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Assessment of the probability of introduction of bovine tuberculosis to Danish cattle farms via imports of live cattle from abroad and immigrant workers

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ABSTRACT

Denmark has been recognized as officially free (OTF) from bovine tuberculosis (bTB) since 1980. In this study, we estimated the annual probability (*PIntro*) of introducing *Mycobacterium bovis* into the Danish cattle population, through (a) imports of cattle and (b) foreign personnel working in Danish cattle herds. Data from 2000 to 2013 with date, number and origin of imported live cattle were obtained from the Danish Cattle Federation. Information on immigrants working in Danish cattle herds was obtained through a questionnaire sent by email to a sample of Danish cattle farmers ($N = 460$). Inputs obtained from data analysis, expert opinion, the questionnaire and literature were fed into three stochastic scenario tree models used to simulate the effect of import trade patterns, and contact between immigrant workers and cattle. We also investigated the opportunity of testing animals imported from OTF countries by tuberculin skin test and animals from non-OTF countries by interferon-γ test (IFN-γ), exemplified by using year 2009 where the number of imported animals was higher than usual.

Results showed that *PIntro* is driven mainly by importation of live cattle. The combined median annual probability of introducing *M. bovis* into the Danish cattle population by either imported live cattle or infectious immigrant workers, ranged from 0.3% (90% prediction interval (P.I.): 0.04%:1.4%) in 2001 to 4.9% (90% P.I.: 0.6%; 19.2%) in 2009. The median of the median *PIntro* estimates from the 14 years was 0.7% (median of 90% P.I.: 0.08%; 3.5%). Hence, on average, at least one introduction each 143 years could be expected, if the annual number of imported animals does not change remarkably in the future.

If the number of imported animals increases, compared to the years we analyzed, additional testing of imported cattle might be considered. For example, in 2009, *PIntro* would have been reduced from 4.9% to 0.8% (90% P.I.: 0.1%; 4.7%) if animals from OTF countries had been tested with the tuberculin skin test and animals from non-OTF countries had been tested with the IFN-γ test.

The presented model could be used easily in other countries with similar bTB status to Denmark, where wildlife represents a negligible probability of infection for domestic cattle and where the imported live cattle represent the main pathway of bTB introduction into the local cattle population.

1. Introduction

Bovine tuberculosis (bTB) is caused mainly by *Mycobacterium bovis* (*M. bovis*) or *M. caprae*, which similarly to other mycobacteria (e.g., *M. tuberculosis*) can cause tuberculosis in humans (O'Reilly and Daborn, 1995; Kubica et al., 2003; de la Rua-Domenech, 2006; Chen et al., 2009).

M. caprae has been defined as a subsp. of *M. bovis* by Niemann et al. (2000, 2002), while Aranaz et al. (2003) classified it as a species apart. Anyway, *M. bovis* and *M. caprae* are very similar with respect to the epidemiology, pathogenesis and from a diagnostic point of view (e.g. in cattle they cause similar reactions to the tuberculin skin test and indistinguishable lesions). In this study we consid-

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ered these two types of *Mycobacteria* altogether in line with EFSA (2013a).[1]

Cattle can also become infected with *M. tuberculosis* (Pavlik et al., 2003; Chen et al., 2009) through contact to infectious persons (Ocepek et al., 2005). Nevertheless, the incidence of *M. tuberculosis* in cattle populations is usually assumed to be very low (Krishnaswami and Mani, 1983; Pavlik et al., 2003; Ocepek et al., 2005).

Generally, few bTB infected animals are found within infected cattle herds (e.g., one to four out of approximately 145) (Menzies and Neill, 2000; Goodchild and Clifton-Hadley, 2001), and purchased cattle represent the main route of disease transmission between herds (Menzies and Neill, 2000; Gilbert et al., 2005). Moreover, *M. bovis* can infect wildlife (e.g., deer, wild boars and badgers) and other domestic ruminants, which can act as sources of the infection for cattle (de la Rua-Domenech, 2006; Pavlik, 2006; Rodríguez-Prieto et al., 2012).

M. bovis is shed in milk, nasal secretions and feces (O'Reilly and Daborn, 1995; Goodchild and Clifton-Hadley, 2001). However, airborne spread over short distances (e.g., within the same airspace) is considered to be the main route of bTB spread between cattle (O'Reilly and Daborn, 1995; de la Rua-Domenech, 2006), and most often, lesions are located in the lymph nodes of the thoracic cavity and of the head (O'Reilly and Daborn, 1995; Goodchild and Clifton-Hadley, 2001).

Genital transmission is rare but may occur during coitus, if *M. bovis* is present in the reproductive organs (Menzies and Neill, 2000). Nevertheless, bulls entering into approved semen collection centers must arrive from bTB free herds and must test negative with the intradermal tuberculin test at admission (Council Directive, 64/432/EEC; Council Directive, 2003/43/EC). The same test (Monaghan et al., 1994; de la Rua-Domenech et al., 2006) must be carried out at least once a year on all bovine animals present in approved semen collection centers (Council Directive, 2003/43/EC). Embryos traded between countries must be bTB free and must have originated from animals and herds free from bTB (OIE, 2013).

