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Parameter estimates to support future risk assessment of *Mycobacterium bovis* in raw milk cheese

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ABSTRACT

Zoonotic tuberculosis, caused by *Mycobacterium bovis*, is mainly linked to the consumption of raw milk from infected cows. In many countries, cases are rare, due to pasteurisation of milk and national programmes to control *M. bovis* infection in cattle. Speciality cheeses, which are often produced using raw milk, present challenges to risk managers in countries where *M. bovis* is endemic or (re-) emerging. A key concern is the potential risk of zoonotic transmission of *M. bovis* via the consumption of dairy products produced using raw milk originating from herds infected with *M. bovis* (bovine tuberculosis, bTB). The aim of this study was to determine parameter estimates to support the future risk assessment of *M. bovis* in raw milk cheese. In this study, the hazard was identified as viable *M. bovis* organisms in raw milk cheese. Parameters of interest in this study related to exposure assessment (the estimated extent of human exposure to viable *M. bovis* organisms) and hazard characterisation (the risk posed to human health following exposure to viable *M. bovis* organisms). The pathway for exposure assessment was visualised using a conceptual framework, which describes the steps through which *M. bovis* may be transferred from an infected animal(s) through manufacturing to the final cheese product. Estimation of most parameters for exposure assessment and hazard characterisation was undertaken using systematic literature reviews. Estimates could be derived for many parameters, but not all. In particular, the number of *M. bovis* organisms excreted in the milk and present in the faeces of infected cattle are unknown. There is zero-tolerance for *M. bovis* in foods of animal origin destined for human consumption in European legislation. This work has highlighted important gaps in knowledge, and areas for further research. For each of the parameters for which estimates are available, we outline the types/sources of uncertainty as reflected in relevant published papers. In any future application of these parameter estimates, care will be needed to reflect the uncertainties associated with these elements of exposure assessment.

1. Introduction

Zoonotic tuberculosis is a form of tuberculosis (TB) in people caused by *Mycobacterium bovis (M. bovis)*, which belongs to the *M. tuberculosis* complex. It is a serious human disease similar to tuberculosis caused by *M. tuberculosis*, but generally indistinguishable on clinical, radiological or pathological grounds humans (de la Rua-Domenech, 2006). The contribution of zoonotic TB caused by *M. bovis* to the global burden of active human TB cases is uncertain, but is at higher incidence in regions where there is a close association between people and cattle, and where milk and dairy products are consumed unpasteurised (Kock et al., 2021). A Roadmap for Zoonotic TB was launched in 2017 to address bovine and zoonotic TB, supported by four partners including the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease. It is built on a One Health approach to address health risks across sections, with a focus on improving the scientific evidence base, reducing transmission at the animal-human interface and strengthening intersectoral and collaborative approaches (FAO, 2017).

In some countries, including Ireland, this disease is primarily of historic interest, however, it was previously endemic in the human population. The consumption of raw (that is, unpasteurised) milk and milk products is recognised as the main vehicle for *M. bovis* transmission from cattle to humans, although other routes (aerosol inhalation or direct contact with mucous membranes and skin abrasions) are possible

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(de la Rua-Domenech, 2006).

The prevalence and presentation of zoonotic tuberculosis is influenced by the prevalence of bovine TB (that is, infection of cattle with *M. bovis*) and the effectiveness of national control (or eradication) efforts, and also by national policies for milk pasteurisation. In the earlier part of the last century, there was a very high bovine TB prevalence in the Irish national cattle population, noting that gross pathology consistent with bovine TB was present in 31–33% of cattle slaughtered at the city abattoirs in Dublin during 1929–38 (Cotter et al., 1996). At this time, and prior to the widespread implementation of milk pasteurisation, zoonotic tuberculosis in children was a common and often serious condition, characterised by infection of the gastrointestinal system and associated organs and tissues (reviewed by de la Rua-Domenech, 2006).

