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SCIENTIFIC OPINION

Statement on a conceptual framework for bovine tuberculosis[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Control and eradication of bovine tuberculosis (bTB) is a challenge owing to the complex interactions between the pathogen, hosts and local environments. Modelling various bTB situations may lead to improved understanding of the ways in which different factor combinations and interactions influence occurrence, surveillance outcomes and control efforts. A conceptual framework would help to outline, from an epidemiological perspective, which factors influence bTB infection, detection and control, and how they might interact in various European situations. The conceptual framework on bTB described in this statement is built around an anchor model describing the interactions between the most important biological and non-biological parameters involved in bTB infection, detection and control. The interactions are examined at three levels, corresponding to three 'units of interest', in the case of bTB the animal, the herd and the area levels. The conceptual framework is intended to help understand the inputs that should be considered when developing a specific component of the episystem (i.e. the ecological context of the epidemiological problem). It should be able to help both in the generation and interpretation of predictive and analytical models (dealing with a specific component of the framework) designed to answer specific questions regarding bTB. The relationship between the conceptual framework and particular examples related to the force of infection, the non-biological context and testing of bTB are explained and discussed in the document.

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KEY WORDS

bovine tuberculosis, cattle, conceptual framework, model, context

[1] On request from EFSA, Question No EFSA-Q-2013-0531, adopted on 14 May 2014.

[2] AHAW Panel members: Edith Authie, Charlotte Berg, Anette Bøtner, Howard Browman, Ilaria Capua, Aline De Koeijer, Klaus Depner, Mariano Domingo, Sandra Edwards, Christine Fourichon, Frank Koenen, Simon More, Mohan Raj, Liisa Sihvonen, Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Ivar Vågsholm, Antonio Velarde, Preben Willeberg and Stéphan Zientara. Correspondence: AHAW@efsa.europa.eu

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SUMMARY

EFSA asked the Panel on Animal Health and Welfare (AHAW) to generate a conceptual framework on bovine tuberculosis (bTB). Control and eradication of bTB is a challenge owing to the complex interactions between the pathogen, hosts and the local environments. As a result, the effect of intervention measures may not always be consistent; similar control measures may result in different outcomes if applied to different epidemiological situations. Modelling various bTB situations may lead to an improved understanding of the ways in which different factor combinations and interactions influence the occurrence, surveillance outcomes and control efforts. A conceptual framework would help to outline, from an epidemiological perspective, which factors influence bTB infection, detection and control, and how they might interact in various European situations.

The conceptual framework on bTB is built around an anchor model describing the interactions between the most important biological and non-biological parameters involved in bTB infection, detection and control. The interactions are examined at three levels, corresponding to three 'units of interest', in the case of bTB the animal, the herd and the area levels. bTB testing aims to determine the infection status at the animal level. These bTB testing data are then used to determine the apparent herd prevalence and status (within a herd) and apparent area prevalence and status (between herds); the latter is subsequently used as the basis for determining the official status of an area. This in turn influences the sampling scheme at both herd and area levels. Compartmental simulation models have been developed concerning 'the force of infection', 'sampling and testing' and 'the non-biological context'.

The conceptual framework described in this statement is not intended for direct translation into a single mathematical model that covers all aspects. It is instead intended to help understand the inputs that should be considered when developing a specific component of the episystem (i.e. the ecological context of the epidemiological problem). The framework should be able to help with both the generation and the interpretation of predictive and analytical models (dealing with a specific component of the framework) designed to answer specific questions regarding bTB. The relationship between the conceptual framework and particular examples related to the force of infection, and the non-biological context and the testing of bTB are explained and discussed in the document.

This statement reflects the conceptual view of the AHAW Panel on the current situation regarding bTB infection, detection and control in Europe. It is not based upon, and does not contain, a (systematic) review of the scientific literature. The intentions of the conceptual framework are to facilitate (1) explaining how a change in one parameter would affect different aspects of bTB infection, detection and control; (2) identifying parameters for which insufficient data are available to estimate their effect on bTB infection, detection and control; (3) making more explicit which aspects influence a specific risk question within the broader context of bTB infection, detection and control should be considered while addressing a specific risk question; (4) clarifying the value of different types and sources of data for future data collection, and for which questions these data are relevant; (5) optimising the formulation of risk questions by formulating them within the context of this framework; and (6) communicating amongst the different actors involved in bTB.

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BACKGROUND AS PROVIDED BY EFSA

Bovine tuberculosis is included as one of the animal diseases to be eradicated in the European Union since 1964. Substantial progress towards eradication has been achieved by the control policies implemented as in 2011, Austria, Belgium, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Latvia, Luxembourg, the Netherlands, Poland, Slovakia, Slovenia, Sweden, Norway and Switzerland were officially tuberculosis free (OTF); Italy has six OTF regions and 13 OTF provinces, and in the United Kingdom, Scotland is also OTF.

However, during the years 2006-2011, the proportion of existing cattle herds infected or positive for *M. bovis* in the EU (all MSs) has remained stable. A low level of infected herds ranging from 0.37 % in 2007 to 0.60 % in 2011 was maintained in the last 5 years. Further decrease has not been observed and re-emergency is being reported in several countries.

Out of the 1,361,555 existing herds in OTF countries, in 2011, 194 herds were positive for *M. bovis*; in Belgium (1 herd), France (173 herds), Germany (three herds), Poland (13 herds) and the Netherlands (four herds). In the non-OTF MSs, the proportion of *M. bovis*-positive herds increased slightly from very low (0.46 %) in 2007 to low (1.12 %) in 2011. In total, the 12 non-OTF MSs reported 1,524,638 existing bovine herds with 17,102 of them (1.12 %) infected with or positive to *M. bovis* in 2011. Furthermore, a number of MSs reported findings of *M. bovis* in animal species other than cattle. The occurrence of *M. bovis* in wildlife and domestic animals other than cattle seems to be directly related to the presence of TB in cattle herds.

The complex situation described above is most likely a reflection of the existence of a range of epidemiological factors operating at a local level, which influences the effectiveness of both surveillance (including monitoring/detection) and control activities. These local activities are guided by national policies and EU-level policies, highlighting the interplay between, and importance of EU and national and local factors in the disease epidemiology.

The Community legal framework on bovine tuberculosis is formed by legislation on trade of bovine animals (Directive 64/432/EEC), legislation on animal products for human consumption (Directive 64/433/EEC, Directive 92/46/EEC and Regulation No. 2004/853/EC) and legislation regarding Community co-financing of eradication programmes (Directive 77/391/EEC and Decision 90/424/EEC). Council Directive 64/432/EEC defines that a MS or part of a MS may be declared OTF if the percentage of bovine herds confirmed as infected with tuberculosis has not exceeded 0.1 % per year of all herds for six consecutive years and at least 99,9 % of herds have achieved OTF status each year for six consecutive years.

Several EFSA opinions have looked at specific aspects of this complex picture and it is felt that such an approach is not optimal for scientific advice to risk managers since it does not contemplate the interactions between animal populations and their environment, and the specificities of testing and culling programs applied in different field situations.

The AHAW Panel wishes to develop a conceptual framework towards holistic approach to bovine tuberculosis. The objective is to establish and maintain a broad understanding of the epidemiology of bovine tuberculosis, relevant to effective surveillance and control, throughout the EU, while addressing specific questions posed from the Commission.

TERMS OF REFERENCE AS PROVIDED BY EFSA

To develop a conceptual framework considering:

- the phases of infection (absence, introduction and early establishment, well-established infection, progress towards eradication, successful eradication),
- the perspective involved (herd and region/country), and

- the programme-related activities (surveillance [detection of infection] and control [management of infection following detection]).

In order to achieve this objective, the Panel considers that it is necessary to compare the epidemiological situations in different MS and different areas within each MS, and to identify key issues that hinder effective bTB surveillance and control or otherwise provide an explanation for differences in surveillance/control effectiveness in different epidemiological contexts.

Other MS risk assessment agencies are developing similar initiatives and collaboration is foreseen for the development of this mandate. Exchange of information and expertise with the French Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), as in the previous EFSA mandate on bovine tuberculosis, will be continued. The EFSA scientific network for risk assessment in AHAW will also be consulted.

EVALUATION

1. Introduction

Considerable efforts towards control and eradication of bovine tuberculosis (bTB) have been made at both national and European levels for decades (e.g. 59 % of the farms were bTB positive in Germany in 1952 (Meyn, 1952) and one out of four herds was infected in France in 1954 (ANSES, 2011)). The control measures put in place by national and European regulations were triggered by the zoonotic character of the pathogen, economic losses in affected farms and the need to develop safer trade across Europe. Despite these efforts, control and eradication of bTB remains a challenge in several European regions or countries. This is in particular due to the complex interactions between the pathogen, the hosts and the local environment. As a result, the effect of individual intervention measures may not always be predictable; similar control measures as laid down by European Union (EU) legislation may result in different outcomes if applied to different epidemiological situations. An example of the former is the effect of badger culling on bTB infections in cattle in England. An example of the latter is that a negative skin test from an animal originating from a high-prevalence area (e.g. some areas in England) has a lower predictive value than one originating from a bTB-free country (e.g. Denmark), owing to the lower prior probability of bTB occurrence in Denmark than in some regions of England. Skin test-positive animals, however, would more often be negative for bTB at post-mortem examination in Denmark than in England. Consequently, such interactions need to be considered when addressing the risk of bTB. Since realistic multifactorial experimental infections in the target species would be impossible to carry out, modelling various bTB situations may lead to an improved understanding of the ways in which different factor combinations and interactions influence occurrence, surveillance outcomes and control efforts.

In agreement with the Terms of Reference, the aim of this statement is to present a conceptual framework that will help to outline, from an epidemiological perspective, which factors influence bTB infection, detection and control, and how they interact in various European situations. This statement reflects the conceptual view of the Panel on Animal Health and Welfare (AHAW) on the current situation regarding bTB infection, detection and control in Europe. It is not based upon, and does not contain, a (systematic) review of the scientific literature. Non-biological aspects are taken into account in this statement when they become factors influencing bTB infection, detection or control.

The conceptual framework could facilitate:

(1) explaining how a change in one parameter would affect different aspects of bTB infection, detection and control;

(2) identifying parameters for which insufficient data are available to estimate their effect on bTB infection, detection and control;

(3) making more explicit which aspects influence a specific risk question within the broader context of bTB infection, detection and control should be considered while addressing a specific risk question;

(4) clarifying the value of different types and sources of data for future data collection, and for which questions these data are relevant;

(5) optimising the formulation of risk questions by formulating them within the context of this framework;

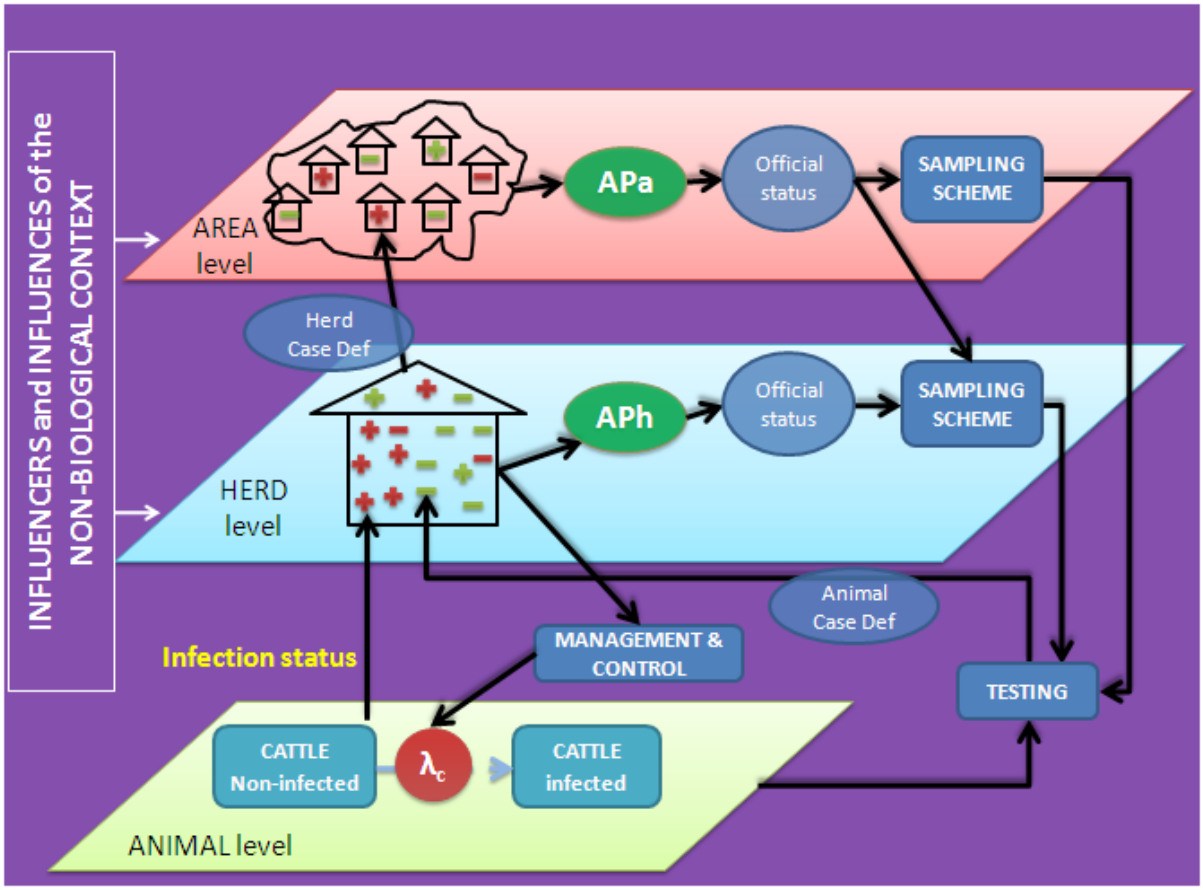
(6) interdisciplinary interaction amongst the different actors involved in bTB to facilitate multidisciplinary approaches.

In the following, the conceptual framework, and how it could be transformed into parameterised models, is described.

2. Description of a conceptual framework on bovine tuberculosis

2.1. Anchor model

The conceptual framework on bTB developed for this statement is built around an anchor model (Figure 1) describing the interactions between the most important biological parameters involved in bTB infection, detection and control (biological context). All of these parameters are affected by actors and influences which define the non-biological context (see section 2.6).



Legend: λ_c, force of infection for cattle; APh, apparent within-herd prevalence; APa, apparent area prevalence; green +, non-infected animal/herd testing positive (false positive); green –, non-infected animal/herd testing negative (true negative); red +, infected animal/herd testing positive (true positive); red –, infected animal/herd testing negative (false negative); , herd.

Figure 1: Anchor model

The interactions are examined at three levels, corresponding to three 'units of interest', in the case of bTB the animal, the herd and the area level. The animal level comprises all characteristics that are specific for each individual (e.g. bTB status, age, breed, immune status). All activities and events taking place 'within a herd' are considered part of the 'herd level', whereas all activities and events taking place 'between herds' are considered part of the 'area level'. In the conceptual framework, 'area' is used in its epidemiological meaning. Depending on the situation one wants to analyse, the area level may be considered at any scale of epidemiological relevance (from a few neighbouring herds to any geographical area), or of relevance in the context of Council Directive 64/432/EEC (part of a Member State's territory which is at least 2 000 km^2 in area and which is subject to inspection by the competent authorities).

Figure 2: Some components of the anchor model (e.g. 'force of infection', 'testing' and 'herd sampling scheme' and 'area sampling scheme') and the biological parameters and variables that may influence them are further explained in subsequent compartmental models (Figures 2–5).

The true bTB status of an animal can be non-infected or infected. The component λ_c represents the force of infection for cattle: the rate (probability per unit of time) at which a non-infected animal within a herd becomes infected with pathogens of the *Mycobacterium tuberculosis* complex (MTBC), that is *M. bovis, M. caprae* or *M. tuberculosis* from all possible sources of infection. At the herd level, this results in a mixture of infected and uninfected animals (represented, respectively, by red- and green-coloured symbols in Figure 1). Through the testing procedure applied, animals are seen as test positive or test negative (represented by '+' and '–' symbols in Figure 1, respectively). Thus, a red '+' is a true-positive animal, whereas a red '–' is false negative; a green '–' is a true-negative animal, whereas a green '+' is false positive. Based on the test results and depending on the case definition that is chosen, an animal may be classified as 'infected' or 'not infected'.

An animal with a positive diagnostic test is suspected of being infected with MTBC, but, owing to the characteristics of the tests, is not always a truly infected individual and vice versa. Thus, an individual animal corresponds to a 'case' (i.e. it should be considered as bTB infected if it has given positive results to a test or a combination of tests meant to detect bTB infection). In most cases, the definition of infection is based on results from a combination of tests carried out on live animals, termed 'pre-slaughter tests' (such as the tuberculin skin test) and on carcasses from the same animals, termed 'post-slaughter tests'. The presence of individual bTB cases in a herd is the basis for defining that herd as infected. Therefore, a misclassification of the animal's bTB status might result in a misclassification of the herd's bTB status.

Case definitions at individual and herd levels are required to design surveillance in cattle and to optimise targeting of 'management and control' measures. These include, for the purpose of this statement, all procedures and actions that may have an effect on the force of infection, including regulatory measures. Some examples are culling of test-positive animals, which reduces within-herd prevalence and thus decreases exposure of remaining animals within a herd; improving biosecurity at herd level by reducing exposure of non-infected animals; epidemiological investigations after a herd breakdown, which will result in measures that will limit the force of infection in the breakdown herd or in contact herds. 'Management and control' measures are applied and have an effect primarily at the animal level (on force of infection, λ_c); however, these actions may be positioned at all three levels in the framework, since several control measures are implemented at the area level, whereas culling, hygiene and biosecurity measures are most often implemented at the herd level. Although control and eradication are two different theoretical disease management concepts, the history of bTB in Europe indicates that in practice there has been a gradual transition from control to eradication in countries that have been successful in eradicating the infection (e.g. Scandinavian countries).

The number of animals that test positive within a herd (represented by the '+' symbol in Figure 1), as a ratio of the total number of animals in the herd, defines the apparent within-herd prevalence (APh). The continued absence of positive test results (APh = 0 %) is the basis for a herd to be granted and to maintain official tuberculosis-free (OTF) status. Any suspicion of bTB infection in the herd will lead to suspension of the OTF status of that herd, and confirmation of infection will lead to withdrawal of the status. The official status of a herd (OTF, suspension of OTF or withdrawal of OTF) will affect the sampling and testing procedures subsequently applied to that herd.

The third, higher, level in the conceptual framework is the area level, which may correspond to a country or any geographical area. The apparent prevalence of infected herds in an area (APa) is used to define the official status of that area regarding bTB, a status which will determine conditions for trade and animal movements within that area and between areas. The OTF status currently applies to areas where the prevalence of bTB is below a certain threshold, considered as reflecting a very low risk of infection. In accordance with EU regulation, the OTF status is granted to a country or part of a country, termed a 'region', if APa is below the prevalence threshold of 0.1 % for six consecutive years

and if at least 99.9 % of the herds within that country or region are OTF. The minimal size of a 'region' is defined by the regulation (2 000 km^2) and 'regions' are based on administrative units in each country. Thus, a country may apply for OTF status at the national level (combining APa in all administrative units) or at a regional level (which should comprise at least one administrative unit). The official status of an area with regard to bTB will in turn influence the surveillance programme implemented in that area, resulting in different regimes of sampling and testing.

2.2. Compartmental model on 'force of infection'

Figure 2 shows the detailed model that describes infection of a previously uninfected bovine. Here, infected animals include both those infectious to other animals and those latently infected, that is those that are not (yet) infectious to other animals. For modelling purposes, it may be necessary to distinguish between them. It is assumed that non-infected cattle become infected at a rate called the 'force of infection' (λ_c). This overall force of infection is the cumulative result of the forces of infection from the various sources of infection present. The force of infection reflects the transmission rate between an infected/infectious animal and the non-infected bovine, as well as the number of infected and infectious animals present and as such can change over time. In Figure 2, cattle are chosen as the central point at which the force of infection is directed. The figure reflects a snapshot in space and time, whereas space and time need to be considered when using the framework.