M. bovis can survive in the environment (e.g., contaminated soil and feces) for a few months, though variations have been observed under different environmental conditions (Duffield and Young, 1985; Menzies and Neill, 2000). Still, Menzies and Neill (2000) argued that environmental contamination should play an insignificant role in the maintenance of *M. bovis* infection within cattle herds.

Humans can become infected with *M. bovis* through consumption of raw milk (Doran et al., 2009), inhalation and by direct contact with mucous membranes and skin cuts/abrasions (Griffith and Munro, 1944; Grange and Yates, 1994; O'Reilly and Daborn, 1995; de la Rua-Domenech, 2006). Transmission of *M. bovis* to cattle by bedding and hay contaminated with urine from infected humans is possible (Grange and Yates, 1994; O'Reilly and Daborn, 1995). Dust and dried sputum are also considered effective to transmit the infection (O'Reilly and Daborn, 1995).

Usually, between 1% and 6% of the human cases of tuberculosis seen world-wide are due to *M. bovis* (Griffith and Munro, 1944; Cutbill and Lynn 1944; Ritacco and de Kantor, 1992; Grange and Yates, 1994; Cosivi et al., 1998; de la Rua-Domenech, 2006; Chen et al., 2009).

Denmark has been recognized as officially free from bTB (OTF) since 1980 (Commission Decision, 80/984/EEC; Reviriego Gordejo and Vermeersch, 2006), and the last outbreak of tuberculosis in cattle occurred in 1988, with infection being of human origin (Danish Ministry of Food Agriculture and Fisheries, 2012). European Union member states can be certified OTF if less than 0.1% of the cattle herds are test-positive for *M. bovis* (Council Directive, 64/432/EEC; Council Directive, 98/46/EC). Danish farmers export animals to other countries, so keeping the OTF status and keeping the cattle population free from bTB is a priority. The objective of this study was to estimate the annual probability (*PIntro*) of introducing *M. bovis* into Danish cattle herds and to determine the effect of risk mitigation measures, such as testing imported cattle, on *PIntro*.

2. Materials and methods

To carry out the risk assessment, we considered as main bTB introduction routes (a) the import of undetected, infected cattle from non-OTF countries, (b) the import of infected cattle from OTF-countries, and (c) the transmission of bTB from infectious immigrant workers to Danish cattle. Wildlife was ruled out, because (to our knowledge) *M. bovis* has never been reported in free-ranging Danish wildlife. Furthermore, bTB has not been detected in farmed deer since 1994 in the on-going meat inspection of all farmed deer (Danish Ministry of Food Agriculture and Fisheries, 2012).

In EU Member States, samples from suspected bTB infected animals must be tested for *M. bovis* (e.g., by bacteriological culture test) and positive cases should be dealt with to substantiate the OTF status. Hence, controlling cattle infections due to *M. tuberculosis*[2] is not implicit in the current EU legislation (Council Directive 64/432/EEC).

For each of the three potential *M. bovis* introduction routes, we made a stochastic scenario tree. The trees were defined as: "non-OTF" for cattle imported from "non-OTF" countries (Fig. 1), OTF for cattle imported from OTF countries, and "W" for immigrant workers (Fig. 2), respectively. The tree used for the OTF animals was the same as for "non-OTF" cattle, reported in Fig. 1, but without the test node, since for those animals tuberculin testing is not required before export (Council Directive 64/432/EEC; Commission Decision, 2000/504/EC). In our scenario trees each node constitutes a point that splits the population of interest into either population proportions with different risk properties, or probabilities of an event, e.g. that an imported animal comes from a truly infected herd, or that an imported animal originating from a truly infected herd is truly infected itself (which is equivalent to the within-herd prevalence). Hence, each imported animal only fits in one of the branches of the tree. The summed probability of all limbs in one scenario tree is always 100%.

Inputs obtained by analysis of available data on imports and from literature were fed into the stochastic scenario trees. For the "W" tree, additional information was obtained through a questionnaire, which was sent to a sample of Danish cattle farmers (Section 2.2.3). The overall annual probability of bTB introduction into Danish cattle herds (*PIntro*) was then estimated by combining outputs of the three scenario trees into Eq. (1):

$$PIntro = 1 - [(1 - P_{non\text{-}OTF}) \times (1 - P_{OTF}) \times (1 - P_W)] \quad (1)$$

where $P_{non\text{-}OTF}$ is the annual probability that at least one infected cattle is introduced from "non-OTF" countries to the Danish cattle population, P_{OTF} is the annual probability that at least one infected cattle is imported from OTF countries, and P_W is the annual probability that at least one Danish cattle is infected by the immigrant worker(s), who shed *M. bovis* (e.g., in sputum, exhaled air or urine). We had to assume that P_W was constant between the considered years (2000–2013), since we could only estimate the number of immigrants working in Danish cattle herds for 2014.

[1] Further in the text, only *M. bovis* is mentioned, but any reference to *M. bovis* also includes *M. caprae* (EFSA, 2013a), unless the contrary is specified.

[2] In another study, we carried out the risk assessment also for *M. tuberculosis*, because it can cause reactions in the tuberculin skin test and lesions in cattle, which could lead to removal of test-positive animals. In this paper we focus on *M. bovis*.