In Ireland, the introduction of pasteurisation of milk in the 1950s resulted in a dramatic reduction in the incidence of zoonotic tuberculosis in humans. Similar experiences were observed in other countries, including the UK (de la Rua-Domenech, 2006). Although *Mycobacteria* are amongst the most heat resistant non-sporulating bacteria, standard high temperature/short time pasteurisation of *M. bovis* leads to 4.1–4.9 $\log_{10}$ reductions at 65 °C, with a holding time of 35 s, which far exceeds the Codex Alimentarius requirements for inactivation of pathogens using this process (Hammer et al., 2015). At this time, the national control program for bovine tuberculosis was introduced in Ireland (c. 1954) leading to a considerable decrease in the prevalence of the disease in the national cattle herd in subsequent years (More and Good, 2006). As a consequence of these two actions, zoonotic tuberculosis is now rare in Ireland. This is consistent with official figures, with an average of 6 notified cases of zoonotic tuberculosis (range 2–12) annually between 2006 and 2018, accounting for 3.2% of all notified tuberculosis cases in 2018 (HPSC, 2021a, 2021b). It is not clear however, if these cases are autochthonous or imported, or whether there is a strong epidemiological link between these few cases and the prevalence of bovine tuberculosis in the Irish cattle herd.

Outbreaks of zoonotic tuberculosis in Ireland and in other developed countries are now rare, but do occur. This is evidenced by an outbreak of *M. bovis* infection in people and cattle on a dairy farm in Ireland that was reported by Doran et al. (2009), highlighting the ongoing risks associated with raw milk consumption. In this case, the consumption of raw milk from a seven-year-old cow with tuberculous mastitis was implicated as the source of infection. During autumn 2004 and spring 2005, 25 of 28 (89%) calves born on the farm were identified as TB reactors (that is, positive to the tuberculin skin test). Milk from the implicated cow had mainly been used to feed calves, and was added on occasion to the bulk tank. The family collected milk from the bulk tank and consumed it without pasteurisation. Five of six family members were positive on the tuberculin skin test, including two young children who suffered severe health impacts. In these children, continuous antibiotic therapy for 19 months was required.

A scientific opinion on the public health risks related to the consumption of raw drinking milk highlighted the risk of zoonotic tuberculosis associated with unpasteurised milk and milk products (EFSA, 2015). In the UK, there is an ongoing risk of *M. bovis* infection for some individuals in the form of continuing on-farm consumption of unpasteurized cow's milk, retail sales by approved establishments of unpasteurized milk and dairy products, and occupational exposure to infectious aerosols from tuberculous animals and their carcasses (de la Rua-Domenech, 2006).

In Europe, the production and consumption of speciality cheeses has a long tradition. In Ireland, speciality cheese are often made on a small scale at the farm where the milk is produced (O'Brien et al., 2009), and there were over 60 Irish farmhouse cheese producers in 2020 (Teagasc, 2020). Speciality cheeses are often produced using raw milk (de Sainte Marie et al., 2020), which presents challenges to risk managers, including potential risks to public health (Hunt et al., 2012). In countries where bovine TB is either endemic or (re-)emerging, including France (Bouchez-Zacria et al., 2018), India (Refaya et al., 2020), Ireland (More, 2019) and the United Kingdom (Godfray et al., 2013), *M. bovis* is one such risk. A key concern is the potential risk of zoonotic tuberculosis infection in humans from consuming dairy products produced using raw milk originating from herds infected with bovine TB.

The assessment and management of risk posed by *M. bovis* in herds that supply raw milk cheese is not straightforward. EU and national legislation is clear with respect to actions to be taken once infection has been diagnosed. As outlined in Regulation 853/2004 and SI 58/2015, milk from reactor (and inconclusive) animals is prohibited from entering the food chain from the time tuberculosis has been diagnosed. Further, milk from a restricted herd must be subjected to the appropriate heat treatment prior to direct consumption or for manufacturing. What is less clear, however, is the safety of raw milk cheese that had been produced prior to the detection of infection, whilst the herd was *"officially tuberculosis free"* (that is, without any restrictions to trade; as defined in Directive 64/432/EEC on animal health problems affecting intra-Community trade in bovine animals). In other words, is the cheese in storage, that was made from raw milk originating from a herd that was officially tuberculosis free at the time of milk collection but subsequently became *"restricted"*, safe for human consumption (free from the risk of zoonotic tuberculosis)? In this context, a herd is restricted following the detection of bovine tuberculosis leading to restrictions on the movement of bovine animals or their products from that holding, as outlined in SI 308/1989 as amended. In Ireland, all animals are tested at least yearly, in an annual full-herd test (Good et al., 2017). Nonetheless, when infection is disclosed, it is typically not possible to know the precise time at which the infection had been introduced. In most animals, a full response to the tuberculin skin test is likely within 3–6 weeks post-infection (de la Rua-Domenech et al., 2006), however, given the interval between testing, it is plausible that infection may have occurred at any time since the most recent negative, full-herd test.