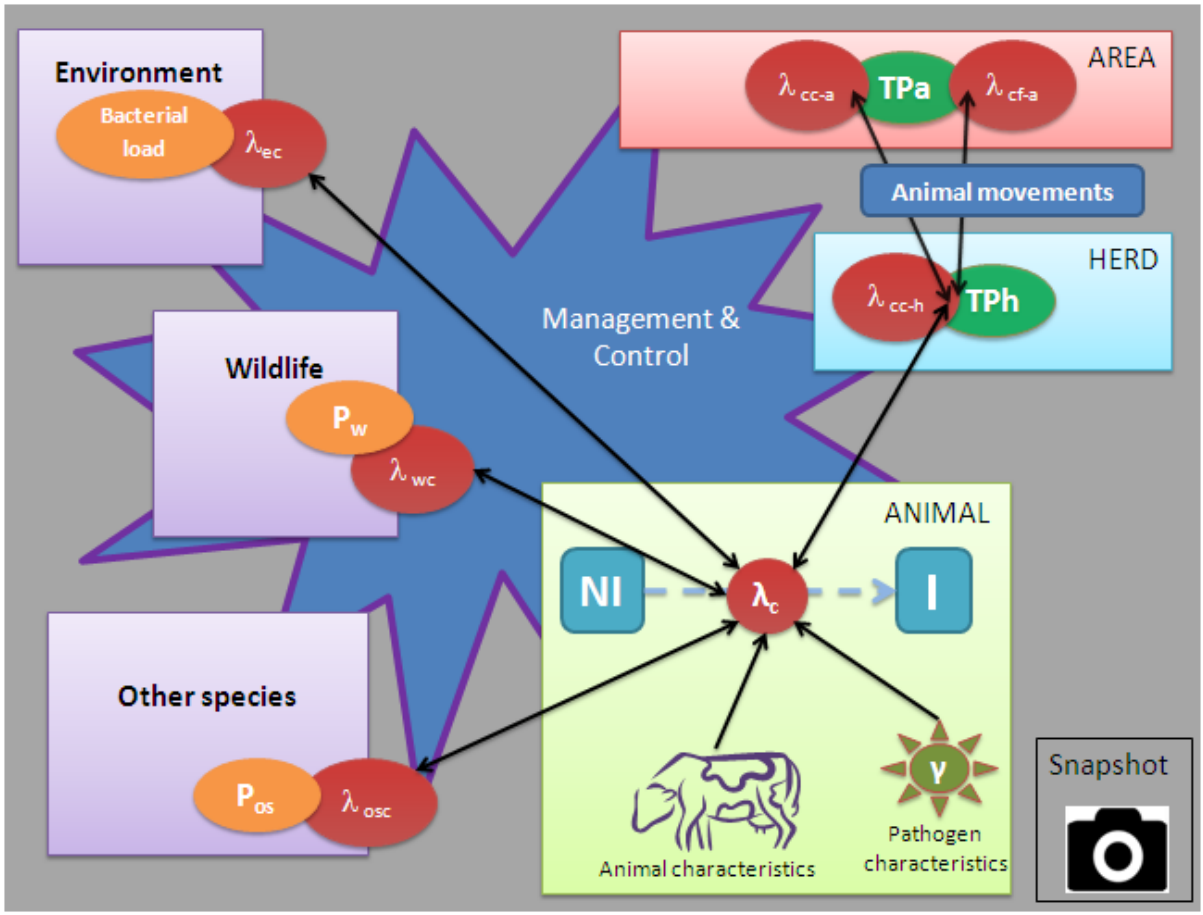
The sources of infection include:

- Cattle in the herd that are infected and infectious (including those that have been introduced from other herds; prevalence shown as true within-herd prevalence (TPh) and force of infection within a herd as $\lambda_{cc\text{-}h}$). A non-infected bovine within a herd may also become infected by direct contact with infected bovines from other herds in the area ($\lambda_{cc\text{-}a}$) (e.g. direct cattle–cattle contact between animals on neighbouring or shared pastures) or by an indirect contact (non-animal, e.g. via fomites or veterinarians) ($\lambda_{cf\text{-}a}$) from other infected herds in the area (prevalence shown as true area prevalence TPa).

- Other domestic species (e.g. sheep, goats, farmed game and humans) infected with MTBC (P_{os}, λ_{osc}) (Broughan et al., 2013a).

- Wildlife (such as badgers or wild boar) infected and infectious with MTBC (P_w, λ_{wc}) (Naranjo et al., 2008; Fitzgerald and Kaneene, 2013).

- The environment contaminated with MTBC (bacterial load, λ_{ec}). The number of bacteria in the environment depends on the number deposited by infected animals previously present (previous outbreaks) and the inactivation rate of the bacteria (Courtenay et al., 2006).



Legend: NI, non-infected animal; I, infected animal; λ_c, force of infection for cattle; TPh, true within-herd prevalence; TPa, true area prevalence; $\lambda_{cc\text{-}h}$, force of infection within a cattle herd; $\lambda_{cc\text{-}a}$, force of infection resulting from animal contacts with other cattle herds in the area; $\lambda_{cf\text{-}a}$, force of infection resulting from fomites of other cattle herds in the area; λ_{osc}, force of infection resulting from other domestic species; λ_{wc}, force of infection resulting from wildlife; λ_{ec}, force of infection resulting from the environment; P_{os}, prevalence in other domestic species; P_w, prevalence in wildlife.

Figure 3: Compartmental model on 'force of infection', where cattle is chosen as the central point and at a specific moment (snapshot)

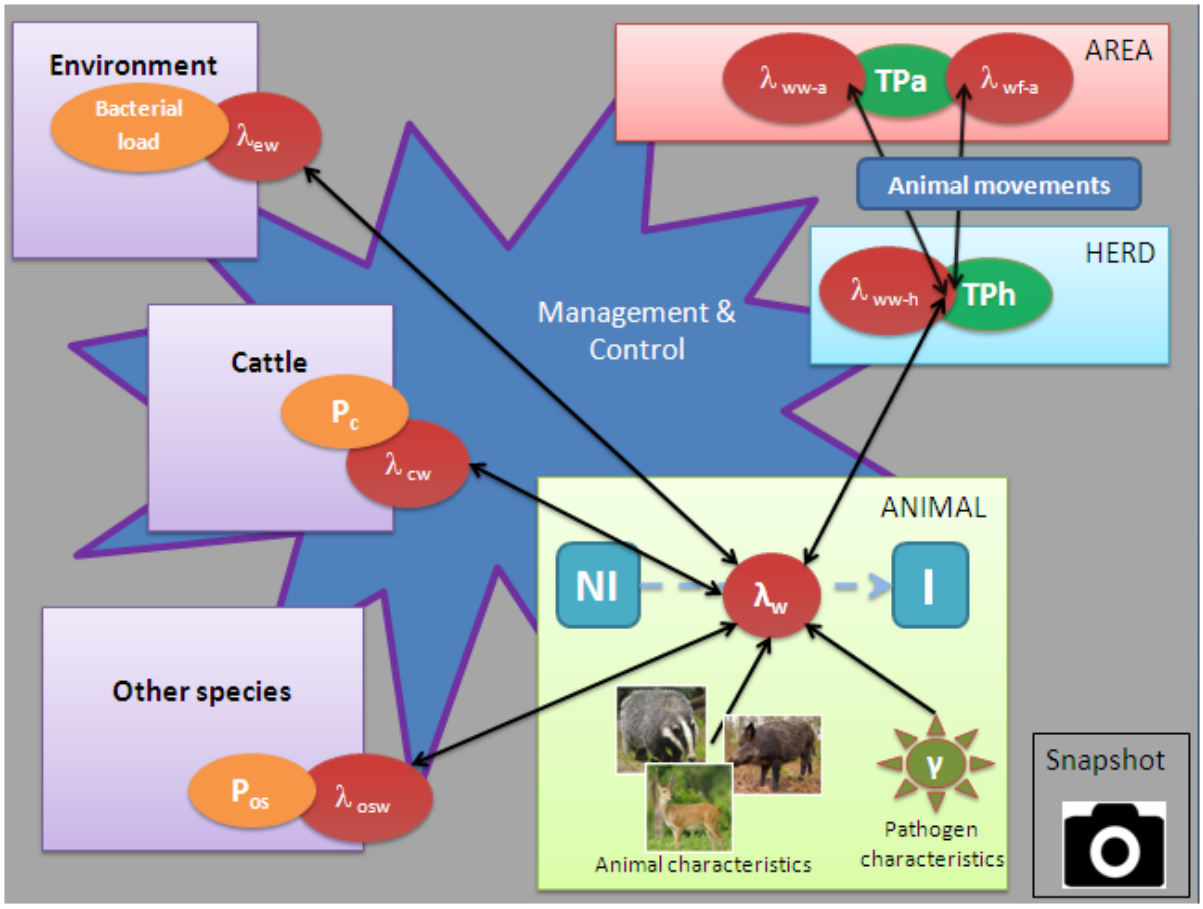
The force of infection may also be influenced by:

- Characteristics of the animal, such as breed, age and immune response (e.g. some animals can become anergic and could become supershedders, whereas others may remain latent for some time as a result of an efficient immune response) (Barthel et al., 2000; Houlihan et al., 2008).

- Characteristics of the pathogen with regard to pathogenicity and virulence of a specific strain. For instance, some *M. bovis* genotypes may lead to a greater proportion of infected animals with lesions, which may increase bacterial shedding in a herd (Wright et al., 2013). On the other hand, concurrent infections with related pathogens such as *M. avium* subsp. *paratuberculosis*, or any microbe with shared epitopes, such as non-tuberculous, environmental mycobacteria, may elicit cross-reactive immune responses which indirectly influence the force of infection (Alvarez et al., 2009).

- Management and control characteristics that may influence the contact rate between bovines (e.g. separation of young stock and adult cattle) and movements/trade of bovines or contacts between cattle and wildlife (e.g. indoor housing of dairy cattle) (Ward et al., 2006; Judge et al., 2011). On the other hand, management and control measures could also affect

transmission among wildlife or among other species. Specific bTB control measures will also have an effect on transmission. For example, culling of infected animals will reduce the within-herd prevalence and, consequently, reduce the force of infection.



Legend: NI, non-infected animal; I, infected animal; λ_w, force of infection for wildlife; TPh, true within-herd prevalence; TPa, true area prevalence; $\lambda_{ww\text{-}h}$, force of infection within a wildlife herd; $\lambda_{ww\text{-}a}$, force of infection resulting from animal contacts with other wildlife herds in the area; $\lambda_{wf\text{-}a}$, force of infection resulting from fomites of other wildlife herds in the area; λ_{osw}, force of infection resulting from other domestic species; λ_{cw}, force of infection resulting from cattle; λ_{ew}, force of infection resulting from the environment; P_{os}, prevalence in other domestic species; P_w, prevalence in wildlife

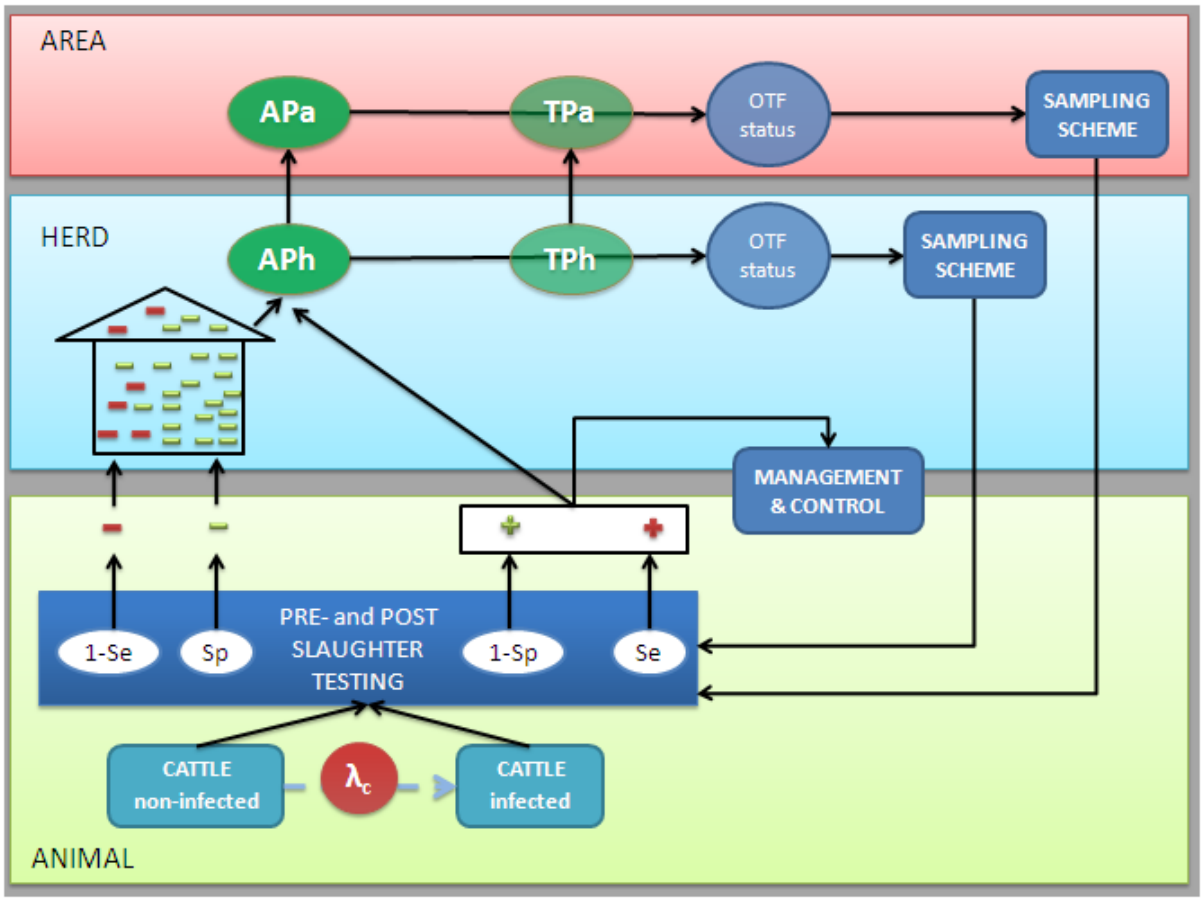
Figure 4: Compartmental model on 'force of infection', where wildlife is chosen as the central point and at a specific moment (snapshot)

For the other domestic species and wildlife, a similar transmission schematic can be constructed. An example is given for wildlife (Figure 3) in which the 'herd level' is considered to represent a group of animals belonging to the same social group.

2.3. Compartmental model on bovine tuberculosis testing

In this statement, 'bTB testing' refers to the testing procedures, composed of one or more diagnostic tests, that are in place in a given area and can be used for surveillance and may also be an integral part of control/eradication efforts. The model of the testing compartment consists of three levels: the animal, within-herd and area levels. At the animal level, non-infected cattle become infected according to the force of infection. In most instances, testing of animals comprises a pre-slaughter step and a post-slaughter step. Animals subjected to pre-slaughter tests (e.g. skin tests, interferon (IFN)-γ assays) are classified as positive, inconclusive or negative reactors, some of which will be falsely classified owing to the imperfect sensitivity and specificity of the testing procedures. Various tests can be combined to increase the bTB testing sensitivity (parallel combination) or specificity (serial

combination). The interpretation of the test results may be adapted (e.g. standard or severe interpretation of a skin test) for achieving the best possible sensitivity without compromising high specificity. Positive reactors to the pre-slaughter tests are slaughtered and investigated using post-slaughter tests (e.g. post-mortem inspection, bacteriology, polymerase chain reaction (PCR), histology) in order to confirm infection.



Legend: λ_c, force of infection; Se, sensitivity; Sp, sensitivity; APh, apparent within-herd prevalence; TPh, true within-herd prevalence, APa, apparent area prevalence; TPa, true area prevalence; OTF, official tuberculosis-free; green +, test-positive non-infected animal; green –, test-negative non-infected animal; red +, test-positive infected animal; red –, test-negative infected animal.

Figure 5: Compartmental model on bovine tuberculosis testing

The testing sensitivity (Se) is related to the probability of finding truly infected animals (red '+' symbol in Figure 4), whereas the specificity (Sp) is related to the probability of finding truly non-infected animals (green '–' symbol in Figure 4). Clearly, the definitions of bTB cases and non-cases determine the applicability, evaluation and comparison of the Se- and Sp-values and, consequently, the number of cases detected.

The proportion of test-positive individuals to tested (or existing) individuals determines the apparent within-herd prevalence (APh), which is time dependent and under the influence of opposite directed forces: within-herd spread (i.e. from infectious to non-infected animals), and ongoing control efforts (e.g. test and slaughter, as well as the removal of infected animals from the herd (natural death, slaughter and sales)).

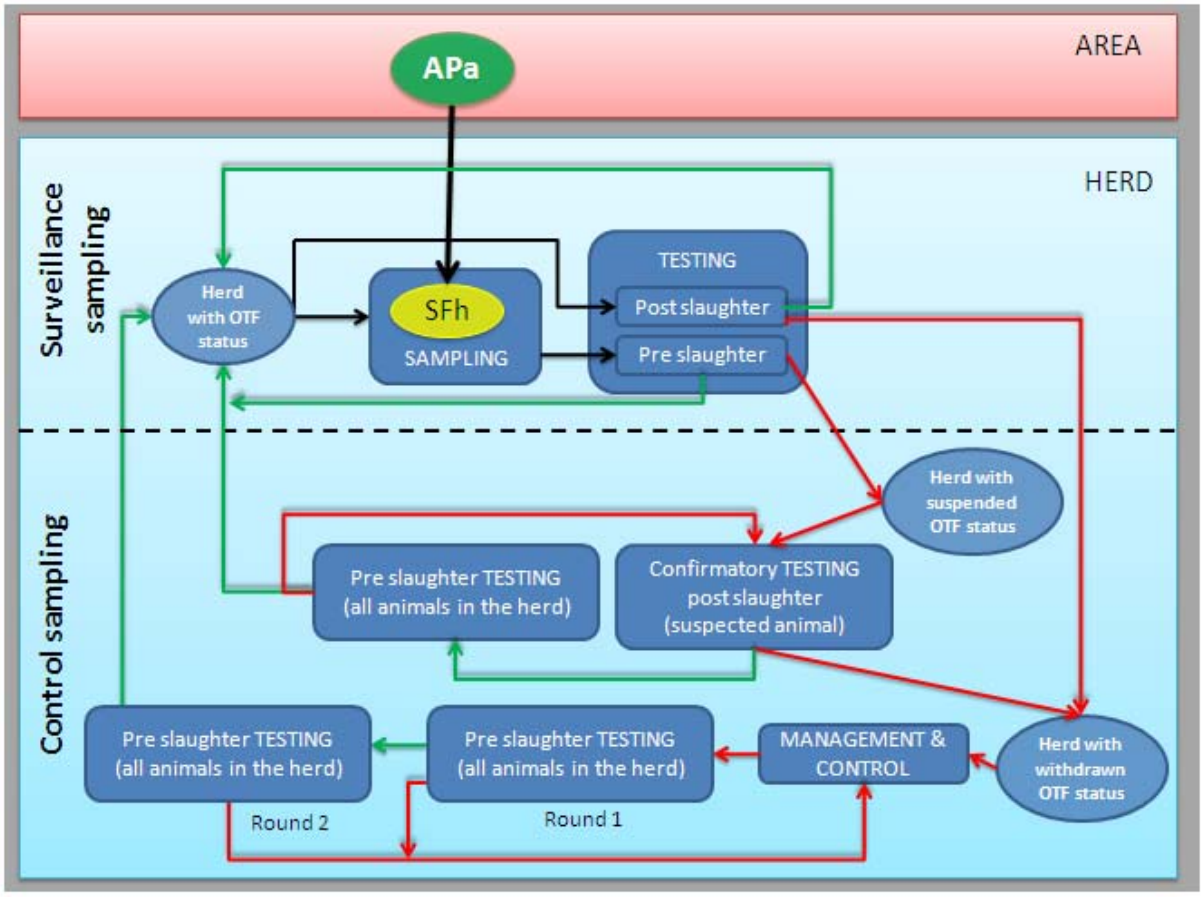
Using estimates of the sensitivity and specificity of the testing system applied, one can approximate the true within-herd prevalence (TPh) of bTB cases as defined by the test at the time of testing, while keeping in mind that the test sensitivity and specificity are themselves dependent on the distribution of

the disease stages in the herd. Similarly, at the area level, the apparent prevalence (APa) of bTB-case herds is determined by the test data collected from all herds in the region, and an estimate of the true prevalence (TPa) of bTB-case herds may be obtained by applying estimates of the overall sensitivity and specificity of the aggregated testing system for the region. Currently, the official status of herds and area (OTF or non-OTF) is based on the results of bTB testing performed according to the eradication programme applied in the corresponding area, which results in apparent prevalence rather than true prevalence estimates.

2.4. Compartmental model on the herd sampling scheme

Figure 5 summarises the processes of 'sampling', 'testing' (i.e. by applying one or more tests) and 'management and control' to maintain or regain the OTF herd status. Consecutive control sampling and testing of the whole herd, and removal of test-positive animals by 'management and control' actions, are applied for eradication of infection in a herd. For that purpose, an infected animal must be removed from the herd before it, on average, infects more than one other animal (i.e. if $R_0 < 1$). If $R_0 < 1$, the infection will fade out from the herd and the pathogen will be eliminated. Therefore, the time interval between different 'sampling and testing' rounds is crucial.

All culled/slaughtered animals are tested for bTB using one or more post-slaughter tests, and living animals of a herd over six weeks old are sampled for testing via one or more pre-slaughter tests at a frequency (SFh) that is dependent on the apparent area prevalence (APa) and the status of the herd (Figure 5). The herd retains its OTF status if all animals are test negative. However, when an animal is positive or has given inconclusive results (unresolved status) in a pre-slaughter test, the herd status will be suspended. The herd can regain the OTF status if post-slaughter testing cannot confirm bTB infection in the suspected animal and all other animals are negative in pre-slaughter testing. The herd OTF status will be withdrawn when an animal is positive for a post-slaughter test, thus confirming bTB infection in that animal, or when an epidemiological enquiry establishes the likelihood of infection or for any other reasons considered necessary. The herd status remains withdrawn until at least two rounds of pre-slaughter testing of all animals in the herd are negative.



