Quantitative decision making in animal health surveillance: Bovine Tuberculosis Surveillance in Belgium as case study

Sarah Welby[1 #], Mickaël Cargnel[1*], Claude Saegerman[2]

[1] Sciensano, Epidemiology and Public Health Directorate, Veterinary Epidemiology Service, Brussels, Belgium

[2]Liège University, Faculty of Veterinary Medicine, Fundamental and Applied Research for Animal and Health (FARAH) Center, Research Unit of Epidemiology & Risk Analysis applied to veterinary sciences, Liège, Belgium.

Disclaimer

[#]Sarah Welby is currently employed GlaxoSmithKline Vaccines. The positions and opinions presented in this article reflect the work carried out during her employment at Sciensano at the time of the study conduct and are not intended to represent the views or scientific works of GlaxoSmithKline.

*Sciensano, Epidemiology and Public Health Directorate, Veterinary Epidemiology Service
Rue Juliette Wytsmanstraat 14 - 1050 Brussels
vet-epi@sciensano.be

Abstract

Introduction: Despite eradication and control measures applied across Europe, bovine tuberculosis (bTB) remains a constant threat. In Belgium, after several years of bTB disease freedom status, routine movement testing, as currently practiced, revealed itself inadequate to detect some sporadic breakdown herds. The aim of this study was to strike the balance between cost and effectiveness of

different surveillance system components to identify sustainable alternatives for early detection and substantiation of freedom of bTB while maintaining acceptance of these amongst the different animal health stakeholders. Methods: Stochastic iteration model was built to simulate, first, the expected current surveillance system performance in terms of sensitivity and specificity of detection. These results were then descriptively compared to observed field results. Secondly, the cost and effectiveness of simulated alternative surveillance components were quantified. To measure impact of key assumptions (i.e. regarding diagnostic tests and true prevalence), sensitivity analysis was performed. Results: Discrepancies between the predicted and observed performance of bTB surveillance in Belgium were observed. Secondly, simulated alternatives revealed that targeted IFN-γ as well serological testing with Antibody ELISA towards risk herds would enable increasing the overall cost and effectiveness of the Belgian bTB surveillance system. Sensitivity analysis showed that results remained constant despite modification of some key assumptions. Discussion: Performance of current bTB surveillance system performance in Belgium was questionable. This exercise highlighted that not only sensitivity, but specificity is a key driver for surveillance performance. The quantitative and participative conceptual framework revealed itself a useful tool to allow evidence-based decision making regarding future tuberculosis surveillance in Belgium, as required by the international standards.

Introduction

Bovine Tuberculosis (bTB) is caused by *Mycobacterium bovis* that affects humans, cattle and other domesticated and wildlife species. Despite efforts made over the last decades to eradicate the disease, bTB is still (re-)emerging in some European Union (EU) Member States (MS) as well as worldwide (EFSA, 2018; Quadri et al 2020; Visavet, 2019). The specific characteristics of the etiological agent, the complex epidemiology of the disease together with limitations of the current diagnostic assays used for bTB and lack of awareness from the different animal health stakeholders following several years of freedom of disease make surveillance and control of bTB a constant and evolving challenge (Downs et al, 2018a,b; Humblet et al., 2009; King et al., 2015; Shiller et al., 2010, 2011). In addition,

bTB control accounts for a large proportion of the Belgium's animal health expenditures which triggers the need for a cost effective and sustainable surveillance program (Drewe et al., 2014).

Following a successful eradication campaign and a constant decrease of the total number of bTB-cases since the end of the nineties, Belgium obtained officially bTB free status in 2003 (EC, 2003). Since then, bTB free status of the cattle population was maintained with annual herd prevalence below 0.1% in accordance with the minimum European legal requirements (EC, 1964, 2003).

Several studies exploring Belgian national animal identification and movement registration system (SANITEL) and merging it with the historical surveillance data revealed that the main risk factors for bTB sporadic breakdown herds in Belgium were previous infection with bTB as well as animal movements Belgium as elsewhere (Humblet et al., 2010; Conlan et al., 2012; Guta et al., 2014; More et al., 2015; Palisson et al., 2016). However, in Belgium over the last decade, mandatory purchase testing did not identify the sporadic breakdown herds, that were detected only at later stage of infection (Calba et al., 2016; Humblet et al., 2010, 2011a, b; Welby et al., 2012). In addition, the sometimes high within-herd prevalence of reactor cattle in a detected breakdown combined with the chronic stage of infection of infected cattle (generalized lesions on organs and carcass of some slaughtered animals and latent infected cattle) raised serious doubts about the current “early warning” aspect of testing at purchase and or slaughter house visual inspection (FASFC, 2020).

While there is a clear need for sustainable cost and effective surveillance systems to detect (re-) emerging diseases for securing public health, animal trade and welfare, criteria and tools to evaluate these systems and allow mutual trust between stakeholders are still lacking (Calba et al., 2015, 2016; Drewe et al.; 2012; Hoinville et al., 2013; Stärk and Häsler, 2015). Following a request from the Belgian scientific food safety committee (FASFC, 2016), a task force, composed of different animal health stakeholders (farmers, veterinarians, agri food sector, regional and central laboratories, animal health control and policy bodies, competent authorities, fund payers), to evaluate the current surveillance system performance and explore possible surveillance alternatives was set up. The overarching aim of this study was to develop a conceptual framework to allow evidence-based decision regarding the future bTB surveillance for disease freedom substantiation as well as early case

detection. For this purpose, a quantitative stochastic iteration model was developed to evaluate the surveillance components performance in terms of cost and effectiveness.