The aim of this study was to determine parameter estimates to support future risk assessment of *M. bovis* in raw milk cheese. This work is of particular relevance to Ireland, where there is a need to determine the public health risk posed by *M. bovis* from the consumption of raw milk cheese originating from herds during the period prior to the detection of *M. bovis* infection.

2. Methods

2.1. Identifying parameters of interest

In this study, the hazard was identified as viable *M. bovis* organisms in raw milk cheese. Parameters of interest in this study related to exposure assessment (the estimated extent of human exposure to viable *M. bovis* organisms) and hazard characterisation (the risk posed to human health following exposure to viable *M. bovis* organisms). The pathway for exposure assessment was visualised using a conceptual framework, adapted from previous work by the Food Safety Authority of Ireland (FSAI, 2008), which describes the steps through which *M. bovis* may be transferred from an infected animal(s) through manufacturing to the final cheese product (Fig. 1). Ireland was used as the context of this work.

This study was informed by the principles and guidelines for the conduct of microbiological risk assessment from the Codex Alimentarius Commission (Food and Agricultural Organization, World Health Organization, 2001), with particular reference to hazard identification, exposure assessment and hazard characterisation.

2.2. Parameter estimation

2.2.1. Exposure assessment

We identified six key parameters within the pathway for exposure assessment, corresponding to Steps 2, 4, 6, 8, 12 and 13 in Fig. 1.