Legend: Green arrow, negative test result; red arrow, positive test result; APa, apparent area prevalence; OTF, official tuberculosis-free; SFh, herd sampling frequency

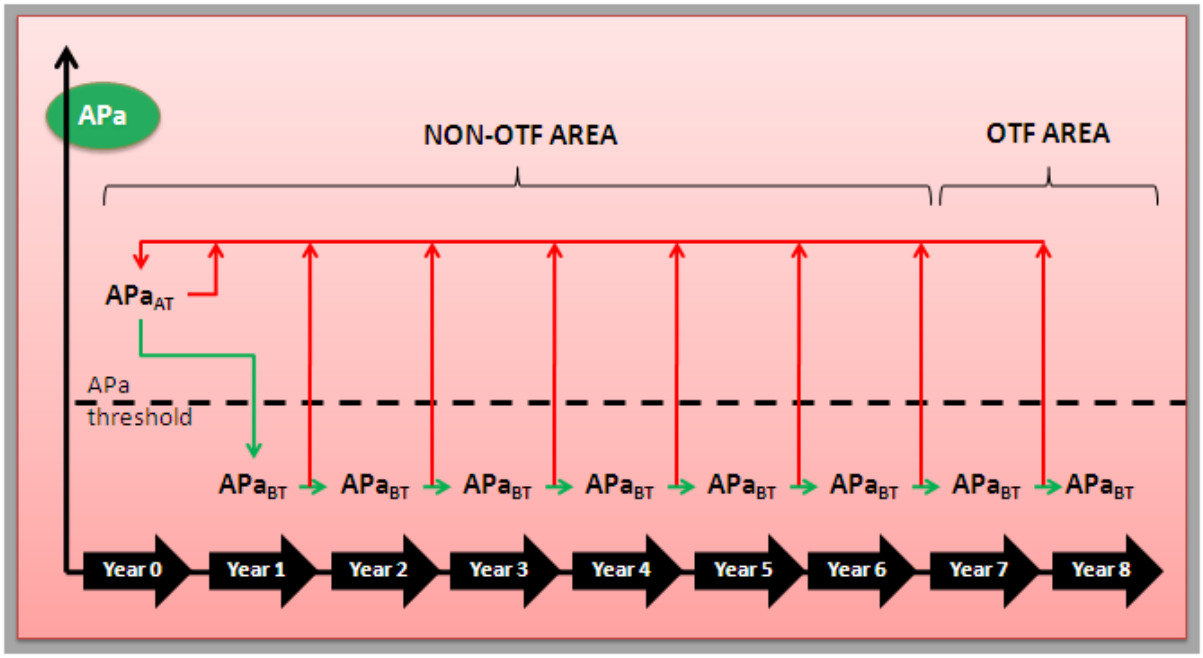
Figure 6: Detailed model on 'herd sampling'

Minimum and maximum intervals between sampling and testing in infected herds take into account the biology of a tuberculosis infection and the cattle immune response to *M. bovis*. Approximately one month after an experimental infection, animals start to become positive in the tests based on cellular immune responses (e.g. IFN-γ assay) and lesions may be detected (Buddle et al., 1995; Cassidy et al., 1998; de la Rua-Domenech et al., 2006). However, it is assumed that natural exposure to MTBC occurs at lower doses and needs more time to become positive in pre- and/or post-slaughter testing. Testing intervals of six months are applied (enough time to develop a detectable immune response in infected animals not captured in the previous testing round, and short enough to minimise the risk of new infections in the herd).

In an OTF area, routine herd testing is usually not applied, or longer intervals between tests are used (e.g. every four years), and surveillance relies mainly on meat inspection (detection of tuberculosis suspected lesions). The sensitivity of surveillance should be good enough to detect (re)introduction of infection in herds at an early stage, and, in the case of a herd breakdown, tracing of in-contact herds should allow prompt control of further spread.

2.5. Compartmental model on the area sampling scheme

Each year, the apparent area prevalence (APa) is determined and is analysed whether it is above or below the threshold (APa_{AT} or APa_{BT}, respectively, in Figure 6), currently defined as 0.1 %. When an area achieves an APa below 0.1 % and a proportion of 99.9 % OTF herds over six consecutive years, it fulfils the officially defined requirements to obtain and retain the area OTF status (Figure 6).



Legend: APa, apparent area prevalence; OTF, official tuberculosis-free; APa_{AT}, APa above the threshold; APa_{BT}, APa below the threshold; green arrow, obtained APa is below the threshold; red arrow, obtained APa is above the threshold

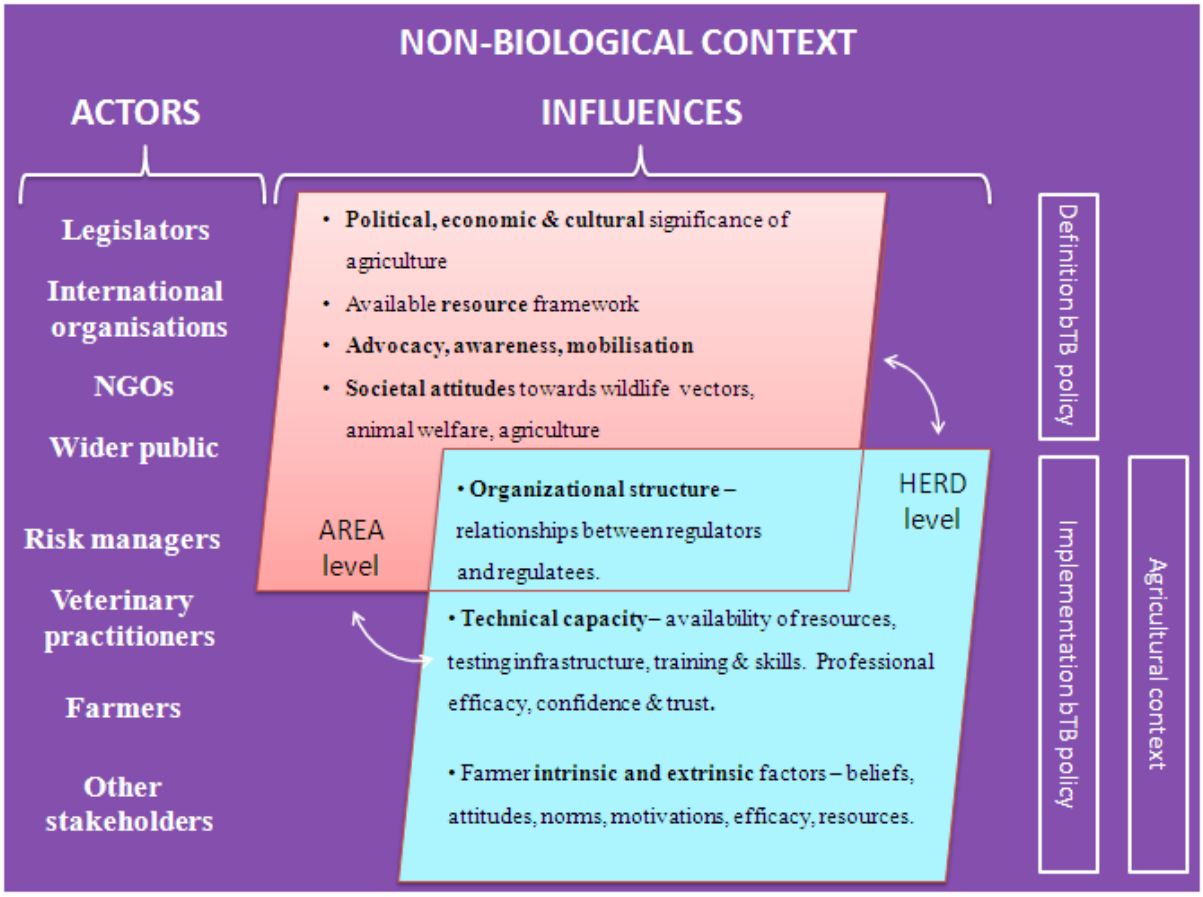
Figure 7: Compartmental model of 'area sampling'

2.6. Non-biological context

Understanding factors affecting bTB control and eradication requires taking into account a broader non-biological, sociological context, as they influence bTB infection, detection and control (Pfeiffer, 2013). Therefore, these factors are taken into account in the conceptual framework and social scientists joined the Working Group to prepare this statement. Figure 7 conceptualises the actors and influences that could affect bTB at the spatial scales of 'area' and 'herd'.

Before official rules defining the bTB policy are adopted, bTB policy is subject to international standards and national rules that are influenced by policy makers, non-governmental actors and public actors. Once official rules regulating the bTB policy are in place, their implementation takes place at the area and herd levels and can be subjected to the direct and indirect influences of the motivations and attitudes of veterinary practitioners, farmers, risk managers and other stakeholders in the daily management of the infection at the herd level. It is also at the herd level that decisions taken by farmers provide the agricultural context for the application of disease control measures. For instance, the animal husbandry infrastructure might have consequences for the implementation of specific biosecurity measures at the farm. Farmers' actions have direct and indirect consequences for disease transmission.

The area and herd levels are related and interconnected, but the influences that determine what happens within the herds will vary between areas. As a result, non-biological factors will lead to variations in the way bTB policy is enacted and implemented. These variations can have positive and negative consequences for bTB, for example the sub-optimal application and interpretation of diagnostic tests, poor-quality disease management and/or effective management of disease in wildlife reservoirs. The remainder of this section provides details of the range of influences and actors at these levels.



Legend: NGOs, non-governmental organisations; bTB, bovine tuberculosis

Figure 8: Actors and influences of the non-biological context on bovine tuberculosis.

2.6.1. Influences at the area level

The design and effectiveness of a bTB eradication plan will be dependent upon a range of interconnected factors.

Firstly, drawing on the political framework of individual countries, the economic and cultural significance of agriculture and the potential impact of its contribution to gross domestic product may affect the significance which governments attach to a disease and its control. Control/eradication programmes, and the resources dedicated to them, may vary with changing political priorities and preferences.

Secondly, the emphasis placed on bTB control programmes may vary as a result of the influence of international organisations. For example, the EU's common agricultural policy has an impact on both the economics of the livestock industry and the farming systems. Where organisations such as the EU can provide additional resources to Member States, these influences may shape the operations of bTB control.

Thirdly, the ability of various non-governmental organisations (NGOs) to influence policy will also affect the shape of the rules regulating the bTB policy. NGOs can play a linking role between the stakeholders and policy makers, and this role is shaped by their advocacy, awareness raising and mobilisation methods.

Fourthly, the societal attitudes of the general public towards wildlife, animal welfare and the wider agricultural context combine to influence the shape and form of policy approaches. For example,

where wildlife vectors play a role in the transmission of bTB, the general public's attitude to different wildlife species may prevent effective implementation of disease control programmes and controlling disease, limit the effectiveness of scientific investigation of different control methods or enhance the influential role of NGOs in shaping policy. This importance and significance that the public gives to wildlife is cultural and therefore may change over time. bTB is no longer a major public health concern, as was the case in the 1950s; therefore, awareness and significance of the infection and its various components among the general public has changed, with possible implications regarding efforts needed for motivation for disease eradication.

2.6.2. Influences at the herd level

The herd is the main level at which policy is implemented. Whilst policy might be designed at the area level, the policy-implementation distance means that, in practice, policies are affected by a range of dynamics and influences when implemented on a day-to-day level. The key factors are as follows:

1. Organisational structure: the way regulators and bTB testers behave is directly related to the relationships that they have with the people they are regulating (regulatees, in this case farmers). For instance, Enticott (2014) described how changes to 'relational distance' in animal disease regulation have led to closer relationships between regulators (veterinarians) and regulatees (farmers), which in turn has led to a departure from standardised disease regulation to approaches that emphasise greater flexibility and judgement. The ways in which policy is interpreted and implemented on the ground will result in varying outcomes, with resulting consequences for the progress of the eradication programmes. For example, the influence of the client–veterinarian relationship can present challenges in the testing environment and may influence testing outcomes (Meskill et al., 2013). The organisational structure may be shaped by the motivations of the various actors—this motivation may change over time, depending on the framing of the disease (within policy and the wider public discourse) as an area of concern, and economic incentives and disincentives at a farm level.

2. Technical capacity: the interpretation and implementation of policy is shaped by the capacity of the technical environment, that is the form and availability of resources in supporting the implementation environment; the testing infrastructure adopted and utilised in the laboratory and field environments; and the training, skills and experience of those involved in the implementation of control strategies in these environments. Perceived deficiencies in skills and training can impact professional self-confidence on efficacy (one's perception of one's ability to complete a course of action), professional levels of confidence and trust in how a disease is being eradicated and, thus, the ability to act collectively in tackling disease eradication at herd and area levels (Meskill et al., 2013). A lack of trust in the diagnostic tests among farmers will undermine the success of the programme. For example, this may result from the absence of correlation between test outcomes and slaughterhouse observations. This is mostly the case in low-prevalence areas, where the tests have a lower positive predictive value, and a number of false positives may be due to infections with non-tuberculous mycobacteria. A concern regarding trust might influence farmer–veterinarian interactions, with farmers often trying to choose specific veterinarians that they believe will produce the desired outcome.

3. Farmer intrinsic and extrinsic influences: the implementation of bTB regulations will also be affected by farmers' beliefs, attitudes and behaviours in relation to them.

Intrinsic influences relate to farmers' own behavioural beliefs and attitudes towards bTB; their perception of social norms of behaviour in relation to disease management and farming in general; and their feelings of self-efficacy—the perception about being able to do anything about the disease—may affect their behavioural commitment to a bTB eradication programme. Typically, farmer motivations are identity oriented, financially oriented and/or animal welfare oriented (Borgen and Skarstad, 2007; DEFRA, 2008; Beekhuis-Gibbon et al., 2011). Sustaining farmer motivations in implementing

biosecurity measures is necessary; however, recurrent outbreaks or absence of outbreaks on a farm may affect the farmer's and veterinarian's motivation levels and, thus, the implementation of efficient on-farm biosecurity measures, such as specific buying and breeding practices.

Farmers' past experiences and their knowledge of disease control options influence their beliefs towards the disease, and its control; experience of a previous bTB outbreak can alter farmers' attitudes to certain bTB-related issues, such as their perceptions of movement controls and the need for more information on control measures. These intrinsic influences combine to shape, for example, farmer attitudes towards specific control measures (Warren et al., 2013). Overall, 'lay epidemiologies'—that is farmers own constructed understanding and interpretation of bTB infection, detection and control based on their social and geographical understandings of disease control—are formed. These are based on farmers' own experiences of bTB, their knowledge of its causes, their interactions and beliefs concerning the experiences of their farming peers and how they perceive and trust the regulatory framework and the effectiveness of biosecurity measures (Enticott, 2008).

The implementation of bTB eradication measures will also draw on the context of farmers' wider attitudes and understanding towards risk, risk management and related non-statutory disease control practices. These inform their perceived sense of responsibility in implementing general biosecurity measures on the farm (Gunn et al., 2008) and, as a result, there will be variation in farmers' behaviour and attitudes between those that are risk takers and those that comply with recommended guidelines. Blame is often attributed to wildlife, testing issues or policy weakness, or is thought of simply as a matter of bad luck for the farm, rather than to gaps in the on-farm implementation or understanding of biosecurity measures. Whilst bTB testing may be compulsory under bTB eradication programmes, the implementation of other forms of non-statutory biosecurity activities may not be. This would include decisions on where to purchase cattle from, stock management decisions and attitudes towards other farming practices that may be risk factors for bTB. Many of these decisions may be taken independently of the disease context.

Independent of individual perceptions, **extrinsic influences** are the external factors that may influence, either by enhancing or inhibiting, the motivation to implement an eradication programme. These could include the availability of economic resources, the industry framework, and community norms of behaviour and stigmas that may also affect the decisions that farmers make. Both the intrinsic and extrinsic circumstances faced by farmers can be said to create an image of 'good farming' or the 'good farmer'; these represent a blending of how farmers perceive and take on the formal rules and practices established by the policy framework with their own intrinsic set of attitudes and beliefs. These influences may shape the implementation of disease control measures in a number of ways: farmers may dedicate more or less resources to handling facilities for disease control purposes; they may fail to isolate reactor cattle; and/or they may delay testing to limit costs and burden rather than optimise disease control conditions.

3. Transition from a conceptual framework to a parameterised model

3.1. Generating parameterised models

Modelling various bTB situations may lead to an improved understanding of the ways in which different factor combinations and interactions influence occurrence, surveillance outcomes and control efforts. The conceptual framework described in this statement is not intended for direct translation into a single mathematical model. However, it could help to understand the inputs that must be considered when assessing a specific risk question. In any given analysis, much of the complexity of the bTB episystem needs to be simplified and these simplifications should be made on the basis of an understanding of their impact on the model results. Similarly, the outputs of the model must also be viewed in context, with an awareness of the impact on other aspects of the episystem, beyond the immediate impact on observed prevalence (e.g. if testing schedules were lengthened, this would give more time for infected animals to become skin test positive, but also allow greater opportunities for transmission). Thus, the framework should be able to help with both the generation and the

interpretation of mathematical and statistical models (dealing with a specific component of the framework) designed to answer specific questions regarding bTB. A full model, incorporating all aspects of the bTB episystem, is unlikely to be robust, owing to the large number of parameters and variables, each of which bring their own uncertainty. Consequently, simple models, either providing a simplified view of large segments of the episystem or describing only part of it based on a specific risk/research question, are more likely to provide useful insights. Starting from the simplest possible models, complexity would be added if needed. The framework would also aid in the identification of the relevant data that must be compiled, in order to fulfil model requirements or to identify what types of modelling approaches would best utilise the data available.

In the sub-sections below, how a particular question relating to the force of infection can be answered in the context of the conceptual framework is described (section 3.2, Figure 8A), with some further illustrations on the non-biological context and testing of bTB (sections 3.3 and 3.4, Figure 8B and C). All examples are examined in greater detail in Appendices A–C. Finally, an example of a methodology is provided for modelling bTB test readings as a continuous dependent variable, which could be applied to examine the effect of 'biological' as well as 'non-biological' parameters (section 3.4 and Appendix D).

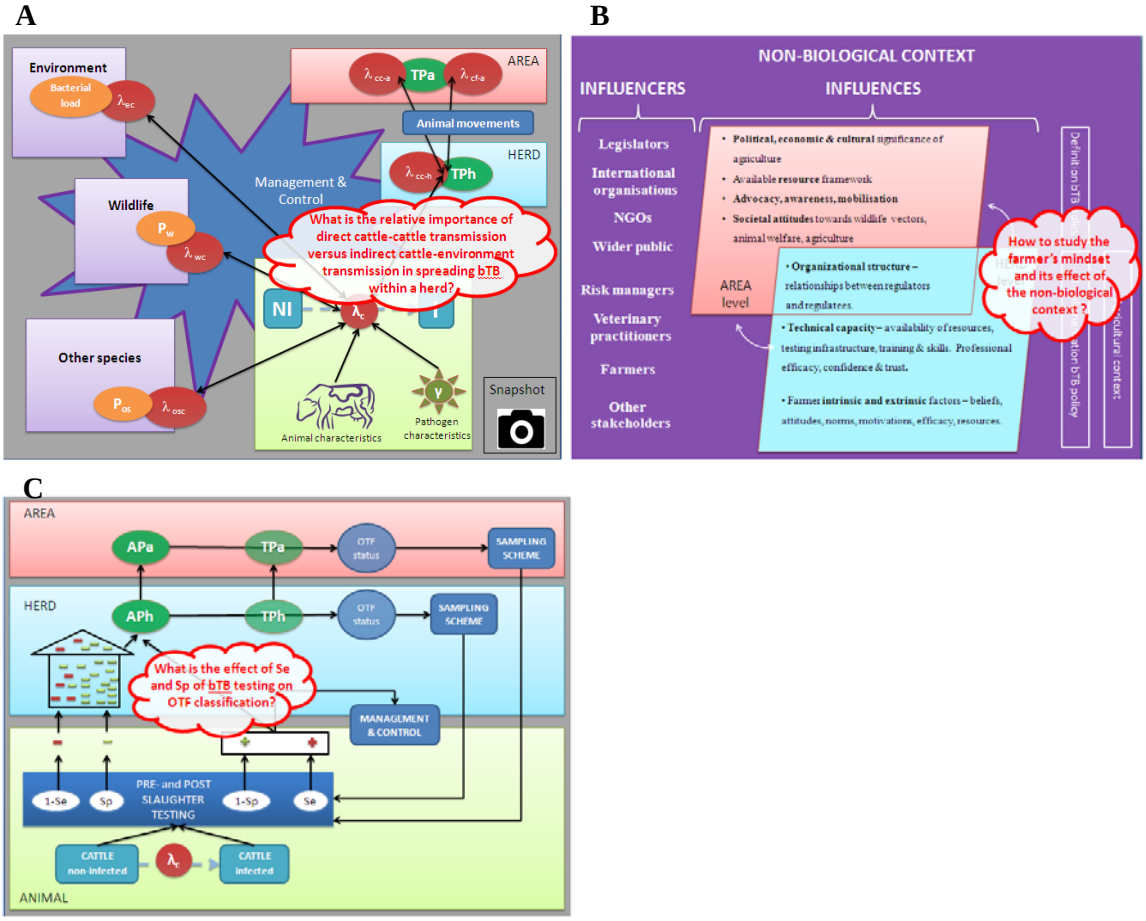


Figure 9: Example questions placed in the compartmental models 'force of infection' (A), the 'non-biological context' (B) and 'bTB testing and sampling' (C)

3.2. Force of infection

The example described in detail in Appendix A deals with the question: 'What is the relative importance of direct cattle–cattle transmission versus indirect cattle–environment transmission in spreading bTB within a herd?'.

The situation may refer to a field outbreak in which the stakeholders wonder if it is likely that an observed outbreak is caused solely by cattle-to-cattle transmission within the herd or if some external source has contributed to the outbreak.

Looking at Figure 2 or Figure 8A, the components involved would be the transmission routes and parameters linking the animal with the herd and with the environment (assuming that no contact to other infected herds has occurred).

The result would help the herd owner, the veterinarian and the authorities to evaluate whether environmental transmission is a contributory source to new herd reactors or if, alternatively, a continued focus on within-herd contacts is a sufficient control strategy. The results of the model question might also help other neighbouring herds served by the veterinarian to estimate the importance of environmental transmission in the locality in question.

3.3. Non-biological context

The model outlined in Appendix B deals with the questions related to the importance of farmers' behaviours to achieve full compliance with the strategy agreed for preventing or eradicating infection at the herd level.

Looking at Figure 7 or Figure 8B, the components involved relate to influences within the non-biological or sociological context at the farm level.

Appendix B describes a framework (Rules Education Social pressure Economic stimuli Tools (RESET)) for changing farmers' behaviours, that was adapted from van Woerkum et al. (1999) and Leeuwis (2004) and was described earlier in a different form regarding mastitis by Lam et al. (2011) and Jansen and Lam (2012). This framework is based on the recognition that influencing farmers' mindsets can be more effective than just providing technical information. The RESET framework focuses on elements of farmers' mindsets that are influential in disease control, including perceived threats (do I have a problem?) and perceived efficacy of preventative measures (can I solve the problem easily?). However, it must be noted that the contexts of mastitis and bTB are different and hence the methodology should be adapted before it can be applied to bTB. Particularly, additional understanding is required with respect to the interface between the rules (i.e. enforcement and compulsory requirements) and the other key components of the framework (i.e. education, applying social pressure, etc.).