Material & Methods

- **Input data**

The surveillance of cattle in Belgium is implemented and coordinated at national level by the Federal Agency for the Safety of the Food Chain (FASFC) in accordance with the guidelines laid down in Council Directive 64/432/EEC and the Royal Decree 17.10.2002 (EC, 1964; Moniteur Belge, 2003). The four ongoing surveillance components of bTB surveillance system in Belgium are (Figure 1):

i) Slaughterhouse (SLGH): all slaughtered cattle undergo a post-mortem inspection at slaughterhouse. This visual inspection detects gross bTB suspected lesions on organs and carcasses and identifies the index bTB cases in Belgium.

The three other components are based on the use of single intradermal tuberculin test (SIT):

ii) Importation (IMP): all imported cattle from non-bTB officially free MS are tested at import. This excludes young fattening calves (FC), which are sent to slaughter at the age of 6 months.

iii) Purchase (PUR): all cattle, except FC, are tested at purchase (national trade).

iv) Winter screening (WS)

a. All cattle older than 6 weeks from herds considered as neighbour or contact herds of a suspected or confirmed bTB positive herd are tested, after tracing-on and tracing-back investigation, during five consecutive years.

b. All females older than 24 months belonging to farms with direct ‘raw milk-selling’ to consumers are tested.

c. Follow-up testing of all imported cattle from non-bTB officially free MS during three consecutive years.

A single intradermal comparative test (SICT) is performed 6 weeks after each non-negative SIT. If a non-negative SICT reactor animal is detected, the herd is under movement restriction. The reactor animal is slaughtered, and visual inspection and palpation/incisions of organs/tissues are carried out.

Suspected gross lesions and selected lymph nodes are sent to the National Reference Laboratory for tuberculosis culture and identification. If these tissues are also confirmed bTB positive at the laboratory, the whole herd is screened by skin testing and all reactor animals are slaughtered. Once bTB is detected in a herd, a thorough tracing-on and tracing-back investigation of all contact animals and herds is carried out and these contact herds are tested for five consecutive years during winter (WS) by SIT.

For the purpose of this study, alternative surveillance components such as targeted cross-sectional screening of herds and cattle identified following tracing-on and -back of bTB breakdown(s) tested with either the IFN-γ test, only SIT, only antibody ELISA (Ab-ELISA) or Ab-ELISA in parallel with IFN-γ) were explored and simulated.

To feed the simulations models below, data regarding all on-farm cattle census data and movements from 01st January 2010 up to 31st December 2015 (births, slaughters, purchases and imports) were collected from SANITEL (the national animal identification and movement database). For each individual cattle and herd, the following variables were compiled: ID cattle, ID herd of origin, ID herd of destination, birth date, movement date, movement type (birth, purchase, import, export, slaughter, rendering plant, market), cattle type1 (fattening calves versus other), cattle type2 (mixed, meat, dairy). Data was merged and concatenated at surveillance component level to get the annual population and tested number of cattle and herds tested in each surveillance component. Data management and analysis was carried out in SAS 9.2.

Annual ongoing surveillance data were obtained from the FASFC and regional laboratories in Belgium (named DGZ and ARSIA) for the years 2010-2015. Data regarding costs of surveillance procedures were obtained from the FASFC and the Sanitary Funds for cattle industry for the years 2010-2015.

The design prevalence at herd level was determined in line with the official bTB design prevalence at herd level (0.1% as described in Directive 64/432/CEE (EC, 2003). Due to the absence of exact information on within herd prevalence, arbitrary prevalence at animal level and within herd level were simulated.

The diagnostic test characteristics (sensitivity and specificity) of the SIT at purchase and visual post-mortem inspection in the slaughterhouse, as well as alternative diagnostic methods were obtained from literature review (Bezos, et al., 2014 ; Casal et al., 2017 ; EFSA,2013 ; Garcia-Saenz et al., 2015 ; Schiller et al., 2010, 2011).

To reflect the uncertainty and variability around the input data estimates, population and surveillance herd and cattle population, test characteristics, as well as minimum legal requirements extracted from above data sources and literature were entered as probability distributions and fed into the stochastic models further described below.

- **Model**

First, the predicted negative and positive results in the tested cattle population given current testing schemes applied in different ongoing surveillance components (SLGH, IMP, PUR, WS) for bTB in Belgium were computed with the following equations (Eq. 1, 2, 3, 4):

$$TP = Se \times P \times n \qquad \text{(Eq 1)}$$

$$TN = Sp \times (1-P) \times n \qquad \text{(Eq 2)}$$

$$FP = (1-Sp) \times (1-P) \times n \qquad \text{(Eq. 3)}$$

$$FN = (1-Se) \times P \times n \qquad \text{(Eq. 4)}$$

Where, the number of expected true positive (TP), true negative (TN), false positive (FP) and false negative (FN) depend on the sensitivity (Se) and the specificity (Sp) of the tests used, the animal level prevalence (P) as well as the number of cattle tested (n). The predicted numbers of TP, TN, FP and FN were computed and used as benchmark to compare with observed annual surveillance data obtained from FASFC and regional animal health organisations in Belgium during the years 2010 until 2015.

Secondly, a simple stochastic model was built to simulate ongoing and alternative surveillance components to explore and determine the most optimal scenario considering its costs and effectiveness.

The effectiveness of each simulated alternative surveillance component was estimated as its probability to limit the further spread of infection by detecting potential infected herds/cattle, measured with its sensitivity using equations described in Martin et al. (2007) (Eq.5, 6).

$$CSe=1-(1-Se_{Herd} \times (n_{Herd}/N_{Herd}))^{(N_{Herd}\times PH)} \quad \text{(Eq. 5)}$$

$$Se_{Herd}=1-(1-Se_{Test} \times (n_{inHerd}/N_{inHerd}))^{(N_{inHerd}\times PA)} \quad \text{(Eq. 6)}$$

Component sensitivity (CSe) (positive result in the component given the population is infected at the specified design prevalence) for each component (i) was estimated taking into account the number of herds present in the population (N_{Herd}) and number of sampled herds (n_{Herd}), expected prevalence at herd level (PH) and herd sensitivity (Se_{Herd}). The mean Se_{Herd} estimate was based on the distribution of number of animals present within a herd (N_{inHerd}) and number of cattle sampled (n_{inHerd}), expected prevalence at within herd level (PA) and within herd sensitivity (Se_{Test}).