Recent systematic literature reviews and meta-analyses were used to

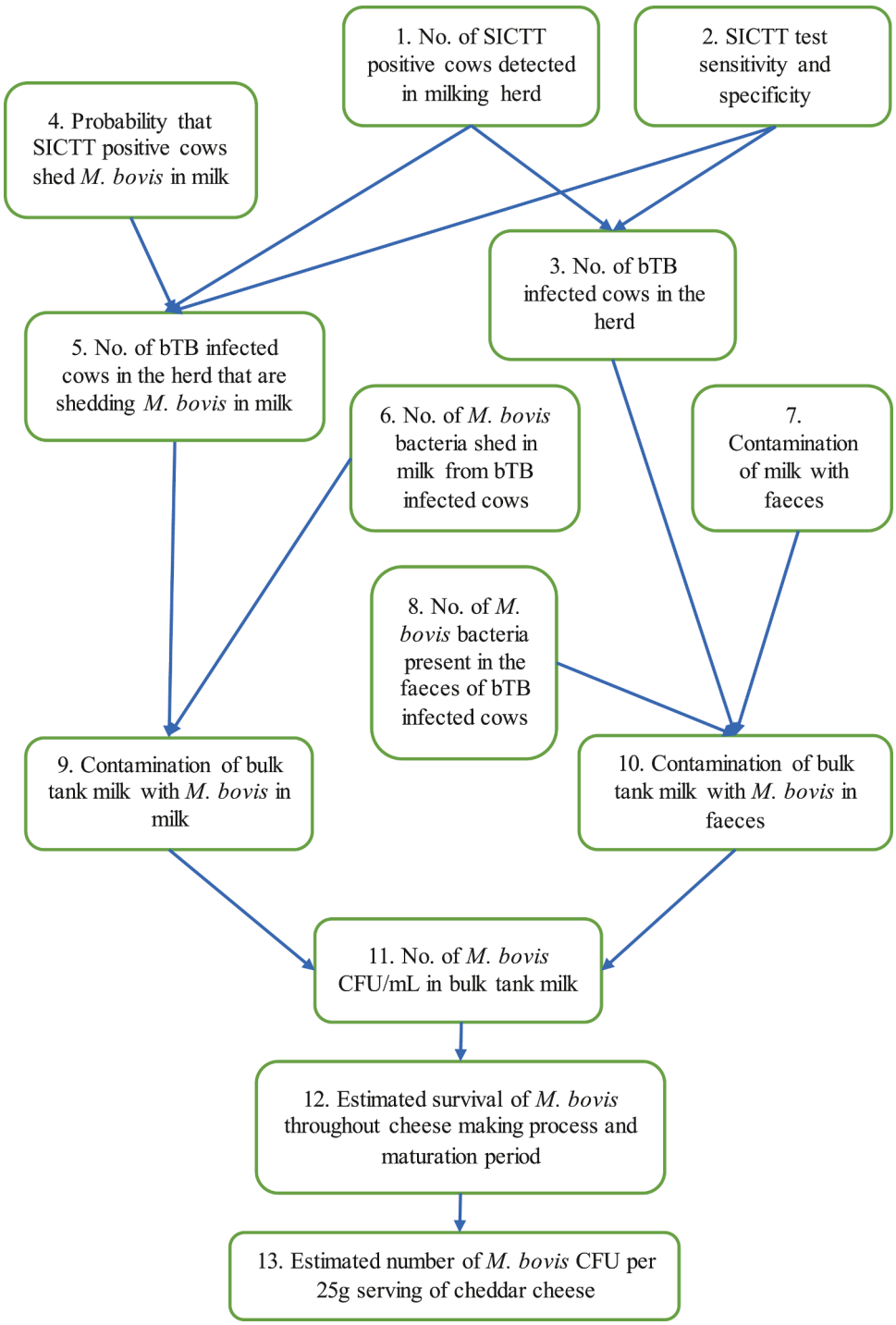

Fig. 1. A pathway for exposure assessment. Abbreviations including SICTT (Single Intradermal Comparative Tuberculin Test), bTB (bovine tuberculosis) and CFU (colony forming units).

estimate the diagnostic sensitivity and specificity of the single intradermal comparative tuberculin test (SICTT) (Step 2 in the pathway), the probability of *M. bovis* shedding in milk in a SICTT positive cow (Step 4) and the number of *M. bovis* bacteria shed in milk of an bTB infected cow (Step 6).

Literature searches were undertaken in the Scopus platform to estimate the number of *M. bovis* bacteria present in the faeces of bTB infected cattle (Step 8) and the survival of *M. bovis* throughout cheese making process and maturation period (Step 12). The Scopus platform was selected because it has a higher journal coverage than similar databases (e.g. Web of Science) and also searches citations from PubMed and Medline (Falagas et al., 2008; Baykoucheva, 2010). No restrictions on language were imposed so long as an English abstract was available.

With respect to Step 8, a literature search was undertaken to identify literature relating to the study of *M. bovis* in bovine faeces. To maximize the sensitivity of the search, any study investigating *M. bovis* in faeces, regardless of the specified study objectives, was considered. A search on titles, abstracts and key words was conducted in Scopus on 02 July 2021 using the following search strategy without limitation by date or language:

(TITLE-ABS-KEY("*Mycobacterium bovis*") OR TITLE-ABS-KEY("bovine tuberculosis") OR TITLE-ABS-KEY("btb") OR TITLE-ABS-KEY ("bovine tb") OR TITLE-ABS-KEY("*m. bovis*") OR TITLE-ABS-KEY("*m. bovis*") AND TITLE-ABS-KEY("faeces") OR TITLE-ABS-KEY ("faeces"))

With respect to Step 12, a literature search was undertaken to identify literature relating to the survival of *M. bovis* in cheese. To maximize the sensitivity of the search, any study investigating *M. bovis* in cheese, regardless of the specified study objectives, was considered. A search on titles, abstracts and key words was conducted in Scopus on 16 June 2021 using the following search strategy without limitation by date of publication or language:

(TITLE-ABS-KEY("*Mycobacterium bovis*") OR TITLE-ABS-KEY("bovine tuberculosis") OR TITLE-ABS-KEY("btb") OR TITLE-ABS-KEY ("bovine tb") OR TITLE-ABS-KEY("*m. bovis*") OR TITLE-ABS-KEY ("*m. bovis*") AND TITLE-ABS-KEY(cheese)).

Relevant to the estimated number of *M. bovis* colony forming units per 25 g serving of cheddar cheese (Step 13), estimates of the typical cheese consumption (g/day) amongst adults and children in Ireland were obtained from the Irish National Adult Nutrition Survey (2008–10) (Irish Universities Nutrition Alliance, 2011) and the National Children's Food Survey II (2017–18) (Irish Universities Nutrition Alliance, 2019).

Reference is made to several studies that have sought to quantify the fecal contamination of bulk tank milk (Step 7), however, a systematic literature review was not undertaken.