3.4. Testing and sampling

The example described in detail in Appendix C deals with questions related to the frequency of false-negative animals and false-negative herds in countries with OTF status, which apply the minimum prescribed test strategy only (meat inspection surveillance).

The example refers to Figure 4 and Figure 8C, in which the green '+' symbols refer to false-positive animal tests and how these enter into the apparent and true prevalence estimates at the herd and area levels. The example also considers how false-negative animal tests relate to the OTF status of herds and areas.

The results would be relevant, for example, to considerations on the possible causes for the continued occurrence of low-prevalence regional bTB outbreaks within some OTF countries.

3.5. Example of a methodology for modelling bovine tuberculosis test readings as a continuous dependent variable, which could be applied to examine 'biological' as well as 'non-biological' parameters

Traditionally, continuous bTB test outcomes such as millimetre skin thickness and optical density (OD) readings from interferon enzyme-linked immunosorbent assay (ELISA) have been converted to dichotomous variables, such as 'positive'/'negative' or 'reactor'/'non-reactor', using specific cut-off

values defined in national or international testing guidelines. This is a necessary procedure, when making cow-side decisions on the management of a potentially infected animal. A common cut-off value, however, is a simplification of reality. When dealing with different herds, regions and countries, for example, the environmental mycobacteria or pathogenic mycobacteria such as *M. paratuberculosis* may have different prevalence and/or immunological effects in cattle. The methodology presented in Appendix D allows for statistical modelling of prevalence data using a continuous variable expressing the bTB test results without the need for a pre-defined common cut-off value. The methodology can be used to assess the effects of biological as well as of non-biological parameters on bTB prevalence.

4. Discussion

4.1. Benefits of a conceptual framework on bovine tuberculosis

This statement describes a conceptual framework that could help to identify the main factors involved in understanding bTB epidemiology, and how these factors interact. The current situation in Europe regarding bTB infection, detection and control is heterogeneous. Some Member States (or parts thereof) are OTF and have not seen bTB for a very long time; some Member States are OTF with sporadic introductions; some Member States are OTF, but experience outbreaks in 'hotspot' areas; some Member States are non-OTF, but are clearly on the way towards OTF; and some Member States are likely to remain non-OTF for the coming years. This heterogeneity is partly a result of the disease (detection and control) history in the country (e.g. Abernethy et al., 2013), but it is also strongly affected by the complexity of the disease epidemiology and variation in human behaviour. Today, advances in surveillance methodology have provided robust and flexible classification alternatives, such as 'substantiation of freedom from disease' based on well-defined statistical procedures and parameters (design prevalence, confidence level, risk of new introduction) (Cameron and Baldock, 1998; Martin et al., 2007a, b; Cameron, 2012).

Irrespective of the local situation, reducing transmission of bTB is essential for controlling or ideally eradicating bTB. Therefore, it is important to identify and subsequently quantify or at least rank the relevant risk factors at each level in order to target management and control of the risk factors with the highest relative importance. Some risk factors for bTB infection are well established and documented (e.g. cattle movements, infected neighbouring farms), whereas others have emerged or have been recognised more recently. The latter risk factors may be associated with local wildlife population dynamics and their interactions with cattle (see below); they may also include the role of other domestic species (e.g. small ruminants) or they may be related to the wide socio-economic changes in Europe over the last 50 years (see non-biological context, section 2.6), which have resulted in increasing farm sizes and changes in farming and husbandry practices. Moreover, it is important to understand how these risk factors interact, and how their relative roles and the size of their interactions may vary in different epidemiological situations.

During a control and eradication programme, the number of infected animals changes. This will affect the probability of new infections and may also change the effect of other risk factors. This requires the availability of good tools to characterise the dynamics of risk factors and to determine the bTB infection status of animals, herds and areas.

This conceptual framework is a tool that can help in understanding the differences in epidemiological situations and answering risk questions, taking account of the biological and non-biological context. Depending on the risk question under consideration, specific parts of this framework could be translated into parameterised models as illustrated in the examples provided in Appendices A, C and D.

The following discussion presents some examples of how this conceptual framework could facilitate a better understanding of important issues relevant to bTB infection, detection and control (wildlife, testing and the non-biological context). It also considers challenges to be addressed during the transition from the conceptual framework to parameterised models. Scientific studies might also

benefit from applying the conceptual framework (e.g. in the planning and supervision of experiments, surveys, field studies, data-register analyses) in order to approach the design of the study, bearing in mind all factors influencing the bTB problem.

4.2. The conceptual framework and the role of wildlife in bovine tuberculosis epidemiology

In the early years of bTB control in Europe (1950–1960s), wildlife was not identified as a significant problem for bTB eradication; the affected wildlife species were mostly considered spill-over hosts. There is a growing body of evidence, from the UK and Ireland initially and from Spain and France more recently, that wildlife plays important roles at the local level in bTB transmission and persistence and, in some instances, may act as a true maintenance reservoir of bTB (reviewed in ANSES 2011; Schoning et al., 2013). The changing role of wildlife in bTB epidemiology has been related to the increasing densities of several wildlife species in Europe over the past 20 years (ANSES, 2011); however, the relative importance of wildlife and cattle in bTB transmission and persistence is not yet clear and is probably highly variable depending on local environmental characteristics. According to the framework, in order to assess this relative role, it would be important to have estimates of transmission rates in the bTB episystem. This includes the transmission rate not only from wildlife to cattle, but also within wildlife and from cattle to wildlife. In combination with the transmission rate within cattle, these estimates will help elucidate the contribution of wildlife to bTB persistence in cattle populations. Prevalence estimates in wildlife are difficult to obtain owing to the limitations of diagnostic tests and limited sampling (Broughan et al., 2013b) and estimates of wildlife species densities and information on interactions between wildlife and cattle are similarly difficult to gather. It should also be noted that, wherever the disease has spread and established in wildlife, achievement of eradication at the area level is in general considered difficult and remains hypothetical in the absence of active and systematic wildlife surveillance and control, including effective biosecurity barriers.

4.3. The conceptual framework and bovine tuberculosis testing

There is a considerable variation across Europe in the design of bTB eradication programmes, which is further complicated by differences in bTB diagnostic test protocols and diagnostic criteria used in different areas and hence differences in sensitivity and specificity of testing regimes used. This is due to differences in technical aspects of testing, but also bespoke adaptations of approaches to local conditions. For instance, in relation to tuberculin skin tests, tuberculins with different potency are being used, with single (only bovine tuberculin) and comparative (using bovine and avian tuberculin) tests being used depending on the local exposure to environmental mycobacteria, and the need for greater specificity (especially important in low-incidence regions) or sensitivity (more important in high-incidence regions). In addition, variation may arise from injection or reading in cattle because the animals are not always easy to handle. On the other hand, the sensitivity and specificity of bTB testing could be affected by the local biological context (e.g. in addition to the prevalence of environmental mycobacteria, concurrent vaccination or disease and genetic predisposition to react to tuberculin skin test may be factors (Amos et al., 2013; Coad et al., 2013)). Thus, even a harmonised bTB testing procedure applied throughout the EU is likely to result in significantly different testing regime characteristics across areas and indeed may also change over time. Interpretation of test results is also influenced by the case definition, the bTB prevalence and the history in the herd and/or area. Taken together, this means that it is difficult to compare data between areas. EFSA collected data in preparation for the scientific opinion on IFN-γ testing, but was not able to combine data from different Member States because of the large variation in test protocols, as this test is not yet standardised at either EU or World Organisation for Animal Health (OIE) level (EFSA AHAW Panel, 2012). The conceptual framework could be helpful to achieve a better description of both the technical and the local testing conditions (taking into account local estimates of test characteristics and true prevalence) and hereby facilitate a better understanding of bTB epidemiology across areas and emphasise the need for caution in interpreting variations in prevalence across Member States.

What is considered optimal for testing regime and their associated sensitivities and specificities depends on the goal of testing, because the latter determines the costs associated with false-positive and false-negative results. High sensitivity is important to guarantee safe trade, but, if associated with

limited specificity, will not document freedom from bTB (because of false-positive findings). Consequently, the requirement for the testing system may be different for different stakeholders. The framework shows how the testing protocols affect, on the one hand, the system of granting and retaining freedom and, on the other hand, the transmission within infected populations (by allowing fewer or more false-negative animals to continue spreading bTB).

The optimal balance of sensitivity and specificity depends on the purpose of the testing. If a herd is infected and minimising the occurrence of bTB is the goal, the test sensitivity is most important, even if this implies that false-positive animals will be culled. To achieve a high sensitivity, tests can be combined in parallel: culling animals when positive in one or both tests. However, when the herd comes close to freedom from bTB, the vast majority of the positive animals will be false positives and turn out negative at post-mortem. At that stage, motivation problems may arise among farmers and veterinarians. Such concerns might be taken into account and the testing specificity could be increased (e.g. use of single intradermal comparative cervical tuberculin (SICCT) serial testing as part of approved experimental protocols). A consequence may be, however, that it takes a long time before the last infected animal is culled. If a herd or an area is bTB free, the rate of false positives has to be low, because otherwise trade will not be possible. From the perspective of the importing area, the predictive value of a negative test result is important, which depends not only on the test performance, but also on the a priori probability of an infected animal being present. The latter depends on the prevalence in the country and varies between infected regions, OTF areas with infections and OTF areas without infections. The framework, when converting the testing part to a parameterised model (taking into account local estimates of test characteristics and true prevalence) would allow for a uniform interpretation of risk estimates across Member States, despite the above-mentioned differences.

As outlined in the beginning of this discussion, advances in surveillance methodology have provided robust and flexible classification procedures for 'substantiation of freedom from disease' based on well-defined statistical procedures and parameters. These principles have been implemented in other EU regulations (e.g. concerning measures for the control of *Echinococcus multilocularis* infection in dogs (Commission delegated regulation (EU) No 1152/2011)). An important difference to the OTF classification is that introducing a 'freedom from disease' approach in bTB control strategies would specify the confidence level required (e.g. 95 %) to achieve and maintain the 'disease-free status' of a Member State or part of a Member State, based on a quantitative evaluation of the surveillance data being sampled. This approach has also been used in the EFSA scientific opinion on the public health hazards to be covered by inspection of meat (bovine animals), in evaluating the effect of visual inspection on the bTB surveillance in OTF countries (EFSA BIOHAZ Panel, 2013). The use of a 'freedom from disease' approach would also introduce a formal distinction between areas with a 'substantiated freedom from disease' as opposed to areas with an apparent prevalence below a specific threshold.

4.4. The conceptual framework and the non-biological context of bovine tuberculosis

Assessments of risk questions related to bTB should consider both the biological and non-biological factors involved, since they are interconnected. For instance, culling of false-positive animals could result in a loss of trust of farmers and veterinarians in diagnostic methods and subsequently influence their motivation to comply with the sampling and testing programme. The limited direct impact of infection on production at herd level (meat and milk) in contrast with the severe indirect economic consequences of a herd breakdown could also influence the farmer's motivation. The non-biological context may also influence bTB transmission within wildlife or the relative importance of wildlife on the rate of bTB infection. For example, providing food and water to wildlife, a frequent management measure implemented by hunters, may result in animal aggregation at points of feeding/watering. Such situations are recognised as an important risk factor for bTB transmission (Schoning et al., 2013). Conversely, distribution by farmers of mineral complements to cattle on pastures may attract some wild species onto the pastures. These examples show that the complex behavioural aspects of different stakeholders in relation to bTB should be investigated by incorporating a social science analysis in

bTB-related risk assessments. This conceptual framework provides a holistic view on bTB that could facilitate (1) the collaboration between biological scientists and social scientists and (2) the communication between scientists, risk assessors, risk managers and all other stakeholders. However, information should be presented in such a manner that policy makers can clearly distinguish the scientific (biological and non-biological) and other (e.g. cultural and political) aspects.

Thus far, examples of social science research include telephone surveys (e.g. to examine farmers' confidence in badger vaccine programmes (Enticott et al., 2012)); face-to-face interviews (e.g. to assess farmers' attitudes towards badger culling (Warren et al., 2013)); focus groups with stakeholders (e.g. to identify challenges to quality testing (Meskill et al., 2013)); semi-structured interviews and participant observation (e.g. to explore farmers' understanding of bTB in farmed cattle and their reasons for biosecurity implementation (Enticott, 2008; Enticott and Vanclay, 2011)); and other surveys (e.g. to gather farmers' responses to pre-movement testing for bTB (Christley et al., 2011)). Other areas of study, such as general biosecurity behaviour, have used both quantitative and qualitative methods to assess attitudes and knowledge of general biosecurity measures. For example, stratified telephone surveys were used to determine the impacts of a priori determinants of biosecurity behaviours of farmers in Great Britain (Toma et al., 2013). A mixed-method approach (focus groups and surveys) was used to measure the different attitudes of farmers, veterinary practitioners and industry members, constraining improvement in biosecurity (Gunn et al., 2008). Other examples are drawn from areas of disease control such as bovine mastitis; in one case, self-reported surveys were used to determine the extent to which farmers' attitudes, as opposed to farmers' behaviours, accounted for variation in mastitis incidence on Dutch dairy farms (Jansen et al., 2009).

4.5. Possible challenges in the transition of the conceptual framework into parameterised models

Whereas this framework describes the relations in the bTB episystem in a qualitative way, for specific risk questions, appropriate elements of the framework can be isolated and translated to a specific model to answer the question. Problems that may arise are mostly related to sparse information available for some parts of the framework, hampering reliable parameterisation of models. As mentioned above, information on the effect of non-biological factors in the models is limited and, moreover, available information does not always allows for straightforward inclusion in risk analysis. In addition, information on transmission parameters is still limited, in particular in relation to wildlife and non-cattle domestic species. Finally, more reliable information on performance characteristics of test systems would increase the possibility to translate the framework into parameterised models.

4.6. Concluding remarks

The conceptual framework as presented here intends to enhance the basic understanding of the complex biological and non-biological background which influences the epidemiology of bTB in Europe and leads to the current diverse levels of bTB in different Member States. The framework aims to facilitate the identification of factors influencing bTB infection, detection and control considering a specific local, a national or the European situation. Future research and risk assessment taking the identified factors into account and using multidisciplinary approaches could enhance the understanding of how these factors interact with each other and determine their relative importance in influencing bTB epidemiology.

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APPENDICES

Appendix A. Example on 'force of infection'

Although there are many routes to model development, the conceptual framework allows the consideration of how a specific quantitative model fits within the general context of bTB persistence. As an example, consider a situation in a bTB-affected area, where there is an unusual, unexplained occurrence of bTB outbreaks, or of bTB individual cases within infected herds. This includes an identification of the scale at which the hypothesis is to be tested. If the observed phenomenon generating the question is the number of outbreaks, then the scale is the 'area'. If it is the size of outbreaks, the scale is the 'herd'. One might ask the question: 'What is causing the increase?' The question can be refined to identify a testable 'hypothesis', formulated in this case as: 'cattle-to-cattle transmission alone is unable to maintain the observed outbreaks in the area' (Figure 8A).

A1. General description of activities related to model development

Model development involves three concurrent activities:

- the interrogation of the conceptual framework for how the hypothesis fits into the general context of bTB;
- examination of the literature to determine what is known about the hypothesis; and
- identification of available or retrievable data that could be used to model the hypothesis (while always asking what the model itself adds to our insight).

A1.1. Interrogation of the conceptual framework

This includes an identification of the scale at which the hypothesis is to be tested. If the observed phenomenon generating the question is the number of outbreaks, then the scale is the 'area'. If it is the size of outbreaks, the scale is the 'herd'.

(a) Identify epidemiological processes that allow the hypothesis to be tested. What are the properties of the system that allow falsification of the hypothesis? A key feature of cattle-to-cattle transmission is that, as the number of cases rise, the force of infection will increase in proportion to the number of infected animals. If we assume that the contribution of the outbreak to an environmental reservoir is small, then the force of infection owing to the environment will not increase as the number of infected cattle increases. This difference identifies a 'signature' that can be tested. Many factors will influence this signature. One of the most important is the impact of time between introduction and detection of infection in the herd (i.e. if the environmental reservoir is primarily responsible for cases, then the increase in the number of cases should be roughly linear in time, whereas if cattle-to-cattle transmission is important, then the number of cases would increase at a greater than linear rate, at least until the number of infected animals is high).

(b) Identify inputs. Once the scale is identified, inputs into the model at that scale are identified (as in Figure 2). Here, these are identified as:

(i) External cattle infection, for example by the movement of livestock, or infection from neighbouring herds, represented abstractedly by $\lambda_{cc\text{-}a}$ and possibly informed by explicit data such as knowledge of movements or spatial locations of herds.

(ii) Fomite transmission between herds (i.e. $\lambda_{cf\text{-}a}$).

(iii) Contact with pre-existing environmental MTBC (λ_e).

(c) Identify relevant demographic and epidemiological factors influencing the force of infection (λ_c) that is experienced by the cattle within the herd. Some considerations could include:

(i) Demographic factors could be identified by statistical analysis. Does outbreak size correlate with herd size? Is there evidence of age-dependent susceptibility?

(ii) For mechanisms of within-herd cattle-to-cattle transmission ($\beta_{cc\text{-}h}$), what is the mode of contact: dependent on the number of infected cattle (i.e. 'density-dependent transmission') or dependent on the fraction of infected cattle (i.e. 'frequency-dependent transmission').

(iii) For environmental contamination (β_{ec}), what will affect the effective bacterial load? Are temperature/humidity likely to have an impact? What is the expected impact of survival duration in the soil? What are the sources of the environmental contamination? Do the cattle within the herd contribute to this (β_{ec})? What about wildlife (β_{ew}) or other domestic species which have access to the same environment (β_{eos})?

A1.2. Literature review

This includes previous mathematical models, field studies and experimental studies, as well as, where appropriate, other sources such as the non-peer-reviewed 'grey literature'. These inform the values of parameters and relevance of the factors identified above. The weight placed on the evidence is dependent on both the relevance (e.g. a field study in the same species versus a study in a related species, parameters measured in the field versus an experimental challenge study), and the scientific weight (e.g. a large field study may have greater scientific weight than a smaller one, or a case-control study may have greater weight than a case report). Some of the relevant literature may include evidence of MTBC environmental contamination (Courtenay et al., 2006), within-herd models of bTB transmission (e.g. Barlow, 1996; Kao et al., 1997; Biek et al., 2012; Conlan et al., 2012) and outbreak studies in OTF countries (Probst et al., 2011).

A1.3. Identify relevant data

Identify relevant data associated with the factors confirmed in section A1.2 above:

(a) What are the available demographic data relevant to the herd structure?

(b) Are the available disease data at a higher density or resolution than the available demographic data, indicating that one or the other defines the resolution of the model?

(c) Assess the relevance of the available data that could inform model parameters, and weigh the evidence for relevance.

(d) Identify which of the 'proxy data' will be used to fit models to (e.g. the number of test reactors at first breakdown and fit a model to this, or the time until herds are removed from restriction, or some combination). This will be a combination of the relevance and the availability and extent of the data.

Model development would then be based on a full awareness of these three considerations, which involves the following four steps: identify the type of model, identify the approach to fitting the model, model selection and parameter sensitivity.

A1.4. Identify the type of model

Identify the type of model that is appropriate for the type of data available. For example, if only simple averages are available, then a deterministic compartmental model may be appropriate. If distributions of measured data are available, a stochastic model may be appropriate. If there are different groups within the herd, a meta-population model could be used. In the example provided below (section A2), the only data required are knowledge of the testing schedule, the size of herd outbreaks at the date of the first positive tests (for previously OTF herds) and the average size of the herd. While further data

may refine the model, provided there are sufficient cases, meaningful estimates of important epidemiological parameters may be obtained.

A1.5. Identify the approach to fitting the model

When a simple model is needed, a regression or maximum likelihood fit may be sufficient. For fitting more complex models, a Bayesian Markov chain Monte Carlo approach may be used. If the number of parameters is large, attempt to reduce their number or, if working in a Bayesian context, identify where strong priors may help reduce the difficulties associated with model fitting. In some cases, model fitting may be unnecessary or inappropriate, although, in this case, underlying parameter values must be well supported, or interpreted as offering guidelines or broad insights into possible underlying processes, rather than specific insights.

A1.6. Model selection

If possible, compare models to determine whether the extra detail in the more complicated model (e.g. including environmental contamination) is justified. If this is not possible, an alternative approach could be to identify qualitative differences in model outputs as a guide to further data collection that could then be used to infer the importance of environmental contamination, or be used to refit models.

A1.7. Parameter sensitivity

If a Bayesian approach is used, identify correlations in parameter posteriors. Otherwise, consider a formal multiparameter sensitivity analysis (e.g. via Latin-hypercube sampling).

A2. Possible modelling approach to assess the following question: 'What is the relative importance of direct cattle–cattle transmission versus indirect cattle–environment transmission in spreading bTB in a herd?'

The conceptual framework allows for identification of critical factors that should be considered in a mathematical model. Here, an examination of the relevant literature would show evidence of MTBC environmental contamination (Courtenay et al., 2006), several models of within-herd bTB transmission (e.g. Barlow 1996; Kao et al., 1997; Biek et al., 2012; Conlan et al., 2012) and outbreak studies in OTF countries (Probst et al., 2011). There is also evidence for the importance of herd size (Brooks-Pollock and Keeling, 2009), while noting that this list is illustrative, rather than comprehensive.