The FN results was also quantified to estimate the risk of missing an infected animal (Eq. 4).

The cost of each simulated alternative scenario ($SCost_i$) was derived considering the number of cattle tested ($n_{AnimalTested}$), the cost of the diagnostic test ($Cost_{Test}$) and the number of herds ($n_{HerdsVisited}$) visited as well as cost of the veterinary visit ($Cost_{VetVisit}$ (times one for serological assays and IFN g and times two for tuberculin skin testing)) (Eq.7).

$$SCost_i= [n_{AnimalTested} \times Cost_{Test}] + [n_{HerdsVisited} \times Cost_{VetVisit}] \quad \text{(Eq. 7)}$$

Additional costs incurring from confirmation testing (with IFN-γ and Ab-ELISA in parallel) of each true and false positive result was considered also by using the same equation Eq.7 where $n_{AnimalTested}$ and $n_{HerdsVisited}$ represented the number of true and false positive reactors and herds.

The outputs generated for each simulated surveillance components were obtained by a stochastic iteration process in @Risk 5.0, with 10,000 iterations per simulation to ensure model convergence.

- **Sensitivity analysis**

To understand the impact of some of the assumptions used in the above modelling exercise, different sensitivity analyses were carried out.

It was argued that the apparent prevalence of bTB in Belgium may be underestimated, due to the current diagnostic constraints. Therefore, sensitivity analysis was carried out to measure the impact of

prevalence (1 infected in 100,000 cattle; 1 infected in 10,000; 1 infected in 1,000) on the purchase testing results while keeping all other parameters fixed.

Because the serological tests target humoral immune responses (i.e. Ab-ELISA), probability of detection will vary depending on stage of infection (acute infection or chronic infection) and prevalence, therefore different scenarios were simulated reflecting varying diagnostic test sensitivity: Ab-ELISA using conventional proteins, Ab-ELISA using specific immune mediated proteins and Ab-ELISA with no prior knowledge of diagnostic test sensitivity value.

Results

- **Data**

Table 1 displays the different input parameters, assumptions together with the respective probability distribution values and sources.

- **Model output**

Firstly, the observed and expected results (mean estimate, minimum and maximum) of different ongoing surveillance components were estimated (Table 2). The predicted SIT false positive results at purchase (38,006 (224-101,042)) were more than 1,000 times higher than observed (9(2-14)). While the observed SIT false positive results during winter screening (390(65-498)), were lying within the expected false positive reaction lower range (23,846(140-63,335)). Observed slaughterhouse inspection lesion notification rate (16(2-86)), though not as high as expected, were lying within the expected range (870(26-4,684)).

Secondly, results of the alternative surveillance components were evaluated (Table 3). Regardless the diagnostic test used, the number of false negative results remained constantly low (0(0-3). The predicted component sensitivity of each alternative testing scheme remained within the same range regardless of each specific test sensitivity meaning that the overall expected sensitivity of the surveillance would not drastically change given the chosen strategy and testing scheme. However, the overall cost (screening + confirmation) was different between the different alternative surveillance components. Depending on the specificity, overall cost could be decreased given less confirmatory

testing would be needed. Similar cost overall was observed for SIT and Ab-ELISA (113,799€ and 119,660€), while cost for IFN (256,594€) were substantially higher mainly due to higher test cost.

- **Sensitivity analysis**

The impact of different simulated animal prevalence (1/1,000; 1/10,000; 1/100,000 infected) during purchase testing are shown in Figures 2. This graph indicates that regardless the design prevalence (very low in disease freedom situation), most of test results will be true negative (around 90%), the false negative rates remained very low (around 0.01%) However, the expected rate of false positive results was high (around 10%).

Table 4 shows the impact of using different Ab-ELISA test sensitivity values. Component sensitivity remained constant and low (given the limited number of cattle herds tested compared to its corresponding herd population size) 9(0.00-0.19).

Discussion

This study highlighted the importance and interplay between sensitivity and specificity when evaluating surveillance performance in terms of cost and effectiveness. Computed predicted positive results given the specificity of diagnostic testing procedures and tested cattle population as well as prevalence enabled benchmarking expected results of the different surveillance components. In line with published results elsewhere (i.e. USDA publishes a minimum expected false positive results rate of 1% using SIT (USDA, 2017)), given expected prevalence of bTB in Belgium, a minimum of 224 of SIT tested bovines at purchase are expected as false positive reactors in Belgium, while in practice, only between 2 (in 2011) and 14 (in 2013) were reported yearly over the last decade. Our study revealed that SIT testing at purchase (in Belgian real life field experience), despite being risk based, showed a more than a 1000-fold lower observed rate of detection than expected and corroborated previous findings (Welby et al., 2012; Humblet et al., 2010). Given the estimated yearly costs of purchase testing of 1,177,462 € (FASFC, personal communication, 2016), its cost-effectiveness could be questioned. Even though the declaration of positive results would result in more confirmatory testing, self-resulting in higher costs, over all because, the overall indirect costs generated by the