2.2.2. Hazard characterisation

A review of the scientific literature was undertaken to identify relevant studies relating to experimental oral inoculation/infection of animals with *M. bovis*. A search on titles, abstracts and key words was conducted in Scopus on 02 July 2021 using the following search strategy without limiting by date of publication or language:

TITLE-ABS-KEY ("*Mycobacterium bovis*" AND "inoculat*" OR "experiment*" OR "dose" OR "challenge" AND "oral" OR "gastrointest*").

In addition, a review of EU and Irish legislation was conducted to determine the legal tolerance for *M. bovis* in foods of animal origin destined for human consumption.

2.3. Uncertainties associated with parameter estimates

The main types/sources of uncertainty affecting inputs to scientific assessments are listed in the principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessment (EFSA Scientific Committee et al., 2018). As outlined in Chapter 8 of that document (*"Identification of sources of uncertainty"*), these include ambiguity, accuracy and precision of the measures, sampling uncertainty, missing data within studies, missing studies, assumptions about inputs, statistical estimates, extrapolation uncertainty, and other uncertainties.

For each parameter estimate (except those where estimates were not available or relevant), all relevant types/sources of uncertainty were identified based on information presented in relevant published papers.

2.4. Past risk assessments

A narrative review was conducted to identify past examples of risk assessments to evaluate the public health risk posed by *Mycobacterium bovis* from the consumption of cheese produced from raw milk originating from infected bovine herds.

3. Results and discussion

3.1. Exposure assessment

3.1.1. Estimating the diagnostic sensitivity and specificity of the SICTT

The systematic review and meta-analysis by Nuñez-Garcia et al. (2018) reported the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. This work was supplemented by Downs et al. (2018) who conducted an evaluation of the methodological quality of each of the contributing studies. In this meta-analysis by Nuñez-Garcia et al. (2018), estimates for SICTT test sensitivity and specificity were modelled to be as relevant as possible to test conditions in Ireland and the UK. The authors report the median SICTT test sensitivity at 0.50 (Bayesian credible intervals, CRL) (95% CRL 0.26–0.78)) and the median specificity at 1.00 (95% CRL 0.99, 1.00).

Several types/sources of uncertainty are apparent, particularly with respect to ambiguity, accuracy and precision of the measures and extrapolation. In their evaluation, Downs et al. (2018) found that most of the reviewed studies had deficiences in design and conduct that could lead to known biases. As examples, in many of the studies there was *"probable awareness of the index test results when performing the reference test"*. Further, *"absence of information about animal withdrawals and uninterpretable study results was another common problem"*. These authors do acknowledge that quality concerns may have arisen because of poor reporting as opposed to methodological deficiencies in the studies. It should also be noted that multiple factors influence the operating characteristics of diagnostic tests, which may impact these estimates. With the SICTT, sensitivity is particularly inflenced by stage of infection and disease, whereas specificity can vary in different locations, depending on the presence of cross-reacting organisms (de la Rua-Domenech et al., 2006). Nuñez-Garcia et al. (2018) did not weigh sensitivity and specificity estimates according to assessed study quality. However, they expressed confidence that publication bias was unlikely, given the effort taken during a comprehensive and systematic search of both the published and grey literature.

Of all the parameters under investigation in the current paper, the estimates for the sensitivity and specificity of the SICTT are likely the most robust. The review by Nuñez-Garcia et al. (2018) is recent, related to the geographic area of interest (Ireland), and conducted using Bayesian methods, which are appropriate given the challenges associated with test assessment in the absence of a gold standard (Johnson et al., 2019; Cheung et al., 2021). The estimates of test sensitivity and specificity needs to be used with care, noting the types/sources of uncertainty that are apparent.

3.1.2. Estimating the probability of M. bovis shedding in milk in a SICTT positive animals

The most relevant literature identified relating to the parameterisation of this model input is the systematic literature review and meta-analysis by Collins et al. (2022). In this study, the authors reported the probability of detecting *M. bovis* in the milk of tuberculin skin test positive animals at 0.08 (95%CI: 0.04–0.13).