These papers (1) suggest that environmental contamination may have a role and (2) identify a model structure that could be used to address our question at the herd level. Having isolated the question to the size of outbreaks in a herd, we consider the various inputs into the force of infection (λ_i) experienced by each animal. We assume here that, although outbreaks are more common than expected based on past experience, they remain rare; therefore, the transmission owing to cattle movements (animal movements) and both direct ($\lambda_{cc\text{-}a}$) and fomite-mediated ($\lambda_{cf\text{-}a}$) contact with nearby cattle herds is low. Furthermore, there is assumed to be no evidence of infection in wildlife (λ_{wc}) or other domesticated species (λ_{osc}). Infection is, therefore, only due to the pre-existing environmental contaminants (λ_{ec}), with force of infection assumed here to be proportional to the farm surface of the herd, and within-herd transmission between cattle ($\lambda_{cc\text{-}h}$). In this simplest version, age of cattle is ignored, but, for example, it might be included should there be evidence of age-dependent susceptibility. All cattle in the herd are assumed to be equally likely to be in contact with the others.

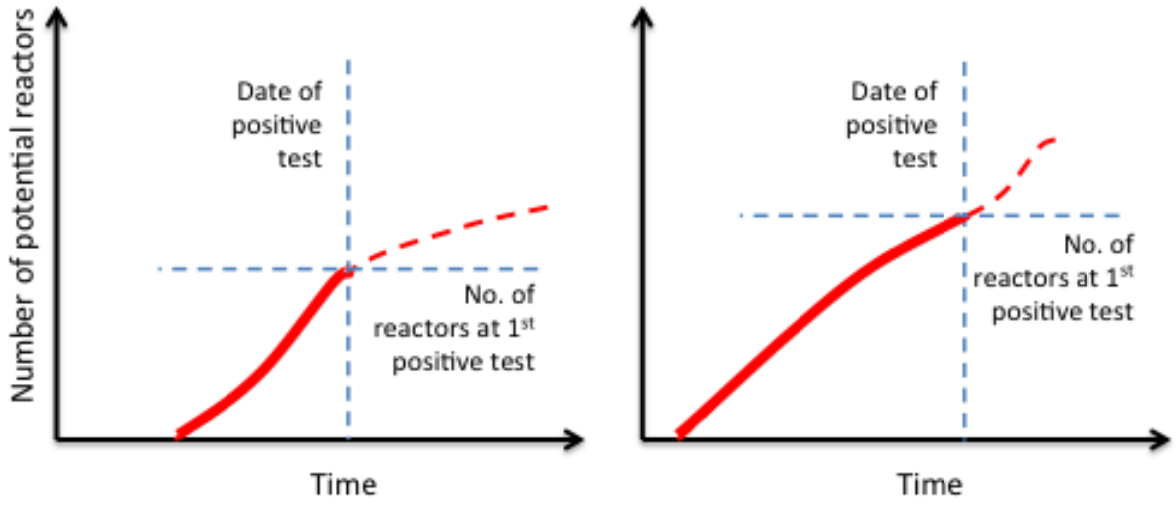
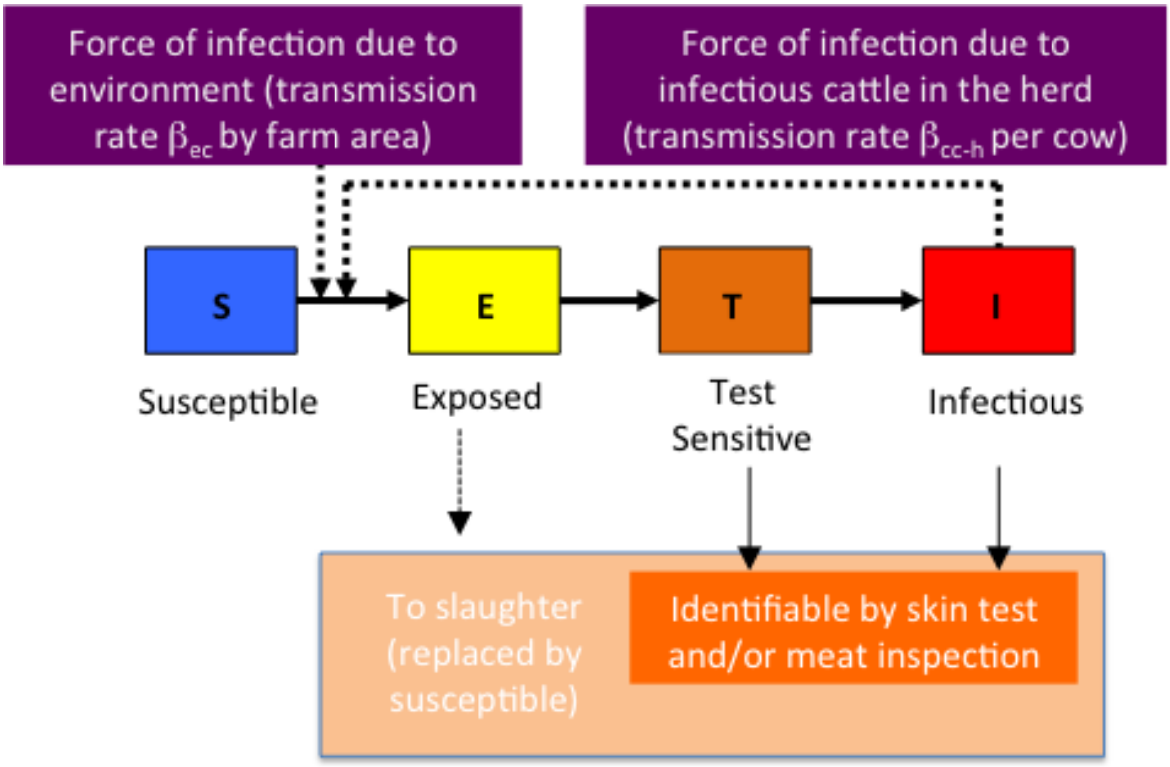


Figure 10: These graphs represent dynamics of infection in two outbreak herds. Each herd outbreak is stopped at a random time, post introduction of infection. Thus, the aggregated distribution of the number of reactors at first breakdown tells us about the progression of outbreaks over time

Any model, now restricted to these two infection components, must therefore distinguish between them. The key difference is that, while the force of infection owing to infectious cattle might be expected to grow as the outbreak progresses, environmental contamination might be expected to grow only slowly, as long as the environmental bacterial load experiences only a slow build-up from infected cattle. The number of cattle infected will be dependent on a combination of the two sources of infection, λ_{ec} and $\lambda_{cc\text{-}h}$; by aggregating this number over multiple outbreaks, the relative contribution of the two factors can be estimated.

Herd outbreak size data are readily available, and so a model that fits these data would be an attractive choice. The number of positive reactors when a herd outbreak is first identified is an indicator of the time since the outbreak started (Figure 9). Simulated stochastic outbreaks are generated using simple cattle-to-cattle transmission models (Kao et al., 1997; Biek et al., 2012; Conlan et al., 2012), in which the outbreak is seeded with a single infected animal, and further infections occur owing to both within-herd transmission and (if appropriate) externally driven transmission. Simulated whole-herd tests and abattoir testing are applied to reflect known schedules, and the model is compared with the distribution of outbreak sizes (Figure 10), using a Bayesian inference approach (see Jewell et al. (2009) for an example with livestock disease). Uniform priors are used, over the range observed in experimental and field data (see O'Hare et al., 2014). More complicated model structures can also be fit, for example assuming that some cattle are highly infectious ('superspreaders', which may include, for example, cattle that are 'supershedders', for which there is some anecdotal support (e.g. Gopal et al., 2006), and evidence in human populations (e.g. Gardy et al., 2011)). Using likelihood ratio tests for model selection, the superspreader model is shown to be a better fit to the data, although the effect is minimal (Figure 11).



$$\frac{dS}{dt} = -(\beta_{ec}A + \beta_{cc-h}I)S$$

$$\frac{dE}{dt} = (\beta_{ec}A + \beta_{cc-h}I)S - (\gamma + \mu)E$$

$$\frac{dT}{dt} = \gamma E - (\delta + \mu)T$$

$$\frac{dI}{dt} = (\delta - \mu)T$$

$\lambda_i = \beta_{ec}A + \beta_{cc-h}I$

N = *herd size* A = *area occupied by herd*

γ = *transition rate to test sensitive*

δ = *transition rate to infectious*

μ = *removal rate*

β_{ec} = *environmental contamination rate*

β_{cc} = *cattle – to – cattle transmission rate*

Figure 11: Compartmental model structure. The model is run stochastically, with simulated testing schedules and meat inspection.

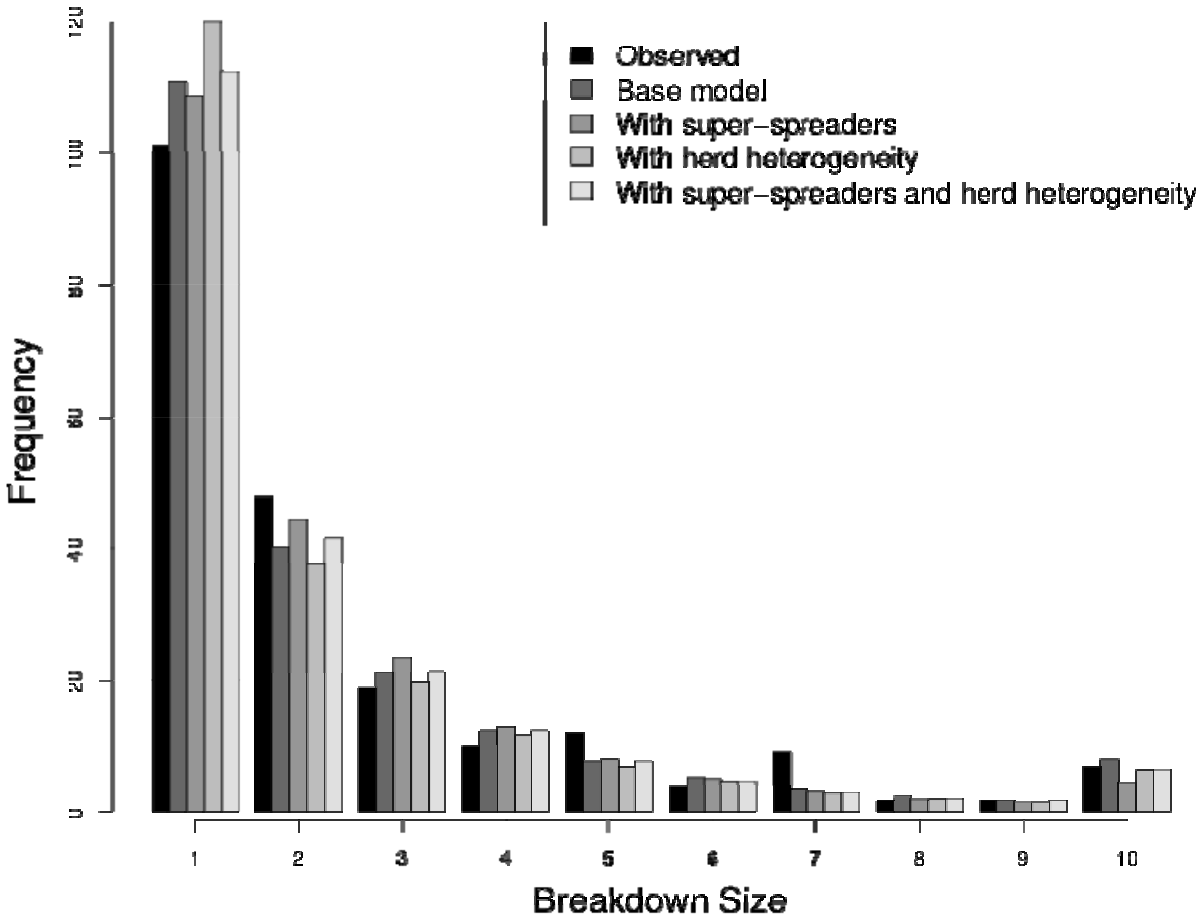


Figure 12: Using an extension of the model described here (incorporating herd age structure), a comparison of data from high-incidence areas in the United Kingdom using the model in Figure 10 is made with models incorporating individual superspreaders, varying infectiousness at the herd level (herd heterogeneity) and both. The model incorporating individual superspreaders is slightly preferred over the other three (from O'Hare et al., 2014)

A critical question regarding moving from a conceptual model, as described in this document, to an analytical model, is one of model selection, that is how much of the detail in the conceptual model needs to be included given the question being asked and can this decision be justified statistically? The analysis outlined here shows that simple models based on summary data can given results consistent with detailed examination of cattle life history data, and that, with appropriate analysis, these summary data can be used to choose between model structures. In this case, the result suggests that overall patterns can largely be described by within-herd processes, without the need to invoke differences between herds to explain them. In contrast, identification of the outcome of the analytical model (in this case, parameters identifying the most likely contribution of the environment compared with cattle-to-cattle transmission) is only a useful contribution if it is understood in context, as provided by the conceptual model. One could ask, for example, whether or not it is worth re-evaluating the analysis considering different categories of outbreaks, based on different environmental risk factors (impact of local climate) or human management risk (e.g. dairy vs. beef) as outlined in Figure 3. Thus, the interaction of model development, model analysis and contextual interpretation in the framework can be iterative.

Appendix B. Example on the non-biological context

Quantification of the non-biological context of bTB in epidemiological models seems difficult. However, this does not mean that the non-biological context cannot be studied. In this appendix, a simple social science model is used to elaborate further on the herd-level context (particularly for farmers' intrinsic and extrinsic beliefs) presented in Figure 7. This model helps the reader understand the farmer's mindset and the effect of the non-biological context at herd level. The description presents an overview of how farmers can be effectively informed and motivated to work towards prevention or eradication of infection on their farms.

The management and control of bTB is, as for many other diseases, partly a matter of people's behaviour. Generally, it is not always known why some farmers, even though it would benefit their results, do not implement effective management practices (Barkema et al., 1999), but it is often assumed that, besides deliberate rational considerations, other farmer mindset factors play a role (Jansen and Lam, 2012). As outlined in section 2.6, under herd-level farmer intrinsic and extrinsic factors, farmer mindset comprises a variety of social psychology constructs. See, for example, the Theory of Planned Behavior (Ajzen and Madden, 1986; Ajzen, 1991; Fishbein and Yzer, 2003) and the Health Belief Model (Janz and Becker, 1984; Garcia and Mann, 2003; Sun et al., 2006), which are both frequently used to explain people's health behaviour (Armitage and Conner, 2001; Noar et al., 2008; Painter et al., 2008).

Based on the results of studies on mastitis control (Jansen et al., 2009, 2010a, b, c), two factors of dairy farmer mindset seem to be the most important behavioural determinants: the perceived threat (i.e. 'do I have a problem?', influenced by perceived susceptibility and perceived severity of the disease) and the perceived efficacy of measures (i.e. 'can I solve the problem easily?', influenced by perceived benefits from, and perceived barriers to prevent and control, the disease). In the case of bTB, one additional factor for consideration might be the perceived responsibility of a farmer in the problem affecting his herd ('how much of the problem do I own?').

Interestingly, the first two factors are also known to be indispensable in motivating people to work on their own health and are included in the so-called Health Belief Model, which is presented in Figure 12 (Rogers, 1983; Janz and Becker, 1984; Griffin et al., 1999; Garcia and Mann, 2003; Sun et al., 2006).

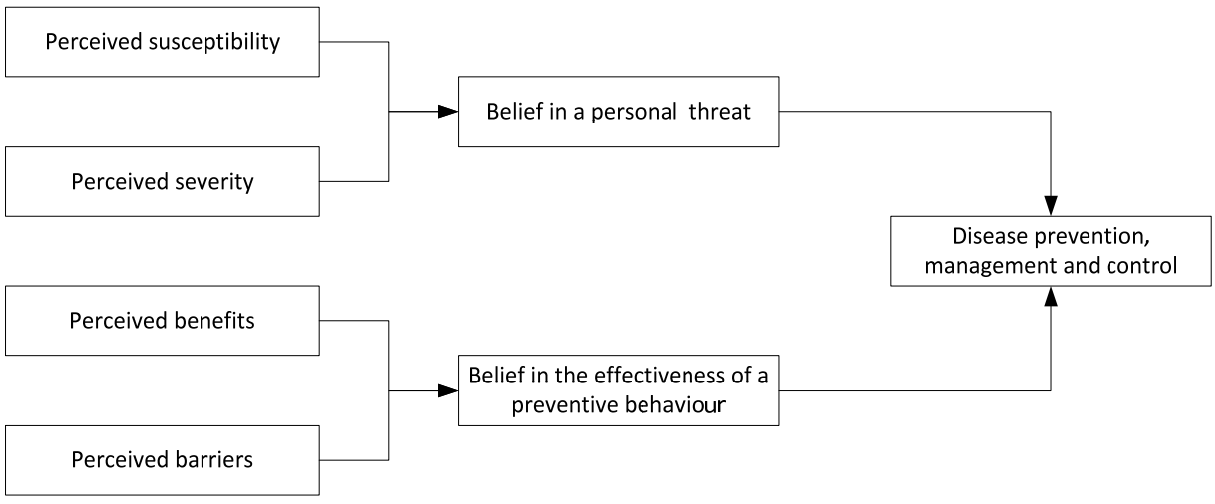


Figure 13: The Health Belief Model (modified and adapted from Janz and Becker, 1984)

The mechanisms behind this Health Belief Model seem to correspond to important behavioural determinants such as attitudes, norms and perceived self-efficacy from the Theory of Planned Behavior (Ajzen and Madden, 1986; Ajzen, 1991; Fishbein and Yzer, 2003; Garcia and Mann, 2003;

Sun et al., 2006). Some of these could probably be applied to bTB and could help understand why some farmers may be more motivated than others to apply biosecurity measures and sampling schemes. If the bTB control measures required are perceived as ineffective owing to uncontrollable wildlife or as hardly resulting in animal health or economic benefits, farmers may also not be motivated to change their management. Understanding these key factors in the mindsets of the involved stakeholders may help to identify communication and policy goals to improve the uptake of bTB management and control measures, as well as help understand why certain policy goals are not achieved.

B1. To RESET the mindset towards bTB

The non-biological context can be summarised by five key factors, using the Rules Education Social pressure Economic stimuli Tools (RESET) model, that was adapted from van Woerkum et al. (1999) and Leeuwis (2004) and was described in a different form earlier regarding mastitis by Lam et al. (2011) and Jansen and Lam (2012). The model shows five main instruments that need to be addressed and studied when a change in behaviour of people is required: R for regulations, E for education, S for social pressure, E for economic incentives and T for tools. As some people are more influenced by negative stimuli, and some more by positive stimuli or social pressure, it is the combination of all this that can make a disease control programme or campaign effective (Jansen and Lam, 2012). Differences between countries with regard to bTB situation may partly result from different RESET strategies, besides major differences in the disease epidemiology.

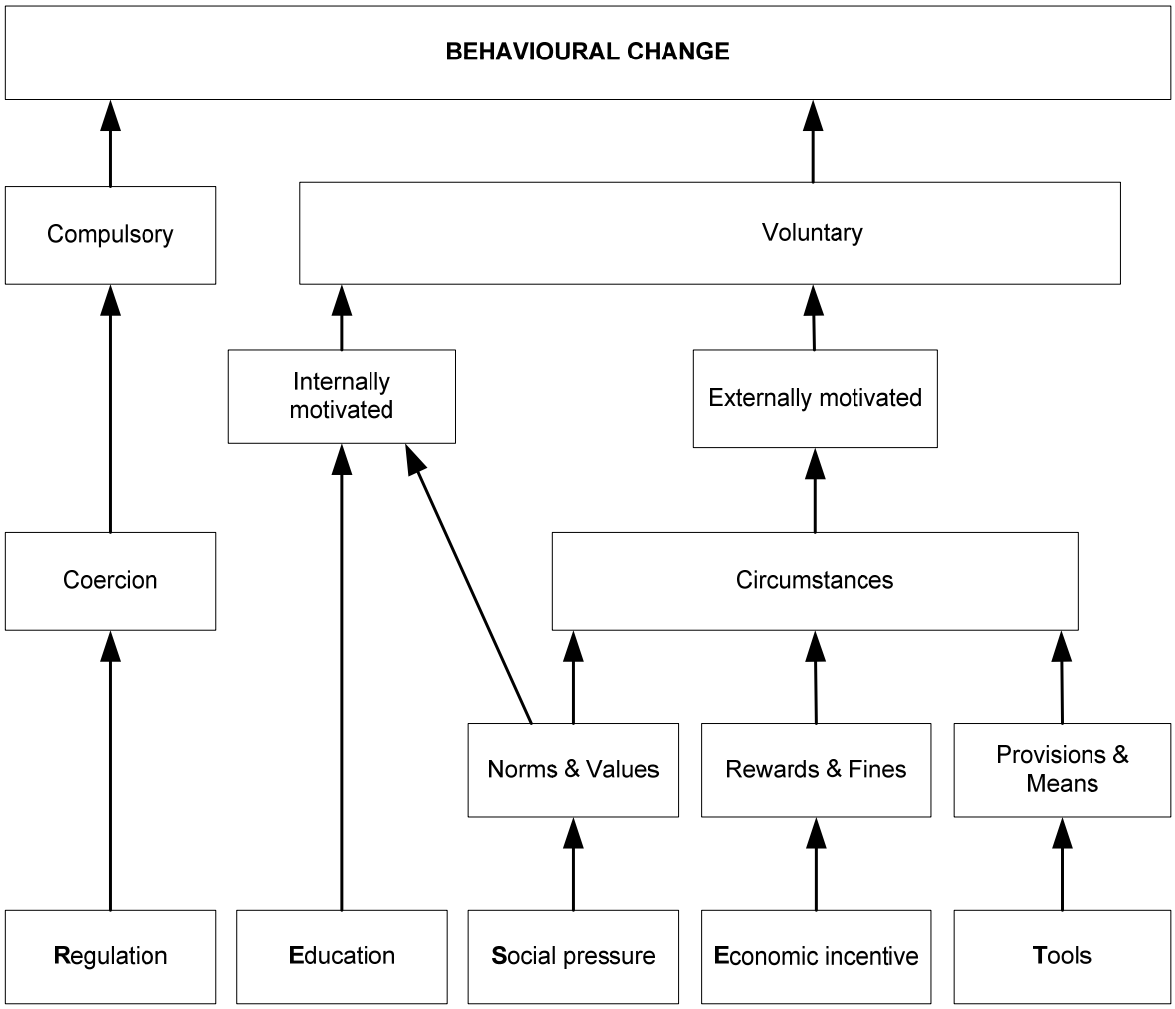


Figure 14: The RESET model: behavioural change by a combination of strategies (adapted from van Woerkum et al., 1999; see also Leeuwis, 2004)

In general, behaviour can be changed in either a voluntary or a compulsory manner. Compulsory behavioural change is facilitated by coercion such as regulations and restrictive provisions (van Woerkum et al., 1999; van Woerkum and Van Meegeren, 1999). It is well known that compulsory behavioural change will probably last only as long as the coercion exists. Therefore, voluntary behavioural change is generally preferable. Voluntary behavioural change can be reached by internal or external motivation. Internal motivation is the most difficult one to influence via a disease control programme, as it relates to age, generation, lifestyle, education and character. External motivation is therefore more suitable, but is mostly underestimated.