234 indemnity/sanitation of breakdown herds (500,000 €/herd) (FASFC personal communication, 2016)
235 discovered only at a rather late stage of infection triggered the need for a sustainable alternative.
236 For slaughterhouse visual inspection, between 26 and 4,684 suspected lesions of annually slaughtered
237 cattle are expected. However, only 16 suspect gross lesions are spontaneously reported yearly.
238 Considering historical data of early 2000, suspicious lesions submission rate was much higher (0.01%-
239 0.08%) and closer to expected results observed in the current study (Saegerman personal
240 communication, 2016).
241 Lack of disease awareness, fear of negative repercussions following notification, logistic constraints
242 (high number of cattle tested, containment of cattle not always appropriate) biological variability, and
243 age (less likely to be infected and/or lower test sensitivity) contribute to the decreased performance
244 and trigger the need for more effective diagnostic testing procedures (Elbers et al., 2010; Humblet et
245 al., 2011a, 2011b; More et al. 2015; Schiller et al., 2010, 2011).
246 Diagnostics assays, such as Ab-ELISA and IFN-γ, gain increasing interest as they allow individual
247 testing as well as general laboratory testing, thereby avoiding subjective interpretation or non-
248 interpretation of testing results and diminishing any pressure of the owners on the veterinarian, and
249 with only single visit and thereby decreasing the financial costs for the farmers. The initial low
250 sensitivity and specificity of these assays have greatly improved over the last years (Bezos et al., 2014;
251 Casal et al., 2017; Saegerman et al., 1995). Current diagnostic tests included in bTB control programs
252 are mainly focussed on cell mediated immune response with the aim of preventing spread of disease at
253 early stage. However, as disease progresses, immunity slowly shifts from cell mediated to antibody
254 response. Therefore, animals missed with current tests targeting cellular response (implemented in its
255 current practices), remain in the herd maintaining and or spreading the disease and producing at last
256 significant economic losses. Hence, it would be advisable to either increase frequency of testing or
257 carry out parallel testing using Ab-ELISA and IFN-γ in high risk herds to increase the sensitivity of
258 the surveillance scheme to enable identification of those latent infected and potential silent bTB
259 spreading animals. This approach would ensure breakdown management (partial or total stamping out)
260 and speed up bTB eradication.

261 The sensitivity analysis revealed that the number of false positive results remained constant and was
262 mainly driven by the specificity of the test, regardless the design prevalence Similarly if the true
263 prevalence was to be higher than the current apparent prevalence the number of eventual missed cases
264 remains the same. To measure impact on the total surveillance performance of the different range
265 distributions of Ab-ELISA diagnostic test sensitivity values, additional simulations were carried out.
266 Surprisingly the impact was not significantly different. The large number of cattle and herds tested
267 probably compensated for the varying values of sensitivity. The number of false negative results,
268 reflecting the probability of missing infected animals, remained substantially low regardless the
269 diagnostic test used. Indeed, the predictive values of each of the considered test were mainly
270 conditioned by the expected prevalence, which is low in Belgium, considering the freedom status of
271 the country. However, validation these tests when used in the epidemiological Belgian field setting is
272 required before incorporating these tests in a routine surveillance.
273 Over the last decade, in general many efforts were made on improving surveillance systems while data
274 quality is often considered as an asset. However, the value of information will be hampered by poor
275 data quality. In Belgium and Europe, the mandatory systematic registration and identification of each
276 animal movement (birth, purchase, import, export, death, …) provides a well of data. But, this study
277 also highlighted the importance of data completeness and quality (standardised formats, harmonised
278 test procedures and applied cut-offs as well and proper coding of diagnostic indication to allow
279 merging between the data sources at regional and national level) as already mentioned elsewhere
280 (FAO, 2011; Stärk and Häsler, 2015).
281 To secure public and animal health and welfare and avoid re-emergence of eradicated diseases, cost-
282 effective and sustainable surveillance systems is a prerequisite. Surveillance should be tailored animal
283 health stakeholders needs and priorities and trade-off between cost and effectiveness for both
284 confidences in freedom context but also for detection of disease should be considered. Because mutual
285 trust between different stakeholder's is key, a bottom up approach involving farmers, veterinarians,
286 agri food sector, regional and central laboratories, animal health control and policy bodies, competent
287 authorities, fund payers is common practice in Belgium to ensure ownership and ultimately sustainable
288 decision making (Dehove et al., 2012; Calba et al., 2016; Hallet et al, 2003). The simulation model

developed enabled quantification of the impact of change in terms of cost and effectiveness and was a useful tool to facilitate the decision making by the different animal health stakeholders regarding the future tuberculosis surveillance in Belgium. It was agreed that testing at purchase using the SIT test currently performed in Belgium was not cost-effective in detecting bTB cases in Belgium. The use of a targeted use of the Ab-ELISA and IFNg tests was identified as an interesting cost-effective alternative to mitigate with the observed weak performance of the SIT in current Belgian real-life field experience (FASFC, 2020). In the light of the evolving national and international regulations (EFSA, 2013, 2014; More et al., 2015; Welby et al., 2012), the conceptual framework developed in the current study revealed itself being a useful tool and provided insight for adapting surveillance systems taking into account heterogeneity in local risk factors, as required by international standards.

Acknowledgments

We thank the Belgian Federal Agency for the Safety of the Food Chain (FASFC) and the Sanitary Fund for cattle for providing data of the ongoing surveillance program in Belgium. We also thank the regional animal health associations and field stakeholders for their valuable help in collecting samples and the experts, for providing valuable information for the estimation of parameters. This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors. The authors declare no conflict of interest.

Ethics

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a research article with no original research data.

Data Availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Bezos, J., Casal C, Romero, B., Schroeder, B., Hardegger, R., Raeber, A.J., López, L., Rueda, P., Domínguez, L. (2014). Current ante-mortem techniques for diagnosis of bovine tuberculosis. Res Vet Sci, 97 Suppl, 44-52.