Several types/sources of uncertainty are apparent, particularly with respect to ambiguity, accuracy and precision of the measures, sampling uncertainty and extrapolation. As reflected by Collins et al. (2022), there are methodolological concerns with the reviewed studies, related to the type of study conducted (few were based on prevalence studies), the limited sample sizes, the limited generalisability, and the potential for bias. Many of the included studies did not adequately describe appropriate sample size calculations or choice of sampling schemes used, leading to potential sample selection bias. Further, most studies included in this synthesis originate in non-English speaking countries, with the potential for bias following the exclusion of non-English articles during initial screening. No studies are available from counties with an epidemiological situation for bovine TB similar to Ireland. Indeed, these

estimates are solely derived from countries with endemic (generally uncontrolled) bovine TB. It is plausible that estimates from Ireland, if available, would be lower, as all bovine animals are subjected to SICTT testing at least yearly, which should limit the number of animals with advanced disease.

In conclusion, the estimates from Collins et al. (2022) are the best currently available. However, there are multiple types/source of uncertainty. Further research in this area is needed to establish the probability of shedding in a context relevant to Ireland.

3.1.3. Estimating the number of *M. bovis* bacteria shed in milk in SICTT positive animals

Similar to estimating the probability of *M. bovis* shedding in milk in SICTT positive animals, the most relevant literature identified relating to the number of *M. bovis* bacteria shed in milk in skin test positive animals is the systematic literature review and meta-analysis undertaken by Collins et al. (2022). In this study, the authors undertook a comprehensive search of the literature investigating *M. bovis* in milk but did not identify any study that quantified the number of *M. bovis* bacteria in raw bovine milk.

No meaningful data are available estimating the number of *M. bovis* bacteria shed in milk of SICTT positive cattle. An Ethiopian study by Mariam (2014), which relates to cows infected with *M. tuberculosis* rather than *M. bovis*, provides the only available parameter estimate, namely 4.7 ± 4.4 log CFU/mL in a pooled milk sample obtained from 30 tuberculin skin-test positive cows. We note that several authors (Phillips et al., 2003; Jordão et al., 2005; de la Rua-Domenech, 2006; Serrano--Moreno et al., 2008; Yesuf, 2012; de Oliveira et al., 2018; Robinson, 2014, 2019) refer to Zanini et al. (1998) when reporting the likely number of *M. bovis* bacteria in raw bovine milk, with Zanini et al. (1998) reporting that *"A typically infected udder may excrete tubercle bacteria to the extent of 5 × 10² − 5 ×10⁵permL of milk"*. When contacted by email for clarification (noting the absence of a reference in support), Zanini et al. (1998) referred to Sinha (1994) who in turn had referred to Wilson (1942). For each of these authors therefore, apart from Mariam (2014), information on the likely number of *M. bovis* bacteria in raw milk can be traced back to Wilson (1942) who reports: *"The number of tubercle bacilli excreted in the milk varies greatly. In the early stages they are very few, but in advanced cases of udder involvement they run into the hundreds of thousands, and even more. Ostermann (1908) [cited by* Wilson, 1942*], for example, found that milk could be diluted 50,000 times and still be infective. Positive results in even higher dilutions were obtained by Ostertag and by Bongert (see Ostermann 1908 [cited by* Wilson, 1942*]). On the average he estimated that 1mL of milk from a cow with a tuberculosis udder contains about 1000 infective doses for a guinea pig. The number of living tubercle bacilli in an infective dose varies somewhat. It is known that a single bacillus may cause infection, but the minimal certain infecting dose is probably rather over 10 bacilli (Schwabacher and Wilson 1937* [cited by Wilson, 1942]*). Adopting a conservative average of 5 bacilli per infective dose, it follows that milk from a cow with a tuberculous udder commonly contains somewhere about 5000 virulent tubercle bacilli per millilitre. Pullinger (1934 [cited by* Wilson, 1942*]), it may be noted, found that two out of three milks from tuberculous udders could be diluted 1 in 1000,000 times and still prove infective for Guinea pigs. The average number of tubercle bacilli excreted by these animals must therefore have been in the neighbourhood of 5000, 000perml"*. On the basis of this information, we express considerable caution with respect to estimates of 5 × 10^3 − 5 × 10^6 bacteria per 10 mL, noting that modern quantification techniques for *M. bovis* were not available to Wilson in the 1940s. Other issues for consideration include the potential for intermittent shedding of *M. bovis* in the milk of TB infected animals, which would impact the number of CFU/mL from milking to milking. Further, *M. bovis* excretion in milk would be expected to vary by stage of infection and disease, and with milk yield. To add further complexity, *M. bovis* may not be homogenously distributed in raw milk.

