posterior probability of freedom (*PostPFree*) (Equation 5), the prior probability of infection (*PriorPInf*) is needed. In this model, the *PriorPInf* was set to 0.1 for the first year of the study period (*i.e.*, 1995), because Denmark obtained the free status of bTB in 1980. After that a lone-standing case of bTB in a cow was detected and removed in 1988, and 16 cases were found in deer between 1988 and 1994. Since then no cases of bTB have been detected so the simulations were started from year 1995. Then the *PostPFree* from bTB was calculated for the first year as:

$$PostPFree_{Tj} = \frac{(1 - PriorPInf)}{(1 - PriorPInf) + (PriorPInf \times (1 - CSe))} \quad (5)$$

The first posterior probability of infection ($PostPInf_{T1}$) was calculated as 1 − *PostPFree*, which was then adjusted for the probability of introduction (*PIntro*) of disease to the SSC in question of 0.1% and 1%, respectively (Equation 6). The adjusted probability of infection ($PostPInf_{Adj}$) was used as the *PriorPInf* for the subsequent year and to calculate the adjusted probability of freedom ($PostPFree_{Adj}$) (Equation 7); this procedure was repeated for each year of the study period. This adjusted probability of freedom is the mean probability of freedom reported in the results section (Figure 1).

$$PostPInf_{Adj} = PostPInf_{T1} + Pintro - (PostPInf_{T1} \times PIntro) \quad (6)$$

$$PostPFree_{Adj} = 1 - PostPInf_{Adj} \quad (7)$$

3.7. Simulations

The models were set up in @Risk 6 (Palisade Corporation®, Middlesex, UK) and run with 10,000 iterations. The input parameters were described by use of distributions to account for the uncertainty in the parameter estimates (Table 2). Sensitivity analysis was performed by regression analysis for each scenario, identifying significant outputs, displayed as "tornado" type charts. Scenario analysis was conducted for two variables of specific interest, *i.e.*, the probability of introduction of bTB into Denmark, the proportion of granulomas detected, submission of detected lesions, design prevalence and prior probability of freedom estimate. Two scenarios were evaluated in the scenario analysis (1) when visual inspection was only used in the 98% of the pig SSC consisting of young slaughter pigs, and (2) where 80% rather than 100% of the adult cattle were visually inspected.

3.8. Modeling of Alternative Surveillance Systems Based on Visual Inspection

A new period was simulated (*i.e.*, 2013–2037), where alternative surveillance systems were assumed to be put in place. The assumption was that the meat inspection would change from the current invasive procedures (*i.e.*, incision and palpation of lymph nodes and palpation of lungs) to a visual inspection only in all or a proportion of units processed in the SCCs. To model the alternative surveillance systems the proportion of granulomas detected was reduced by 50% in units inspected visually. This reduction was only relevant for the domestic cattle and pig SSCs, due to the descriptions of the detection nodes in section 2.3. Two alternative surveillance systems were evaluated: Scenario 1 in which all domestic cattle were inspected visually and Scenario 2 where all pigs were inspected visually.

To estimate the probability of freedom for the alternative surveillance programme, the $PostPFree_{Adj}$ estimate of the last year (2012) of the previous study period was used to calculate the *PriorPInf* for the first year of the following study period, assuming that the alternative surveillance programme started in 2013.

4. Conclusions

In conclusion, for a country considered free from bTB, lowering the intensity of surveillance e.g., by introducing visual meat inspection of cattle and pigs, will not reduce the confidence in freedom from bTB markedly providing the probability of introduction of the infection can be kept low. However, with such a slow spreading infection with a long incubation time, new introduction might take a long time to be detected. Hence, both continued surveillance and rigid import testing and quarantine practices are recommended to acquire a negligible risk of introduction of bTB.

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