Calba, C., Goutard, F.L., Hoinville, L., Hendrikx, P., Lindberg, A., Saegerman, C., Peyre, M. (2015). Surveillance systems evaluation: a systematic review of the existing approaches. BMC Public Health, 15, 448.

Calba, C., Goutard, F.L., Vanholme, L., Antoine-Moussiaux, N., Hendrikx, P., Saegerman, C. (2016). The Added-Value of Using Participatory Approaches to Assess the Acceptability of Surveillance Systems: The Case of Bovine Tuberculosis in Belgium. PLoS One, 11(7):e0159041. doi: 10.1371/journal.pone.0159041. eCollection 2016.

Casal, C., Infantes,J.A., Risalde, M.A., Díez-Guerrier, A., Domínguez, M., Moreno, I., Romero, B, de Juan, L., Sáez, J.L., Juste, R, Gortázar, C., Domínguez, L., Bezos, J. (2017). Antibody detection tests improve the sensitivity of tuberculosis diagnosis in cattle. Res Vet Sci, 112, 214-221. doi: 10.1016/j.rvsc.2017.05.012.

Conlan, A.J., McKinley, T.J., Karolemeas, K., Pollock, E.B., Goodchild, A.V., Mitchell, A.P., Birch, C.P., Clifton-Hadley, R.S., Wood, J.L. (2012). Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain. PLoS Comput Biol, 8(10): e1002730 doi:10.1371/journal.pcbi.1002730.

Dehove, A., Commault, J., Petitclerc, M., Teissier, M., Macé, J. (2012). Economic analysis and costing of animal health: a literature review of methods and importance. Rev Sci Tech, 31(2), 605-617.

Drewe, J.A., Hoinville, L.J., Cook, A.J., Floyd, T., Stärk, K.D. (2012). Evaluation of animal and public health surveillance systems: a systematic review. Epidemiol Infect, 140(4):575-590.

Drewe, J.A., Häsler, B., Rushton, J., Stärk, K.D. (2014). Assessing the expenditure distribution of animal health surveillance: the case of Great Britain. Vet Rec, 174(1), 16.

Downs, S.H., Parry, J.E., Upton, P.A., Broughan, J.M., Goodchild, A.V., Nuñez-Garcia, J., Greiner M., Abernethy, D.A., Cameron, A.R., Cook, A.J., de la Rua-Domenech, R., Gunn, J., Pritchard, E., Rhodes, S., Rolfe, S., Sharp, M., Vordermeier, H.M., Watson, E., Welsh, M., Whelan, A.O., Woolliams, J.A., More, S.J., Clifton-Hadley, R.S. (2018a). Methodology and preliminary results of a systematic literature review of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis. Prev Vet Med, 153, 117-126. doi: 10.1016/j.prevetmed.2017.11.004. Epub 2017 Nov 9.

Downs, S.H., More, S.J., Goodchild, A.V., Whelan, A.O., Abernethy, D.A., Broughan, J.M., Cameron, A., Cook, A.J., Ricardo de la Rua-Domenech, R., Greiner, M., Gunn, J., Nuñez-Garcia, J., Rhodes, S., Rolfe, S.B., Sharp, M., Upton, P., Watson, E., Welsh, M., Woolliams, J.A., Clifton-Hadley, R.S., Parry, J.E. (2018b). Evaluation of the methodological quality of studies of theperformance of diagnostic tests for bovine tuberculosis using QUADAS. Prev Vet Med, 153:108-116. doi: 10.1016/j.prevetmed.2017.03.006.

Elbers, A.R.W., Gorgievski-Duijvesteijn, M.J., van der Velden, P.G., Loeffen, W.L.A., Zarafshani, K. (2010). A socio-phychological investigation into limitations and incentives concerning reporting a clinically suspect situation aimed at improving early detection of classical swine fever outbreaks. Prev Vet Med, 142, 108–118.

European Food Safety Agency (EFSA) (2013). Modelling the impact of a change in meat inspection sensitivity on the surveillance of bovine tuberculosis at country level. Retrieved 16.10.2015, from http://www.efsa.europa.eu/en/supporting/pub/450e.htm.

369 European Food Safety Agency (EFSA) (2014). Statement of a conceptual framework for bovine
370 tuberculosis. Retrieved 16.10.2015, from http://www.efsa.europa.eu/en/efsajournal/pub/3711.htm .
371
372 European Food Safety Agency (EFSA) (2018). The European Union summary report on trends and
373 sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 16(12):e05500.
374
375 European Commission (EC) (1964). Council Directive of 26 June 1964 on animal health problems
376 affecting intra-Community trade in bovine animals and swine 64/432/EEC. Official Journal 121: 1977.
377
378 European Commission (EC) (2003). Commission Decision of 23 June 2003 establishing the official
379 tuberculosis, brucellosis, and enzootic bovine leucosis free status of certain Member States and
380 regions of Member States as regards bovine herds 2003/467/EC. Official Journal, L 156: 74.
381
382 Food and Agriculture Organisation (FAO) (2011). Challenges of animal health information systems
383 and surveillance for
384 animal diseases and zoonoses. Retrieved 14.07.2014, from
385 http://www.fao.org/docrep/014/i2415e/i2415e00.htm.
386
387 Federal Agency for the Safety of the Food Chain (FASFC) (2016). Scientific committee opinion
388 2015/11: Evaluation of the Belgian bovine tuberculosis control program. Retrieved 12.12.2017, from
389 http://www.afsca.be/scientificcommittee/opinions/2016/_documents/Opinion12-
390 2016_Tuberculose.pdf.
391
392 Federal Agency for the Safety of the Food Chain (FASFC) (2020). Santé animale: Tuberculose
393 bovine. Retrieved 21.12.2020, from http://www.favv-afsca.be/santeanimale/tuberculose/
394