B1.1. R for regulations

Figure 7 briefly presents the relationship between the area and herd level and how this influences the design and delivery of regulatory policies. It is well known that regulations (see area level, Figure 7) force people to behave in a preferred way. This mechanism works via coercion. It is known that a change in legislation has a direct effect on farmer behaviour and disease incidence, provided that there is proper surveillance and that consequences of not complying are consequently communicated. Regarding bTB, there are regulations at EU level and national level, but also other laws and regulations may influence farm management directly, for example on housing, quota systems and environmental regulations. These also need to be taken into account when understanding people's decision making.

B1.2. E for education

Applicable to both the area and the herd level, education is one of the most used intervention instruments, but is often overestimated. Education can be more effective if the information is offered and adapted to the different learning styles of people (Lam et al., 2011). To understand bTB prevalence, it is important to determine how knowledge on prevention and control is shared and understood between all stakeholders involved at both the area and the herd level.

B1.3. S for social pressure

The application of social pressure in achieving behavioural change is applicable to the herd level—particularly within the organisation structure and for farmer intrinsic and extrinsic belief systems. Social pressure influences farmers' and veterinarians' norms and values, and can therefore have a long-term effect on internal motivation as well. As humans need social cohesion to be successful, the application of social pressure can therefore be one of the most powerful tools used in intervention strategies. The success of study groups, where farmers influence each other, is mostly based on this principle. Veterinarians and other herd health advisers are important for increasing social pressure and setting a frame of reference about what is normal, and what is not. It is also important to acknowledge that others may influence the farmer, such as family members, peers or staff. If the social pressure is high enough in an opposite direction, the other elements of the RESET model may have no or less effect.

B1.4. E for economic incentives

Typically, economic incentives fall into the extrinsic factors at herd level that farmers draw upon to explain their attitudes and behaviours. External motivation can be accomplished by financial stimuli such as bonuses and penalties. People will change their behaviour by these penalties, not just through some sort of coercion, but mostly because they set a social norm. A good herd health status can be rewarded economically, but also by rewards that apply to the farmers sense of pride and status (social pressure), such as best quality milk. Finally, economic incentives can work for implementing control measures, by showing farmers the economic benefits of implementing measures, by proper compensation of culled animals or by making certain measures much cheaper, such as sampling schemes.

B1.5. T for tools

Tools are important for supporting the technical capacity of policy implementation at herd level and for supporting feelings of self-efficacy in achieving a set of actions at herd level (for farmers, veterinarians and inspectors). Tools, such as technical provisions, means and methods, can stimulate farmers to perform certain behaviours. Tools can make the desired behaviour much easier to perform (e.g. the possibility for easy sampling, or the fact that the premises are easy to protect from wildlife). Tools only work if they are used properly and in combination with the other intervention instruments.

Tools can also help people to unconsciously perform their behaviour the right way. Scientists are more and more aware of the effect of automatic unconscious behaviour in daily life. Rational approaches via education of farmers and veterinarians may not be enough to make farmers use some tools (Jansen et al., 2010a).

B2. Concluding remarks

To be effective at herd level, a disease control programme should do more than distribute technical information about best management practices to dairy farmers. Prevention of complex diseases, such as bTB, requires customised strategies as well as an integrated approach between various stakeholders and different scientific disciplines.

Elements of farmer mindset are important determinants in disease control, including the perceived threat (i.e. 'do I have a problem?') and the perceived efficacy of preventative measures (i.e. 'can I solve the problem easily?'). These issues can be addressed in disease control strategies using the RESET model as a framework and can even be used as a guide to evaluate strategies. Studying both mindset and RESET factors of stakeholders involved may enhance the understanding of bTB occurrence.

Appendix C. Example on 'testing'

Central to our interpretation of bTB prevalence is the need to understand the relationship between surveillance sensitivity and specificity, and true and apparent prevalence. The statistical model presented in full in this appendix allows for the estimation of the potential discrepancies between true and apparent herd and animal prevalence and the influences of the testing system, by considering the relationship between individual test outcomes, the probability of a herd breakdown and the combined sensitivity and specificity of the tuberculin test and meat inspection. Figures 4 and 5 show the complex process which underlies testing. The example presented below considers the construction of a simulation-based statistical model that builds up from individual-level information to herd-level prevalence and subsequent OTF classification. Although the example is simple (incorporating only test parameters), it potentially could include greater complexity, for example by including herd structure and possible differences in test administration.

Hypothetical risk questions addressed in this example:

- In an OTF country with some infected herds, how many infected animals are missed by the testing procedure?
- In the same conditions, what is the probability of missing at least one infected herd by the testing procedure in place (one round of testing)?

The strategy adopted was to generate realistic data in order to mimic a hypothetical representative country (HRC) with a limited number of infected herds compatible with OTF status at the country level.

C1. Data

C1.1. Number of herds (H)

The number of herds per Member State was retrieved from EUROSTAT (2007). The median number of herds (40 840 herds) was used for HRC.

C1.2. Number of animals per herd eligible for testing[4] (a)

A Weibull distribution was fitted to the available data on herd size and its moments calculated. This distribution was used to draw the herd sizes in the HRC simulation. Figure 14 shows the result of the single drawn performed to assign the size to each of the herds in the HRC.

[4] In a herd, not all animals are tested (e.g. the tuberculin skin test is not performed until animals are at least six weeks old). The figures in this example do not include the animals that are not tested.

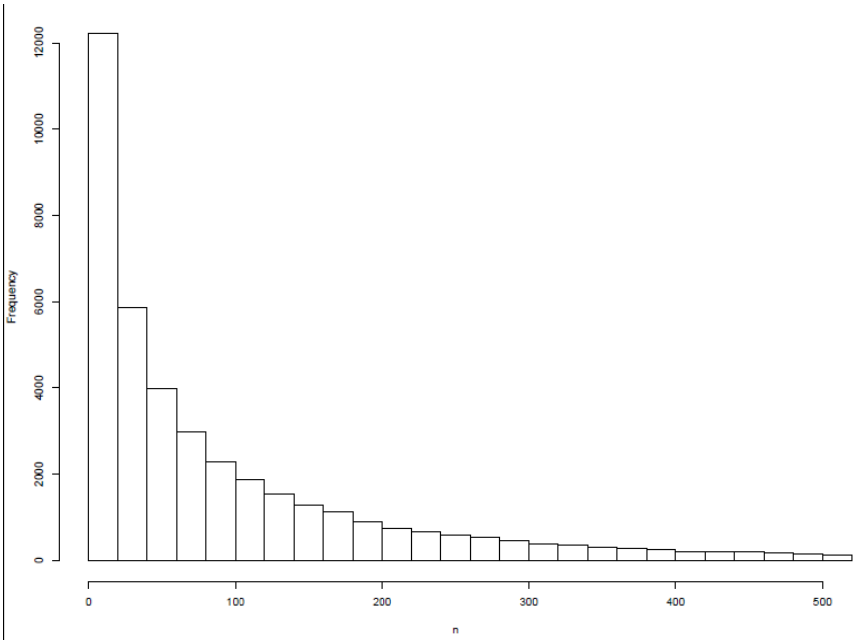


Figure 15: Frequency distribution of the herd size in the HRC; a_i, infected animals

C1.3. Infected herds (IH) and infected animals in each infected herd (*IH_ia*$_i$)

The assessment was performed by simulation of a HRC with infected herds. It has to be noted that no spatial parameter is taken into consideration. Therefore, the infected herds can be either evenly distributed across the HRC or concentrated in regions with higher prevalence values. The parameters used in the following example are:

(Overall) Prevalence of infected herds in the HRC = 8/10 000 = 0.0008 = **0.08 %**

Prevalence of infected animals within the infected herds = 2/100 = **0.02** = 2 %

In the simulated HRC with 40 840 herds, this gives:

where a_i represents the number of animals in herd *i*. The number of infected animals in the infected herds ( ) was estimated to be between 52 and 165 for the 1 000 simulations run.

Equation C1

C2. Testing the population in a hypothetical representative country

C2.1. Number of infected animals testing negative

The number of infected animals testing negative (false negatives) is calculated in two steps, as follows:

- Estimation of the number of infected animals testing positive

Equation C2

where *TSe* is the test sensitivity. Simulations were made with *TSe* values of 0.5, 0.7 and 0.9.

- Calculation of the number of false-negative animals

Equation C3

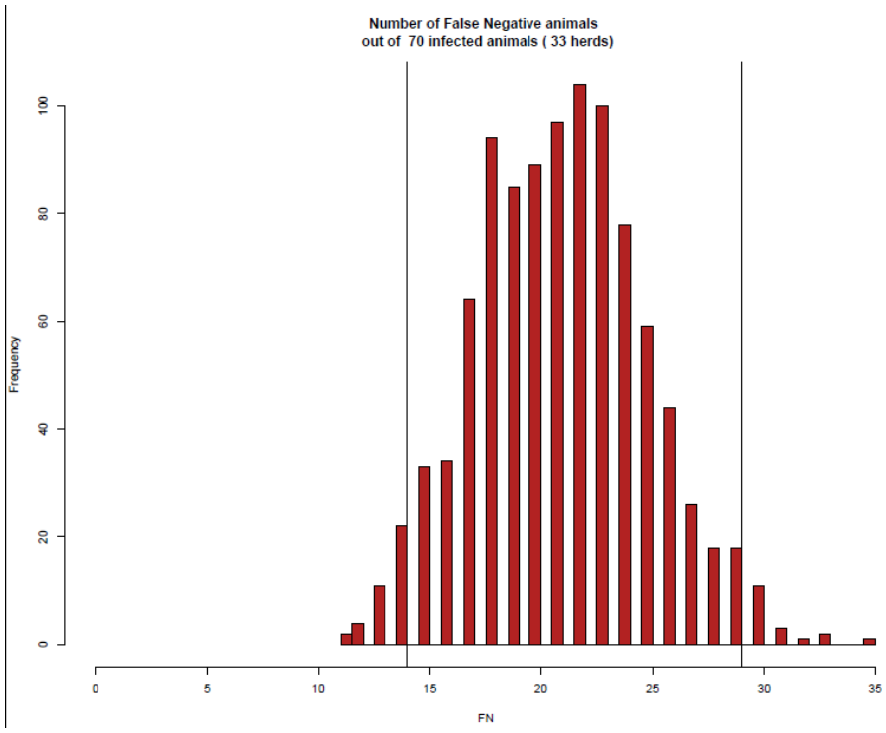


Figure 16: Number of infected animals that were not detected by the testing (neg_IH_ia, number of false negatives) out of the total number of infected animals (IH_ia; 70 in this specific iteration) for 1 000 iterations

Figure 15 shows the outcome of a specific iteration out of the 1 000 runs for the whole simulation exercise. In this case, the testing procedure sensitivity was set to 0.7 and the total number of infected animals was 70. Results show that the number of false-negative animals is between 14 and 28 (CL = 95 %).

Of course, a point estimate could be calculated analytically: as the testing procedure has a sensitivity equal to 0.7, the probability that an infected animal is not detected is equal to 0.3. Thus, the number of false-negative animals can be calculated as follows:

If the same values as in the iteration showed in Figure 15 are used (i.e. with 70 infected animals), the number of false-negative animals would be equal to 21 (which is also the mean value obtained in this particular iteration).

C2.2. Number of infected herds testing negative

C2.1.1. The infected animals in each infected herd (false negatives)

Step	Description	Analytical expression
1	Estimation of the number of infected animals testing positive in each herd (TSe = 0.7)	$pos_IH_ia_i \sim Binomial(IH_ia_i, TSe)$
2	Calculation of the false negatives in each herd (number of infected animals *minus* the number of animals correctly testing positive)	$neg_IH_ia_i = IH_ia_i - pos_IH_ia_i$
3	Quantification of the herds where the number of false negatives is equal to the number of infected animals (**complete failure**)	(logical) $neg_IH_ia_i = IH_ia_i$
4	Calculation of the probability of **complete failure** per herd	See Figure 16

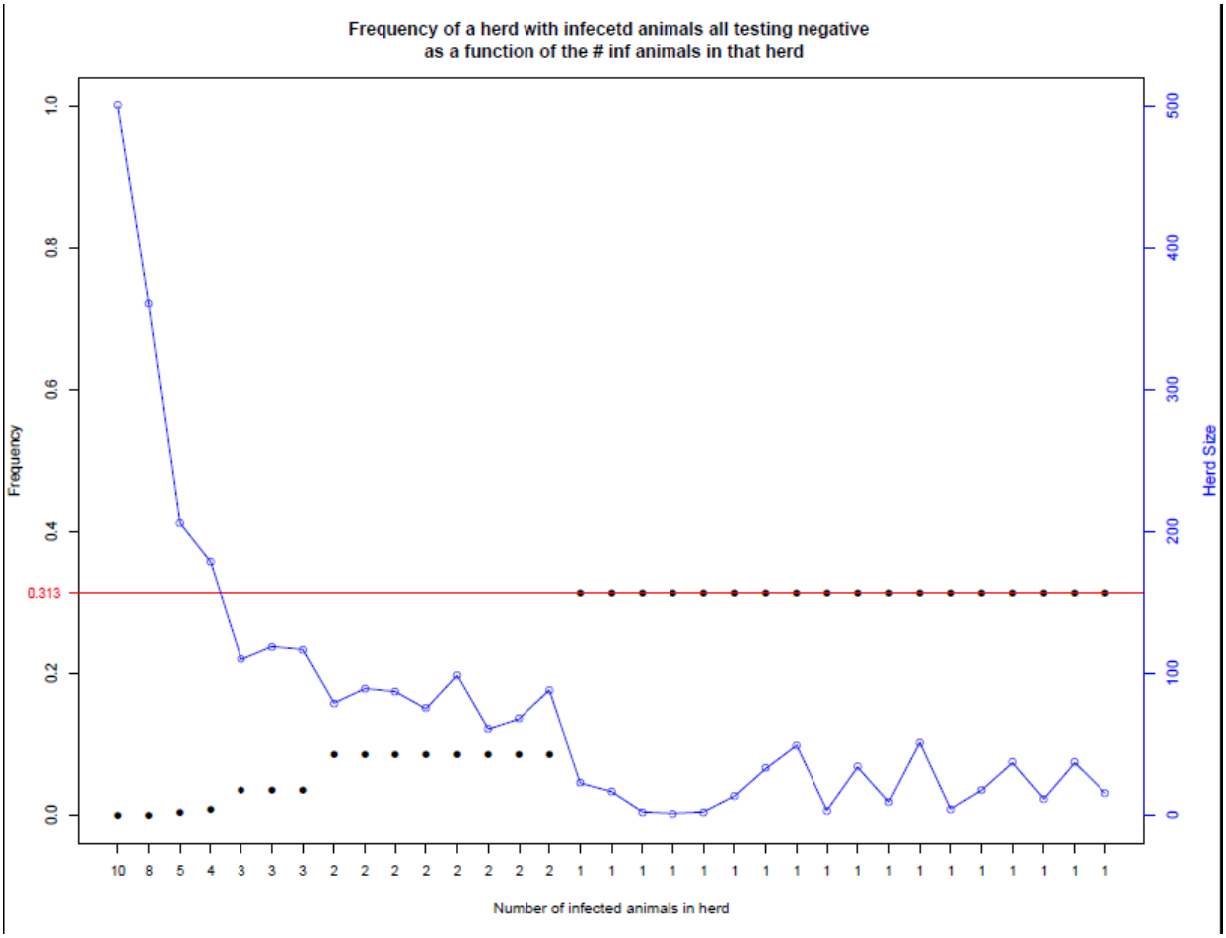


Figure 17: Black dots, frequency at which all infected animals (x-axis) tested negative; red line, max failure rate; blue line, herd size of each herd (see section C1.2)

Figure 16 is for illustrative purposes only. Looking at the results, it can be concluded that herds with less than 50 animals and only one infected animal are the most problematic, with a probability of not detecting the infected animal equal to 0.313. This probability almost halves when the number of infected animals is two.

C2.2.2. The non-infected animals in each infected herd (true negatives)

Step	Description	Analytical expression
1	Calculation of the non-infected animals (nia) in each infected herd (IH)	
2	Estimation of the number of non-infected animals (nia) testing negative (neg) in each infected herd (IH) referring to test specificity (TSp = 0.97)	
3	Quantification of the herds where the number of true negatives is equal to the number of non-infected animals (**perfect classification**)	(logical)
4	Calculation of the frequency of **perfect classification** (all non-infected animals tested negative) of each herd	See Figure 17

As expected, the greater the number of non-infected animals, the lower the frequency (empirical probability) that all of them test negative. In fact, when this number is greater than 170, the probability of perfect classification is almost 0.

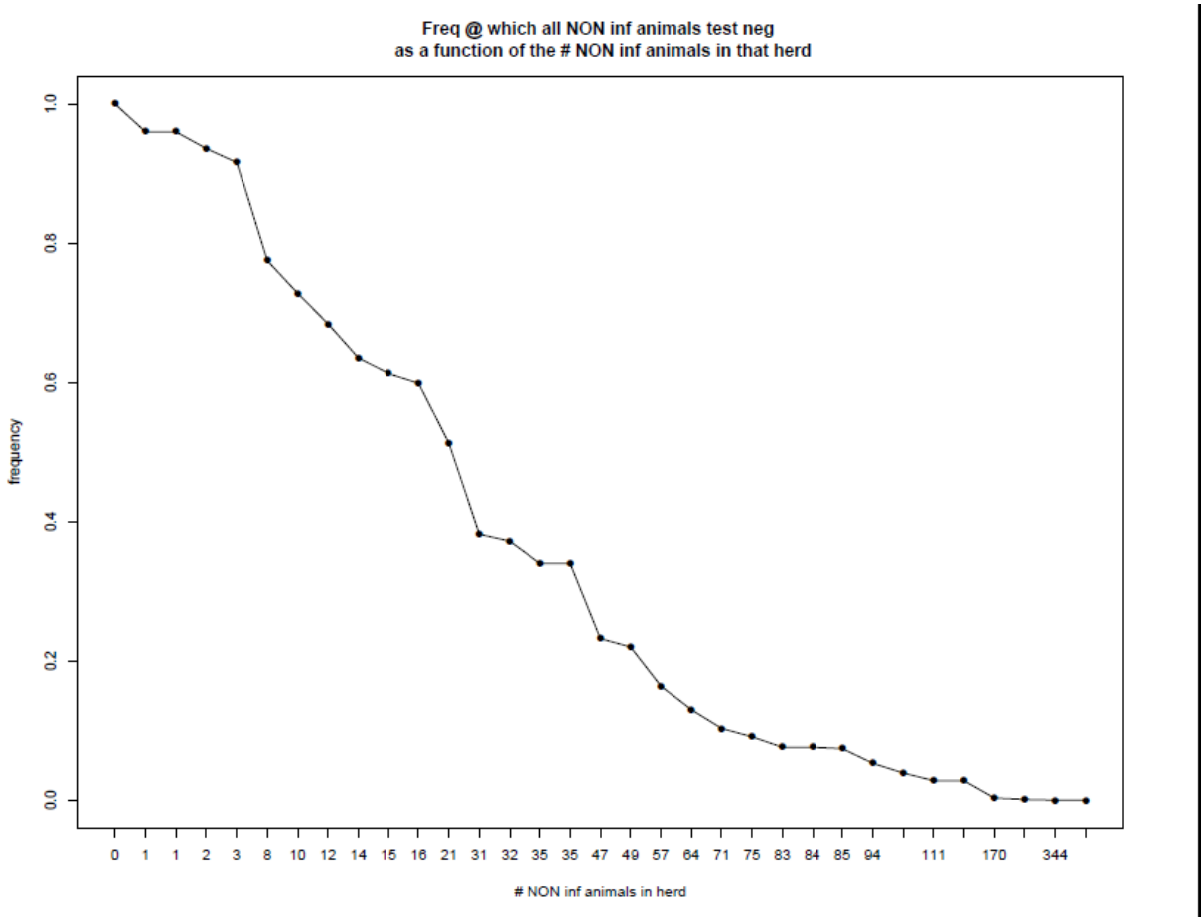


Figure 18: Probability of all non-infected animals (x-axis) testing negative as a function of the number of non-infected animals in the herd

C2.2.3. Combining infected and non-infected animals in infected herds

The probability that all animals in a given infected herd test negative is given by the following equation:

Equation C4

with going from 1 to the number of infected herds ( )

The probability of a single herd testing free from bTB is given by the product of the empirical probability, that all infected animals test negative (complete failure) and the empirical probability that all non-infected animals test negative (perfect classification).