395 Garcia-Saenz, A., Napp, S., Lopez, S., Casal, J., Allepuz, A. (2015). Estimation of the individual
396 slaughterhouse surveillance sensitivity for bovine tuberculosis in Catalonia (North-Eastern Spain).
397 Prev Vet Med, 121(3-4), 332-337. doi: 10.1016/j.prevetmed.2015.08.008.
398
399 Guta, S., Casal, J., Napp, S., Saez, J.L., Garcia-Saenz, A., Perez de Val, B., Romero, B., Alvarez, J.,
400 Allepuz, A. (2014). Epidemiological Investigation of Bovine Tuberculosis Herd Breakdowns in Spain
401 2009/2011. PLoS One, 9(8): e104383. doi:10.1371/journal.pone.0104383.
402
403 Hallet, L. (2003). Collaboration between official veterinarians, private veterinarians and livestock
404 producer organisations Rev sci tech Off int Epiz, 22 (2), 523-532
405
406 Humblet, M.F., Boschiroli, M.L., Saegerman, C. (2009). Classification of worldwide bovine
407 tuberculosis risk factors in cattle: a stratified approach. Vet Res, 40(5): 50.
408
409 Humblet, M.F., Gilbert, M., Govaerts, M., Fauville-Dufaux, M., Walravens, K., Saegerman, C. (2010).
410 New assessment of bovine tuberculosis risk factors in Belgium based on nationwide molecular
411 epidemiology. J Clin Microbiol, 48(8), 2802-2808.
412
413 Humblet, M.F., Walravens, K., Salandre, O., Boschiroli, M.L., Gilbert, M., Berkvens, D., Fauville-
414 Dufaux, M., Godfroid, J., Dufey, J., Raskin, A., Vanholme, L., Saegerman, C. (2011a.) Monitoring of
415 the intra-dermal tuberculosis skin test performed by Belgian field practitioners. Res Vet Sci 91, 199–
416 207.
417
418 Humblet, M.F., Moyen, J.L., Bardoux, P., Boschiroli, M.L., Saegerman, C. (2011b). The importance
419 of awareness for veterinarians involved in cattle tuberculosis skin testing. Transbound Emerg Dis,
420 58(6)
421 , 531-536.
422

King, H.C., Murphy, A., James, P., Travis, E., Porter, D., Hung, Y.J., Sawyer, J., Cork, J., Delahay, R.J., Gaze, W., Courtenay, O., Wellington, E.M. (2015).The variability and seasonality of the environmental reservoir of Mycobacterium bovis shed by wild European badgers. Sci Rep, 6;5:12318. doi: 10.1038/srep12318.

Martin, P.A., Cameron, A.R., Greiner, M. (2007).Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. Prev Vet Med 79(2-4), 71-97.

Moniteur Belge (MB) (2003). Arrêté royal du 17 Octobre 2002 relatif à la lutte contre la tuberculose bovine. Moniteur Belge 2003022000 : 12448.

More, S.J., Radunz, B., Glanville, R.J. (2015). Lessons learned during the successful eradication of bovine tuberculosis from Australia. Vet Rec 177(9), 224-232.

Palisson, A., Courcoul, A., Durand, B. (2016). Role of Cattle Movements in Bovine Tuberculosis Spread in France between 2005 and 2014. PLoS One 11(3), e0152578.

Praud, A., Boschiroli, M.L., Meyer, L., Garin-Bastuji, B., Dufour, B. (2015). Assessment of the sensitivity of the gamma-interferon test and the single intradermal comparative cervical test for the diagnosis of bovine tuberculosis under field conditions. Epidemiol Infect, 143(1), 157-166.

Quadri NS, Brihn A, Shah JA, Kirsch JD. Bovine Tuberculosis: A Re-emerging Zoonotic Infection. J Agromedicine. 2020 Jun 1:1-6. doi: 10.1080/1059924X.2020.1771497. Epub ahead of print. PMID: 32478614.

Saegerman C, Delville J, De Waele L, Gilson D. (1995). Serological and cutaneous testing of bovine tuberculosis with the A60 antigen complex from Mycobacterium bovis, strain Calmette-Guérin. Prev Vet Med, 1995, 23, 239-248.

Schiller, I., Oesch, B., Vordermeier, H.M., Palmer, M.V., Harris, B.N., Orloski, K.A., Buddle, B.M., Thacker, T.C., Lyashchenko, K.P., Waters, W.R. (2010). Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. Transbound Emerg Dis, 57(4),205-220.

Schiller, I., Waters, W.R., Vordermeier, H.M., Jemmi, T., Welsh, M., Keck, N., Whelan, A., Gormley, E., Boschiroli, M.L., Moyen, J.L., Vela, C., Cagiola, M., Buddle, B.M., Palmer, M., Thacker, T., Oesch, B. (2011). Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: trade, surveillance and diagnostics. In: Fairbrother, P., Crews, K., Livingstone, P., Buddle, B., de Lisle, G., Collins, D., Nugent, G. (Eds.), Special Issue: 5th International Conference on Mycobacterium Bovis. Vet Microbiol, 151, 153–159.

Stärk, K.D., Häsler, B. (2015). The value of information: Current challenges in surveillance implementation. Prev Vet Med, 122(1-2), 229-234. doi: 10.1016/j.prevetmed.2015.05.002. Epub 2015 May 14.

United States Department of Agriculture (USDA) (2017). BovineTuberculosis: Caudal Fold Testing and Reporting. Retrieved 02.08.2017, from https://aglearn.usda.gov/customcontent/APHIS/APHIS-VS-BovineTuberculosis-01/scopage_dir/cftreport/cftreport.html.

Welby, S., Govaerts, M., Vanholme, L., Hooyberghs, J., Mennens, K., Maes, L., Van Der Stede, Y., (2012). Bovine tuberculosis surveillance alternatives in Belgium. Prev Vet Med, 106(2),152-161.

VISAVET (2019). Bovine tuberculosis eradication in Europe. Retrieved 01.02.2020, from https://www.visavet.es/bovinetuberculosis/bovine-tb/eradication.php

Figures and Tables

Table 1. Model input parameters and assumptions values and sources (mode (min-max))

Parameter	Value	Sources
Yearly cattle herd population size	24,000 (22,000-25,000)	National animal identification and movement registration system, Federal Agency Food Safety Chain, Sanitary Fund
Yearly cattle population size	2,500,000(2,200,000-2,700,000)	
Herd Size	53 (8-143)	
Yearly purchased cattle size	345,298 (338,392-352,066)	
Yearly slaughtered cattle size	501,189 (491,165-511,012)	
Yearly tracing outbreak cattle tested during winter screening size	216,643(212,310-220,889)	
Yearly tracing import and dairy tested cattle during winter screening Size	81,653(80,021-83,253)	
Simulated RBS screening Number of sampled herds	215	
Simulated RBS screening Number of sampled cattle	13000	
Sensitivity Ab-ELISA	0.56(0.04- 0.98)	Bezos, et al., 2014 ; Casal et al., 2014 ; EFSA, 2013 ; Garcia-
Specificity Ab-ELISA	0.92(0.81-0.97)	
Sensitivity tuberculin skin test	0.94(0.49-1)	
Specificity tuberculin skin test	0.91(0.7-1)	

Sensitivity IFN-γ	0.77(0.61-0.89)	Saenz et al., 2015 ; Schiller et al., 2010, 2011 Federal Food Safety Agency, Sanitary Fund
Specificity IFN-γ	0.98(0.95-0.99)	
Sensitivity abattoir	0.71(0.38-0.92)	
Specificity abattoir	1(0.99-1)	
Cost Ab-ELISA (€)	4(3-5)	
Cost tuberculin skin test (€)	2(1-3)	
Cost IFN-γ (€)	17(15-25)	
Cost of farm visit by the vet (€)	30.13	
Animal Prevalence	0.0001	Simulated
Herd prevalence	0.0010	64/432/CEE
Within-herd prevalence	0.100	Simulated

481 NA: Not applicable

482 RBS: random based sampling

483

484 **Table 2. Number observed and expected positive results (true and false**
485 **positives) within the different bovine tuberculosis surveillance components**
486 **ongoing in Belgium using the single intradermal tuberculin test or post**
487 **mortem visual inspection at slaughterhouse (mode (min-max) values)**
488

Components	Data source	Observed	Predicted
Purchase	FASFC 2010-2015	9 (2-14)	38,006 (224-101,042)
Slaughter		16 (2-86)	870(26-4,684)
Winter screening:		390(65-498)	23,846(140-63,335)
• **Tracing outbreak**			
• **Tracing import**			

• **On farm delivery dairy farms**		817(172-1486)	8,987(52-23,816)

Table 3. Simulation results of alternative bTB surveillance scenarios (random cross-sectional screening testing with the IFN-γ, tuberculin skin test or Ab-ELISA test): true positives (TP) and false positives (FP), true negatives (TN) and false negatives (FN), component sensitivity screening and confirmation testing price (mode (min-max) values)

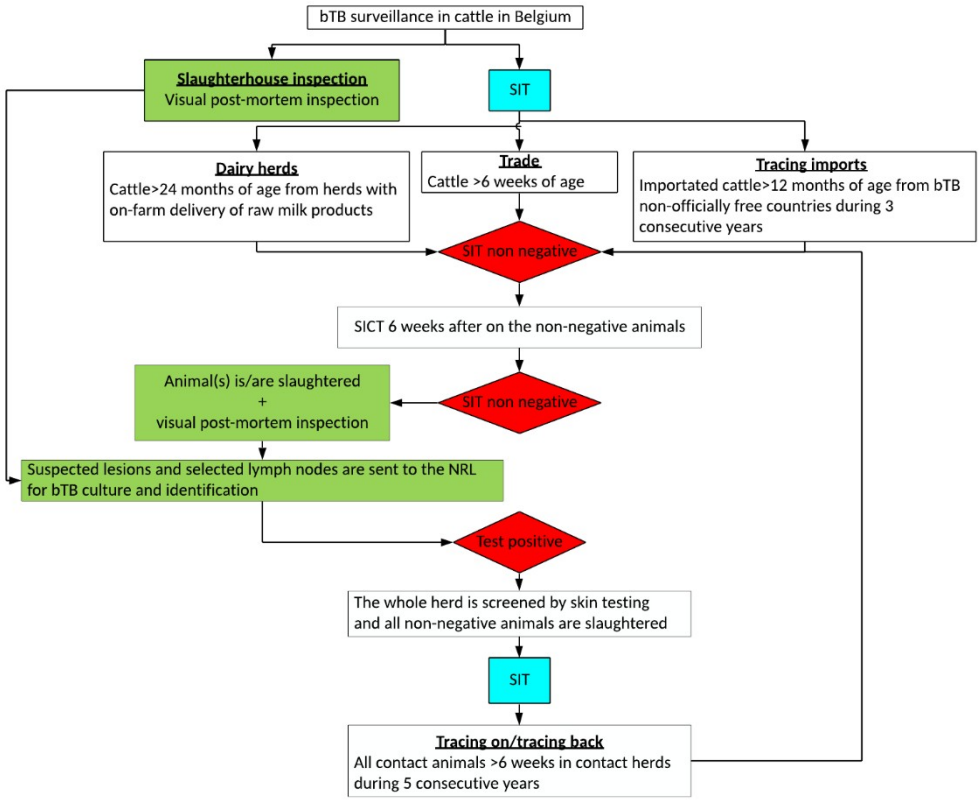
	Screening with tuberculin skin test (Vet Visit *2)	**Screening with IFN-γ test**	**Screening with Ab-ELISA test**	**Screening with IFN-γ + Ab-ELISA test**
TP	1 (0-3)	1 (0-3)	1 (0-2)	1 (0-3)
FN	0 (0-1)	0 (0-1)	1 (0-2)	1 (0-3)
FP	1,434 (5-7,055)	303 (28-1,136)	1,172 (82-4,667)	1,448 (132-5302)
TN	11,572 (1,679-27,232)	12,703 (1,856-28,692)	11,834 (1,746-26,486)	11,572 (1,679-27,232)
Component sensitivity	0.14 (0.03-0.19)	0.11 (0.02-0.18)	0.08 (0.01-0.19)	0.14 (0.03-0.19)
Price screening(€)	38,951 (16,114-88,874)	240,753 (36,622-625,026)	58,519 (13,576-138,831)	292,794 (43,719-713,194)
Price confirmation testing (€)	74,848 (315-370,670)	15,841 (1,425-60,708)	61,141 (4,430-235,328)	75,530 (7,138-267,419)