It is now possible to estimate the overall probability that a randomly selected infected herd tests free from bTB (see Equation C5).

Equation C5

with going from 1 to the number of infected herds ( )

The steps were repeated 1 000 times and the full results are shown in Figure 18.

C3. Simulation results

As previously anticipated, three testing procedure sensitivity values were explored (0.5, 0.7 and 0.9).

It is worth noting that the three investigated values do not refer simply to the sensitivity of a specific diagnostic test performed at an individual level. In fact, the testing procedure sensitivity may refer to a series of different tests, including meat inspection and serial skin testing procedures.

It can be seen that, on average, around 17 % of the infected herds would appear to be free from bTB. In the specific situation with 30 infected herds, five of them would keep their OTF status.

With TSe = 0.7, on average, around 10 % of the infected herds would appear to be free from bTB, so among 30 infected herds, three of them would keep their OTF status.

With TSe = 0.9, on average, around 3 % of the infected herds would appear to be free from bTB, so among 30 infected herds, one of them would keep its OTF status.

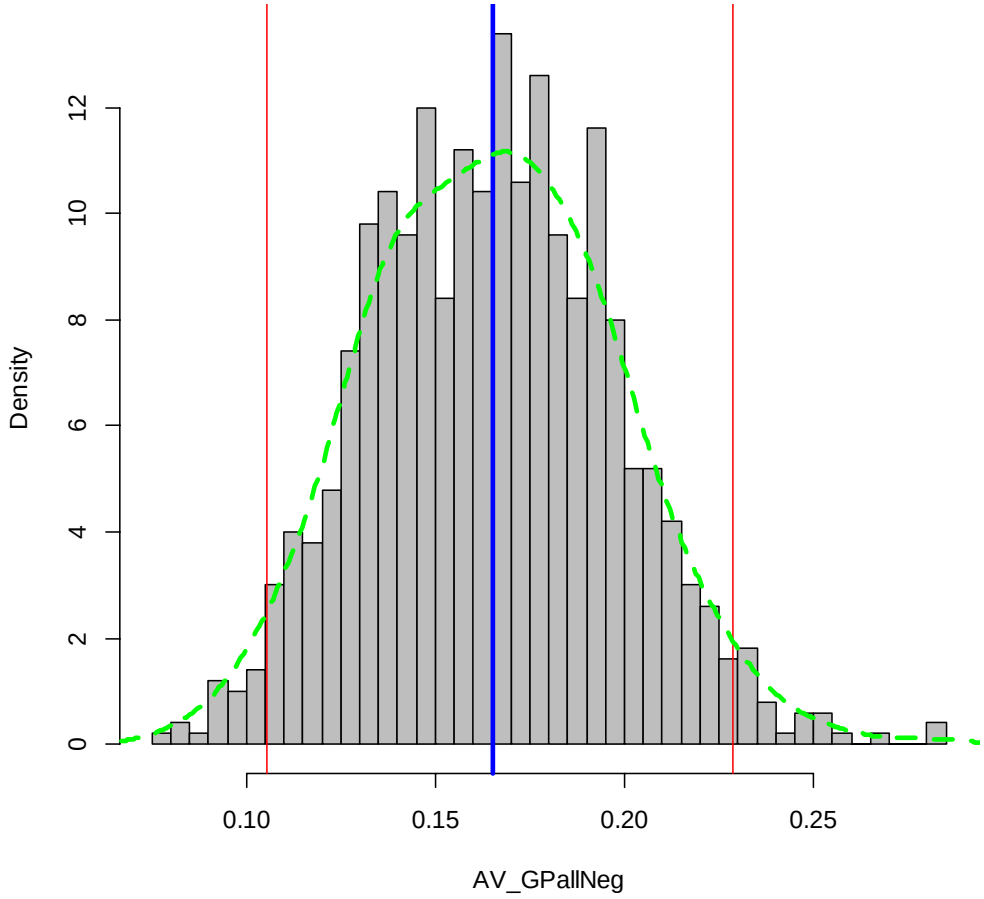


Figure 19: Empirical probability that, among the infected herds, at least one of them tested free from bovine tuberculosis (no positive animal in the herd), where test sensitivity is 0.5

C4. Conclusion

The simulated examples illustrate the potential biasing effect of less than perfect sensitivity of the testing procedure on the OTF classification of herds. Other simulation examples might be considered to also illustrate the effect of the less than perfect specificity of testing procedures, which results in culling of false-positive animals or herds.

Appendix D. Technical description of a methodology for modelling bovine tuberculosis test readings measured as a continuous dependent variable, which could be applied to examine the effects of 'biological' as well as 'non-biological' parameters

D1. Summary

This technical appendix describes a **method that could be used to assess the effect of given influencing factors on the apparent prevalence of bTB infection** in an area. For each animal included in the analysis, the following data are required: continuous bTB test result (e.g. skin thickness in mm) and two variables related to the non-biological context of bTB testing (e.g. variable for 'organisational structure' that has been assigned to the farm (Z1 in Equation D5) and variable for the technical capacity that has been assigned to veterinarian working in the farm (private vs. official veterinarian, described as Z2 in Equation D5)).

The modelling methodology will indicate whether or not one or both possible influencing factor(s) has a statistical significant effect on bTB prevalence. Prevalence is the proportion of infected animals in a given population ('area level' if more than one farm is included in the analysis).

Once the prevalence is estimated, in the simplest model, the effect of the influencing factor(s) on the force of infection at 'animal level' could be calculated (using Equation D6).

The methodology can be used to assess the effect of biological and non-biological parameters on bTB prevalence. For example, additional levels of complexity can then be considered by introducing further explanatory variables (e.g. area, local prevalence of breakdown herds) that may account for any differences in positive test rates. This methodology may help understand bTB epidemiology but will not have an impact on the practical applications of bTB testing.

D2. Mixture models

As discussed in the main text, here we consider the simple example of assessing the potential effect of plausible influencing factors (e.g. biological and/or non-biological factors) on the prevalence. This is possible especially if a cut-off is not set beforehand. In this case, the classification of the infection status of individual animals can be based on a particular test with a continuous test outcome (e.g. the swelling thickness measured in a tuberculin or skin test). Although typically interpreted in terms of a test cut-off value (i.e. swellings above a fixed measure are considered to be reactors), a more realistic approach is to consider the probability that, given a measured thickness, an animal has a certain infection status (e.g. for a swelling of 2 mm, what is the probability that a cow is infected?). This methodology also allows for estimation of the potential cut-off according to the specific needs (e.g. high sensitivity or cut-off that maximise the sensitivity and the specificity), as well as the test characteristics based on the estimated cut-off.

Mixture distributions are encountered in practical problems when the measurements of a random variable are taken under different conditions (or can be considered as coming from different populations). This methodology offers more elaborate probabilistic modelling when direct modelling is done using classical distributions such as normal, binomial, Poisson and gamma, among others, which are too limited to be able to accommodate the observed phenomenon (McLachan and Basford, 1988; Lavine and West, 1992).

In order to avoid the need to base the classification of the infectious status of a given unit of interest on a pre-specified cut-off point, a modelling approach based on mixture modelling was first proposed by Greiner et al. (1994) and refined by Gay (1996).

A certain population can be divided into K sub-populations that might represent, for example, cattle belonging to different infection statuses (e.g. uninfected, infected but not infectious, infectious, anergic). Mixture modelling is a data-driven approach that aims to assign the observations to these K sub-populations (components) and, unlike cluster analysis methods, allows the characterisation of not

just cut-off values, but probability distributions within the obtained clusters. Furthermore, Grün and Leisch (2008) discussed a finite mixture of regression models, where the means of the mixture components and/or the mixture probabilities depend on a set of fixed and random effects, which are estimated using a Bayesian approach (Evans and Erlandson, 2004; Ødegård et al., 2005; Nielsen et al., 2007; Hardelid et al., 2008).

The basic idea of the mixture model is to consider an indicator (j), that specifies the sub-population from which the samples are supposed to come, and the assumption that the distribution of the sample is a mixture of distributions with k components. The probability density, $g(x)$, of the sample can then be approximated as follows:

$$g(x) \approx \hat{g}(x) = \sum_{j=1}^{k} p_j f(x|\theta_j) \qquad \text{(Equation D1)}$$

Equation D1 is referred to as the general finite mixture model, with p_j being the mixing probability. In this specific framework, p_j represents the prevalence of sub-population j, with the additional assumption that

$$\sum_{j=1}^{k} p_j = 1 \text{ and } f(x|\theta_j)$$

is any parametric distribution (continuous, discrete or a mixture of both).

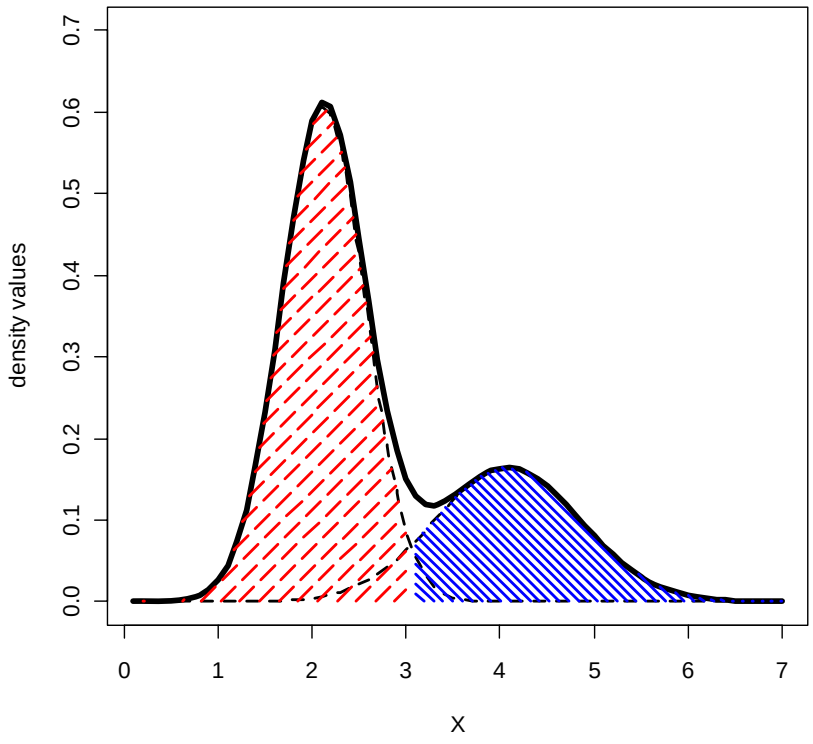


Figure 20: Graphical representation of a two-component mixture model. The colour red represents the density function of a given test outcome on a continuous scale (e.g. OD from serological test) in the non-infected population; the blue colour represents the density function for the infected population

To simplify the model (Equation D1) specifically for our setting, it is assumed that the number of components, **k**, in the finite mixture is two, with one component corresponding to the infected animals and the other to the non-infected animals (see Figure 19). In its most general form, the parametric

distributions for each observation and/or each component can be different, but for simplicity we assumed identical parametric distribution for each observation and each component. The model (Equation D1) can now be written as:

$$g(x) \approx \hat{g}(x) = \sum_{j=1}^{2} p_j f(x|\theta_j)$$

(Equation D2)

The choice of the parametric distribution depends on the study context as well as beliefs about the biological mechanism generating the data.

As noted earlier, the test results for bTB are allowed to be a continuous random variable (e.g. mm skin thickening for skin test, but with an equivalent interpretation in other cases, e.g. for the IFN-γ test). It is then plausible to assume a continuous distribution, such as normal distribution (or a transformation of this could be approximated by a normal distribution).

The aim is to maximise the complete data likelihood as follows (see Equation D3):

$$L(\psi|y, z) = \prod_{i=1}^{N} \left[(1 - p_i) \cdot f(y_i|\theta_1)\right]^{z_i} \cdot \left[p_i \cdot f(y_i|\theta_2)\right]^{z_i}$$

(Equation D3)

The vector ψ is the vector containing all unknown parameters in the model to be estimated with $\psi = (p', \theta')$ and $z_i \sim \text{Bern}(p_i)$, where z is the unknown infection status (also in the statistic literature called latent infection status) and p is the probability that an animal subjected to bTB testing is truly positive. Equation D3 was then derived by introducing the latent class indicator z_i in the observed data likelihood.

An extension to this model could place test results in the context of non-biological (e.g. social) parameters and their influence on the prevalence of bTB. We are interested in modelling the probability for a single animal of belonging to one of the two components (infected/non-infected) as a function of a set of possible non-biological influencing factors, such as the technical capacity of the (state or private) veterinarian implementing the test, or other factors defined in section 2.6.

This concept is analytically expressed in the following equation:

$$p_i = h(\text{Influencing Parameters})$$

(Equation D4)

The function h in Equation D4 allows the effect of a given influencing factor of interest on the individual probability of being infected to be estimated. The value p_i is a probability and consequently is between 0 and 1, ensured by imposing the functional form for h also called a link function in the statistical field (examples of the function h are the logit link, probit link, etc.).

D3. Use of mixture models in the bovine tuberculosis context

In order to illustrate how the methodology could be used in a non-biological context, it is assumed that the mean of one of the components (in this example, the 'infected' component, μ_2) was considered larger than the mean of the other component (the 'non-infected' component, μ_1), which is $\mu_2 = \mu_1 + \delta$, where $\delta > 0$..

Equation D4 can then be written as an additive effect model of the potential influencing factors of interest, for example the organisational structure assigned to the farms and the technical capacity that has been assigned to the veterinarian working on the farm (private vs. official veterinarian). Equation D4 then becomes:

$$p_i = h(\mu + \gamma_1 \cdot \text{Organization Structure} + \gamma_2 \cdot \text{Technical Capacity})$$ (Equation D5)

In this illustration, we are considering that the potential influencing factors are dichotomous variables (indicating one of the two categories), but it could be extended to more complex situations without losing generality. The parameters (γ_1 and γ_2) associated with the potential influencing factors could be used to test the effect of the potential influencing factors on the prevalence in the population ($H_0 : \gamma_j = 0$ vs. $H_A : \gamma_j \neq 0$). In case of rejection of H_0 (hypothesis of no effect of the potential influencing factor on the prevalence), it could then be tested whether the effects of the potential influencing factors on the prevalence are increasing or decreasing the prevalence in the population under study.

This methodology can be used to deal with estimation of two important parameters when studying the epidemiology of infectious diseases, such as the prevalence (p) and the force of infection (λ). The former is the proportion of diseased/infected animals in a given population, whereas the latter is the individual risk of a non-infected animal to naturally acquire an infection.

Once the prevalence is estimated, using mixture model methodology, the force of infection could be directly derived (Farrington, 1990; Shkedy et al., 2003, 2006), as follows:

$$\lambda(t) = \frac{1}{1 - p(t)} \cdot \frac{\partial p(t)}{\partial t}$$

(Equation D6)

In order to illustrate how the methodology works, a 'dummy' example is considered, where skin test results (mm) from 5 000 animals during a 50-week period have been reported by private or official veterinarians, and two different organisation structures (description A and description B) have been assigned to the farms; for each individual animal, data were generated. The data generated are presented in Figure 20. The aim of the study is to investigate and quantify the potential effects of the organisation structure and the technical capacity (considering official or private veterinarians) on the prevalence of bTB in the area under consideration, as well as potential differences regarding infection pressure.

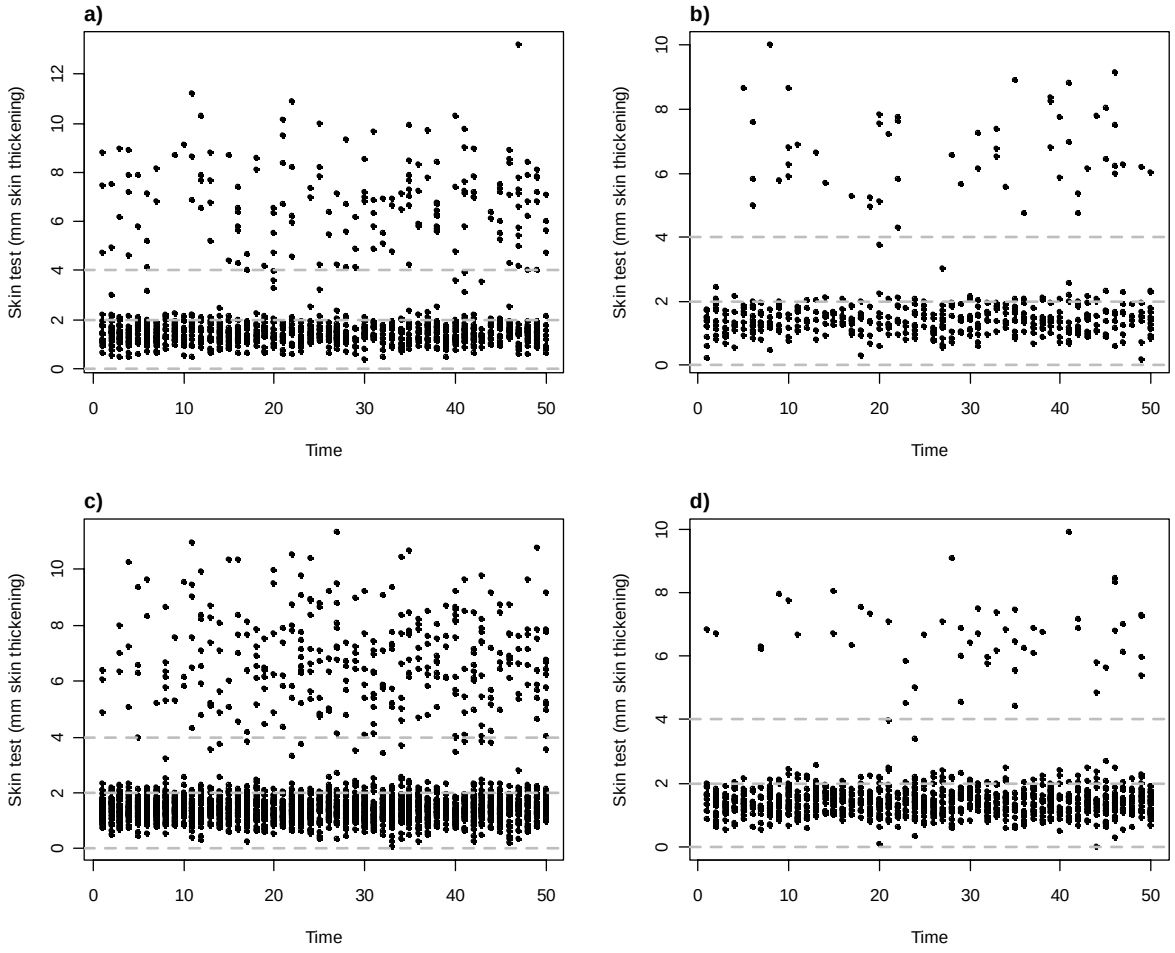


Figure 21: Individual readings of the skin test for the four possible combinations of organisation structure and technical capacity: (a) the animal readings taken by official veterinarians in organisational structure A; (b) the animal readings taken by private veterinarians for organisational structure A; (c) the animal readings taken by official veterinarians for organisational structure B; and (d) the animal readings taken by private veterinarians for organisational structure B

It is clear that the number of reactors in Figure 20c differs from the other combinations, but differences between Figures 20b and 20d are less apparent. The impact of time of test is also difficult to see. In such situations, the model previously described provides the appropriate tools to assess and quantify such differences. In this particular situation, the model described in Equation D5 is used, considering the function *h* to be the inverse of the logit function, as follows:

$$\mathbf{p_i} = \frac{\mathbf{Exp}\left(\mu + \gamma_1 \cdot \mathbf{Organization\ Structure} + \gamma_2 \cdot \mathbf{Technical\ Capacity} + \gamma_3 \cdot \mathbf{Time}\right)}{\mathbf{1 + Exp}\left(\mu + \gamma_1 \cdot \mathbf{Organization\ Structure} + \gamma_2 \cdot \mathbf{Technical\ Capacity} + \gamma_3 \cdot \mathbf{Time}\right)}$$

In this illustration, p_i represents the prevalence, whereas γ_1, γ_2 and γ_3 represent the parameters multiplying the factor of interest (organisation structure and technical capacity). Those latter parameters will allow us to quantify and test the effect of the factor of interest on the prevalence, and consequently their effect on the force of infection. Figure 21 shows the histograms of the skin test readings for each of the combinations, illustrating the potential large peak corresponding to the non-infected animals centred around 1 and a second much less pronounced peak potentially representing the infected animals, with a larger variation centred on 6.5. Using the Bayesian paradigm, the mixture model was fitted to the data generated and the resulting credible intervals are:

- for μ is (–2.36; –1.92);
- for γ_1 is (–0.62; –0.26), for γ_2 is (–0.94; –0.52), for γ_3 is (0.02; 0.03);
- for μ_1 is (1.39; 1.42), for μ_2 is (6.49; 6.78);
- for σ_1 is (0.14; 0.16) and for σ_2 is (2.59; 3.34).

It is clear that credible intervals associated with the parameters γ_1, γ_2 and γ_3 do not contain the zero indicating a significant effect on the prevalence. In particular:

- The odds between organisational structure B versus A being 0.64, the results indicate a reduction in the probability of being infected when belonging to structure B.
- The odds between private and official veterinarians is estimated to be between 0.39 and 0.59, also indicating a potential underestimation of the prevalence when private veterinarians assess the animals.
- The temporal dependence of prevalence is clearly illustrated in Figure 22. The mixture model, fit together with the histogram of the observed skin test readings, is shown in Figure 23. The vertical line indicates the potential cut-off that maximises the sum of the sensitivity and specificity of the skin test, an estimate of which is shown at the end of this section.

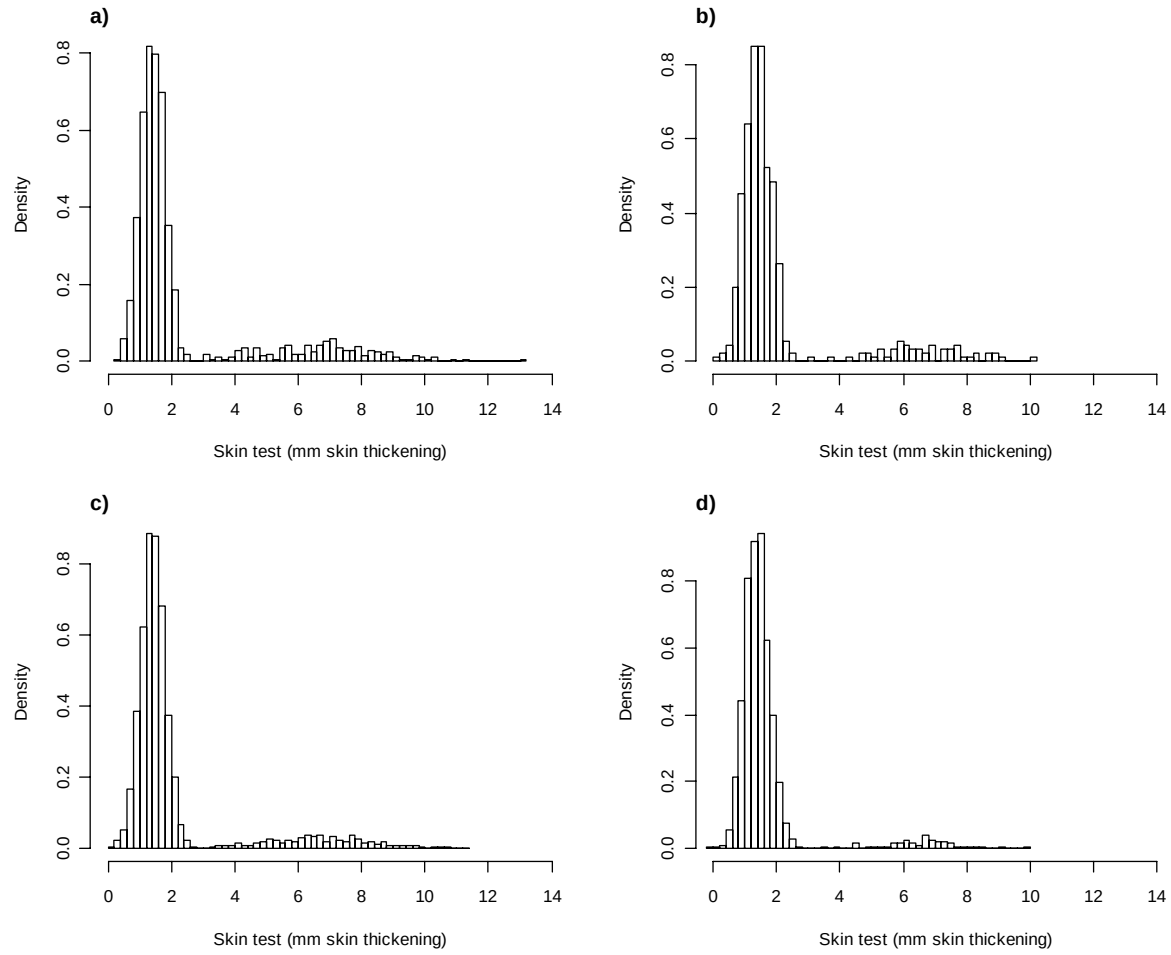


Figure 22: Histogram of the individual skin test readings for the four possible combinations of organisational structure and technical capacity: (a) the animal readings taken by official veterinarians in organisational structure A; (b) the animal readings taken by private veterinarians for organisational structure A; (c) the animal readings taken by official veterinarians for organisational structure B; and (d) the animal readings taken by private veterinarians for organisational structure B

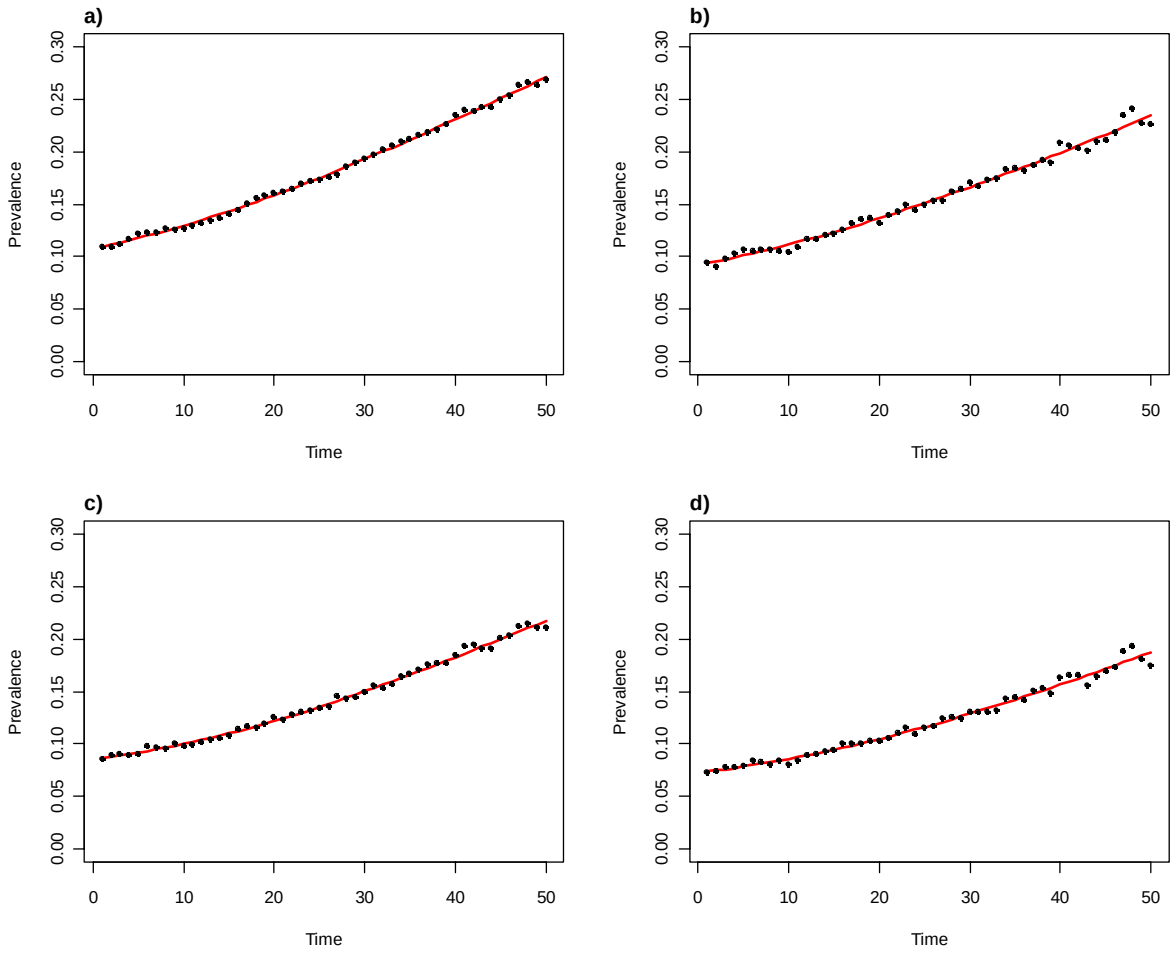


Figure 23: Prevalence estimation based on skin test readings for the four possible combinations of organisational structure and technical capacity: (a) the animal readings taken by official veterinarians in organisational structure A; (b) the animal readings taken by private veterinarians for organisational structure A; (c) the animal readings taken by official veterinarians for organisational structure B; and (d) the animal readings taken by private veterinarians for organisational structure B

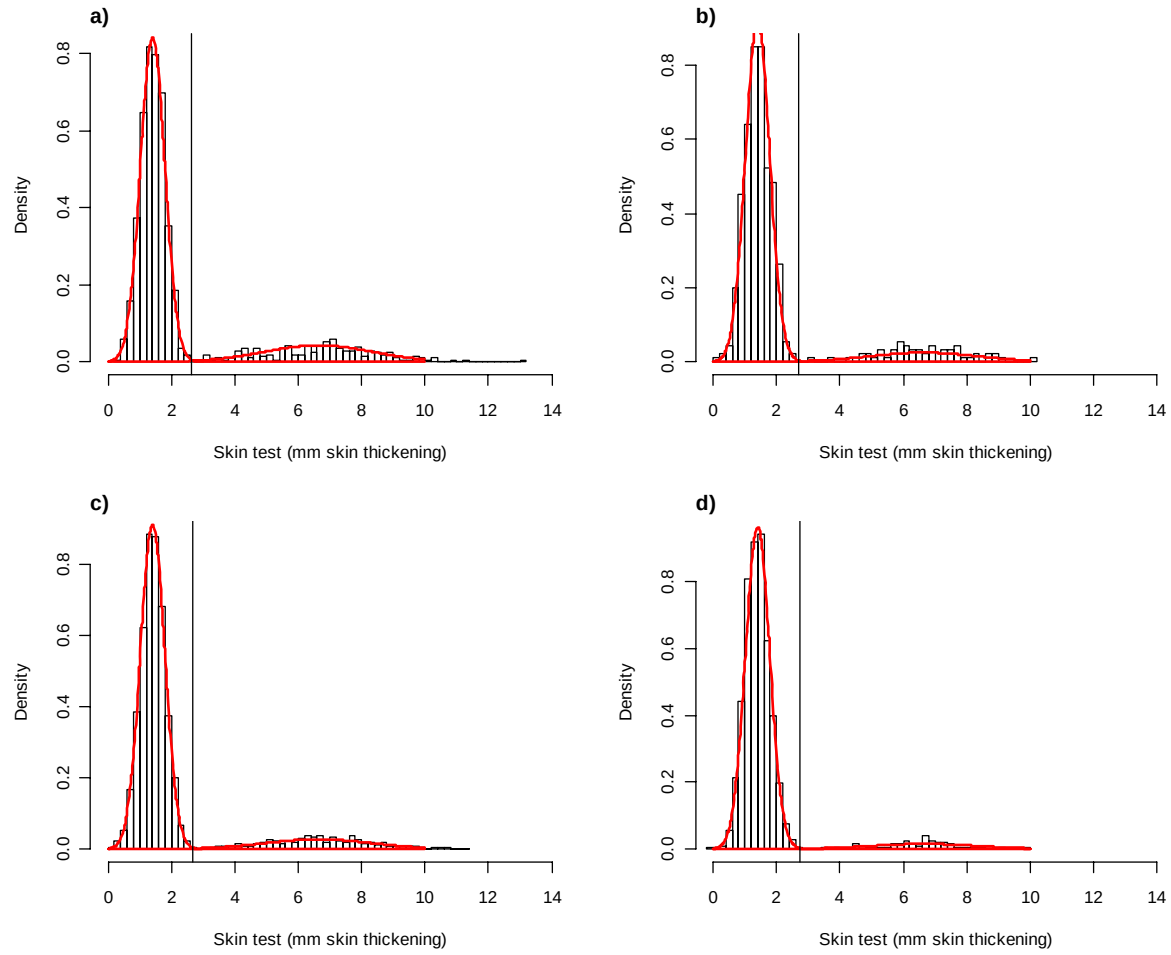


Figure 24: Histogram of observed skin test readings together with the model fitted and potential cut-off value for the four possible combinations of organisational structure and technical capacity: (a) the animal readings taken by official veterinarians in organisational structure A; (b) the animal readings taken by private veterinarians for organisational structure A; (c) the animal readings taken by official veterinarians for organisational structure B; and (d) the animal readings taken by private veterinarians for organisational structure B

The dependency of prevalence on non-biological factors is not unique for bTB, but it is common in many infectious diseases and so similar studies (as in the example previously presented) have been conducted in other fields. In general, it is expected that the risk of infection might depend on social behavioural factors. Examples of such dependency have been studied for hepatitis C among injected-drug users (Mathëi et al., 2006; del Fava et al., 2011). Mathëi and colleagues presented the way to model p_{i1} as a function of injection time and social behavioural risk factors (such as sharing syringes and other paraphernalia). In this case, the probability p_{i1} is modelled as:

$$p_{i1} = h\left(\mu + \beta \log\left(t_i\right) + \gamma_1 Z_1 + \gamma_2 Z_2\right)$$

where t is the exposure time, Z_1 represents the indicator variable for needle sharing and Z_2 represents the indicator variable when individuals were sharing other materials. The parameters γ_1 and γ_2 can be used to assess the impact of social behaviour factors on the prevalence of the disease.

D4. Use of mixture models to classify individual animals as non-infected or infected

This methodology could also be used to construct a classification rule that could be used in practice to classify individual animals as non-infected or infected.

As an example, group 1 is the non-infected component (dashed red) and group 2 (full blue) makes up the infected animals. Let us now suppose that we need to assess what the optimal cut-off is for a given test in order to maximise the sum of the sensitivity and specificity (minimising the overall misclassification error). Figure 24 illustrates how the mixture model could be used to obtain such an optimal value. It is important to mention that the definition of the classification rule is dependent on the goal that needs to be achieved.

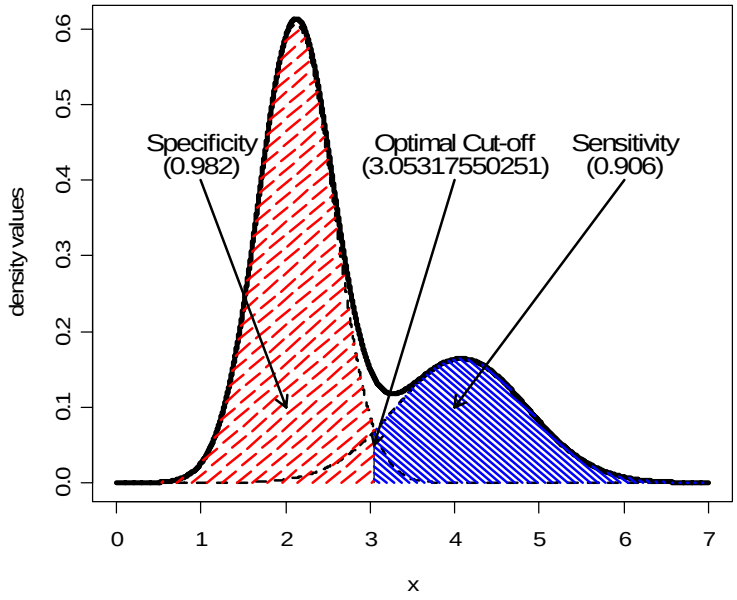


Figure 25: Illustration of use of the mixture model to obtain the optimal cut-off value for a given test

Analytically speaking, in order to estimate the optimal cut-off, the test value for which both densities cross (x_{opt}) (maximum value for the sum of sensitivity and specificity) must be calculated. In order to do so, the following equation has to be solved:

$$f(x|\theta_1) = f(x|\theta_2), \quad \text{where } f(x|\theta_j) = \frac{1}{\sqrt{2 \cdot \pi} \cdot \sigma_j} \cdot e^{-\frac{(x-\mu_j)^2}{2 \cdot \sigma_j^2}} \text{ and } j = 1,2$$

Note also that the mean of one of the components was rewritten as a sum of the mean from the other component plus some strictly positive term ($\mu_2 = \mu_1 + \delta$).

The solution that maximises the sum of sensitivity and specificity for this equation is:

$$\frac{(\mu_1 + \delta)\sigma_1^2 - \mu_1\sigma_2^2 - \sigma_1\sigma_2\sqrt{\delta^2 + (\text{Log}[\sigma_1] - \text{Log}[\sigma_2] + 2\text{Log}[(1-p_1)] - 2\text{Log}[p_1])(\sigma_1^2 - \sigma_2^2)}}{\sigma_1^2 - \sigma_2^2}$$

ABBREVIATIONS

+ (green plus)	test-positive, non-infected animal (false-positive test result)
– (green minus)	test-negative, non-infected animal (true-negative test result)
+ (red plus)	test-positive, infected animal (true-positive test result)
– (red minus)	test-negative, infected animal (false-negative test result)
λ_c	force of infection for cattle
$\lambda_{cc\text{-}a}$	force of infection resulting from animal contacts with other cattle herds in the area
$\lambda_{cc\text{-}h}$	force of infection within a cattle herd
$\lambda_{cf\text{-}a}$	force of infection resulting from fomites of other cattle herds in the area
λ_{cw}	force of infection resulting from cattle to wildlife
λ_{ec}	force of infection resulting from the environment to cattle
λ_{ew}	force of infection resulting from the environment to wildlife
λ_{osc}	force of infection resulting from other domestic species to cattle
λ_w	force of infection for wildlife
λ_{wc}	force of infection resulting from wildlife to cattle
$\lambda_{wf\text{-}a}$	force of infection resulting from fomites of other wildlife herds in the area
$\lambda_{ww\text{-}a}$	force of infection resulting from animal contacts with other wildlife herds in the area
$\lambda_{ww\text{-}h}$	force of infection within a wildlife herd
λ_{osw}	force of infection resulting from other domestic species to wildlife
a_i	the number of animals in herd *i*
APa	apparent bTB prevalence in an area
APa_{AT}	apparent bTB prevalence in an area, above the officially defined threshold
APa_{BT}	apparent bTB prevalence in an area, below the officially defined threshold
APh	apparent bTB prevalence in a herd
$\beta_{cc\text{-}h}$	within-herd cattle-to-cattle transmission
β_{ec}	transmission from the environment to cattle
β_{eos}	transmission from the environment to other domestic species

β_{ew}	transmission from the environment to wildlife
bTB	bovine tuberculosis
CL	confidence level
ELISA	enzyme-linked immunosorbent assay
IFN-γ	gamma interferon
IH	infected herds
IH_ia	the number of infected animals in the infected herds
IH_ia_i	the number of infected animals in each infected herd
MTBC	pathogens of the *Mycobacterium tuberculosis* complex (MTBC), that is *Mycobacterium bovis, M. caprae* or *M. tuberculosis*
OD	optical density
OIE	World Organisation for Animal Health
OTF	officially tuberculosis-free
R_o	basic reproductive number
Pa	bTB prevalence in an area
PCR	polymerase chain reaction
Ph	bTB prevalence in a herd
P_{os}	bTB prevalence in other domestic species
P_w	bTB prevalence in wildlife species
Se	sensitivity
SFh	herd sampling frequency
SICCT	single intradermal comparative cervical tuberculin
Sp	specificity
TPa	potential estimation of true area/country prevalence of affected herds considering the sensitivity and specificity of the area/country surveillance system
TPh	potential estimation of true within-herd prevalence considering the sensitivity (Se) and specificity (Sp) of the within-herd surveillance system
TSe	test sensitivity
TSp	test specificity

GLOSSARY

Actor	Any person involved in shaping the non-biological context of bTB
Anchor model	Description of the interactions between the most important biological parameters involved in bTB infection, detection and control (biological context) and which are affected by actors and influences which define the non-biological context
Animal characteristics	Parameters of the animal associated with transmission of infection
Animal level	All characteristics that are specific for each individual animal (e.g. bTB status, age, breed, immune status)
Animal movements	Movement of live animals between herds (within as well as between regions)
Area level	All activities and events taking place 'between herds'. The term is used in its epidemiological meaning. Depending on the situation one wants to analyse, the area level may be considered at any scale of epidemiological relevance (from a few neighbouring herds to any geographical area), or of relevance in the context of the Council Directive 64/432/EEC (a Member State or part of its territory which is at least a region of 2 000 km^2)
Bacterial load	The number of *Mycobacterium* organisms in the environment
Breakdown herd	Herd in which cattle tested positive in bTB testing resulting in animals being slaughtered and the herd being placed under temporary movement restrictions
Compartmental model of the conceptual framework	Detailed representation of one component of the anchor model
Component of the conceptual framework	The elements of the anchor model such as testing, animal case definition, herd case definition, herd OTF status, area OTF status, herd sampling, area sampling or management, and control and non-biological context
Environment	The surroundings in which MTBC could be present
Episystem	The ecological context of the epidemiological problem
Influencing factor	Factors of the non-biological context of bTB that could affect bTB infection, detection and control
Force of infection	The rate at which a non-infected animal becomes infected
Framing (a disease)	Term used in social sciences to refer to a set of concepts and theoretical perspectives on how individuals, groups and societies organise, perceive and communicate about reality (e.g. a disease)
Herd	An animal or group of animals kept on a holding (within the meaning of Article 2(b) of Directive 92/102/EEC) as an epidemiological unit, whereas, for wildlife, a 'herd' is considered to represent a group of animals belonging to the same social group
Herd level	All activities and events taking place 'within a herd'

Infectious animal	An animal that is infected with bTB and sheds the pathogen
Latently infected animal	An animal that is infected with bTB, but the pathogen is not multiplying
Management and control	Farm characteristics, procedures and actions that can influence the risk factors influencing the transmission of bTB
Non-infected animal	An animal that not has been infected
Level in the conceptual framework	The animal, herd or area level
OTF status	Officially tuberculosis-free status as defined by the Council Directive 64/432/EEC
Other (domestic) species	Non-bovine domestic species
Pathogen characteristics	Parameters related to pathogenicity and virulence of a specific strain (genome sequence, expressed/secreted proteins)
Policy–implementation distance	The difference between policy and its perfect implementation
Post-slaughter testing	One or a combination of bTB tests performed on a dead animal (e.g. post-mortem inspection for lesions, bacteriology, histology, PCR)
Pre-slaughter testing	One or a combination of bTB tests performed on a live animal (e.g. skin test, IFN-γ assay, serology)
Testing	The testing procedure, composed of one or more diagnostic tests, that is in place in a given area and can be used both for surveillance and as an integral part of control efforts
Testing and sampling	All actions and processes related to taking samples and performing the testing procedure
Wildlife	Free-ranging animal species