*If tuberculin test is carried out in accordance with gold standard

Table 4. Impact of using different distributions and values of Ab-ELISA test on bovine tuberculosis random cross-sectional surveillance: expected test results (true positives (TP) and false positives (FP), true negatives (TN) and false negatives (FN)), component sensitivity, testing cost (screening + confirmation) (mode (min-max) values)

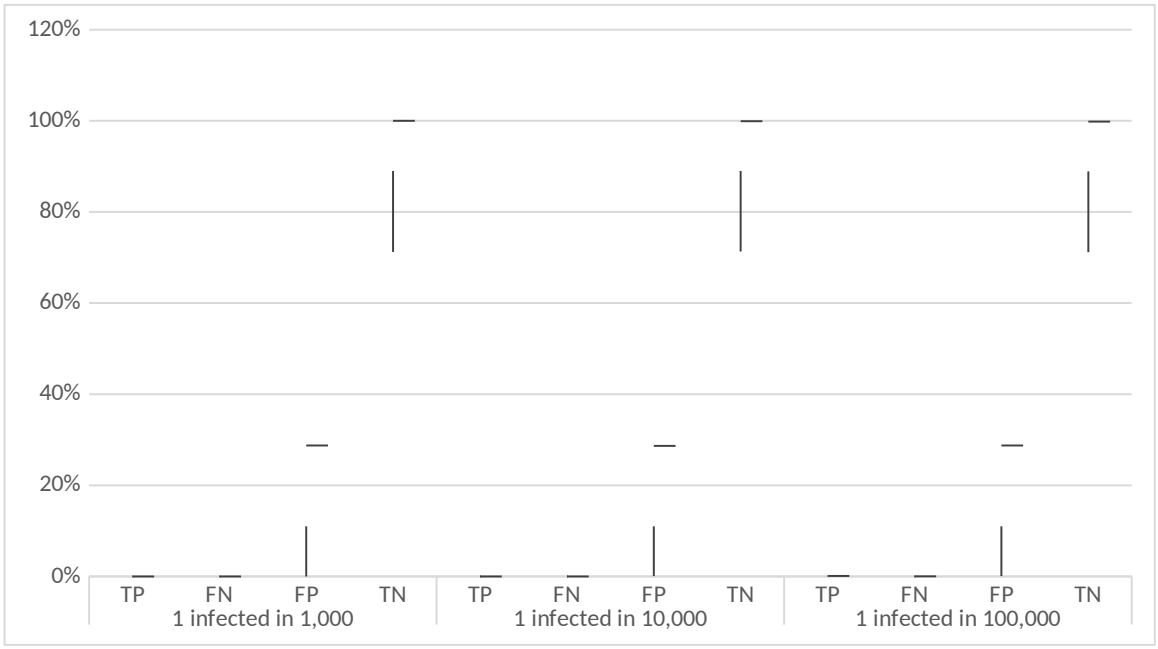
	Pert distribution (0.04,0.56,0.98)	Beta distribution (79,62) Casal et al., 2017	Beta distribution (112,9) Casal et al., 2017	Beta distribution (2,2)
TP	1 (0-2)	1 (0-2)	1 (0-3)	1 (0-2)
FN	1 (0-2)	1 (0-2)	0 (0-0)	1 (0-2)
FP	1,172 (82-4,667)	1,170 (99-4,292)	1,172 (98-4,465)	1,172 (96-4,713)
TN	11,834 (17,46-26,486)	11,836 (1,733-27,290)	11,834 (1,614-27,865)	11,834 (1,790-27,298)
Component sensitivity	0.08 (0.00-0.19)	0.08 (0.018-0.15)	0.15 (0.04-0.19)	0.07 (0.00-0.19)
Price screening (€)	58,519 (13,576-138,831)	58,539 (14074-134450)	58,524 (13,510-141,338)	58,515 (13,292-144,526)
Price confirmation testing (€)	61,141 (4,430-235,328)	61,065 (5352-243202)	61,144 (5,151-239,445)	61,141 (4,814-238,972)

505



506

507 Figure 1. **The main components of bTB surveillance in Belgium. bTB: bovine**
508 **tuberculosis; SIT: single intradermal test; SICT: single intradermal comparative**
509 **test; NRL: national reference national laboratory.**



510

Figure 2. Simulated results (true positives (TP) and false positives (FP), true negatives (TN) and false negatives (FN)) for varying prevalence during purchase testing with tuberculin skin test