An assessment of the types/sources of uncertainty was not undertaken.

In conclusion, no meaningful estimates of this parameter are currently available. Further research in this area is needed to quantify *M. bovis* shed in the milk of SICTT positive animals.

3.1.4. Estimating the number of *M. bovis* bacteria present in the faeces of bTB infected cattle

A master list of 199 results was created and saved to a list in Scopus. All articles were screened; however, none quantified the number of *M. bovis* bacteria present in the faeces of bTB infected bovine animals, suggesting that the number of *M. bovis* bacteria present in bovine faces is currently unknown.

Several articles provide a qualitative insight that are relevant to this issue. Fine et al. (2011) investigated the persistence of the Michigan strain of *M. bovis* in typical environmental substrates (corn, hay, soil, and water) exposed to natural weather conditions in Michigan, USA. In this study, samples were inoculated with 50,000 colony forming units (CFU) *M. bovis*, with the authors reporting "*The 50,000 CFU inoculums used is thought to be indicative of the amount of* M. bovis *that could be deposited by a bovine TB-infected and shedding animal*" (Fine et al., 2011). It was reported that infected badgers can shed between 1 × 10^3 − 4 × 10^5 M. *bovis* cells per gram of badger faeces (King et al., 2015). In addition to Fine et al. (2011) and King et al. (2015), Xu et al. (2021) examined fecal samples for *M. bovis* from animals with advanced disease (defined as: *"In serial testing, positive results from two or more assays (including ELISA) were considered as advanced infection"*) in China. The authors reported that of the 52 fecal samples, "M. bovis *nucleic acid was detected in all 8 samples from fecal pool and 34 faeces samples from bTB-positive cattle. However, no* M. bovis *was successfully isolated* [cultured] *from these 54 faeces samples [..]. These results unravelled the possibility of gastrointestinal transmission mode of EPTB [extra-pulmonary tuberculosis] in dairy farms"*.

An assessment of the types/sources of uncertainty was not undertaken.

Similar to the situation in milk, very limited information is available on the number of *M. bovis* bacteria shed in faeces in bTB infected animals. Estimates are presented of *M. bovis* shedding in the faeces of badgers (King et al., 2015), however the relevance of this information to cattle is unknown. Research is needed.

3.1.5. Quantifying contamination of bulk tank milk with faeces

Fecal contamination of bulk tank milk can occur as a result of contamination of the teats, following the dropping of milking clusters leading to contamination from the environmental pool, or following contamination of the bulk tank post-milking (Clough et al., 2006, 2009). Several methods have been used to quantify fecal contamination of bulk tank milk, including a consensus opinion from an expert workshop in the UK (Clough et al., 2006, 2009) and a biomarker study conducted in the Netherlands (Vissers et al., 2007). Clough et al. (2009) concluded that the minimum total fecal contamination of the bulk tank was, on average, likely to be close to 0 g on very clean farms, rising to 10 g on dirty farms, with the most likely amount around 2 g. These authors used a Gamma distribution with shape parameter 0.05 and scale parameter 600 (mean=30, SD= 134.2) to provide estimates of total fecal contamination in the bulk tank (minimum 160, mean 2200, maximum 10,280 mg), given an average lactating group size of 75 animals. For individual animals, they note that a Gamma (0.05, 600) distribution has 95% of its density between 0 and 340 mg per animal. Using spores of mesophilic aerobic bacteria as a biomarker to quantify the amount of dirt (comprised of faeces, bedding material and soil) transmitted to milk via the exterior of teats, Vissers et al. (2007) estimated that the amount of dirt transmitted to milk varied in 11 randomly selected Dutch farms, from approximately 3 to 300 mg/L, with an average of 59 mg/L.

Despite substantial methodological differences, the estimates from the two reported studies are broadly consistent. The main types/sources of uncertainty relate to the very small number of studies reported here.

As a consequence, a systematic literature review may be warranted, and further research is recommended.

3.1.6. Estimating the survival of *M. bovis* throughout cheese making process and maturation period

A master list of 25 results was created and saved to a list in Scopus. All 25 full texts were screened and six were included in this synthesis. An additional six articles were identified after searching bibliographies. Three articles relating to the survival of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cheese were also included.

Several studies reported the detection of *M. bovis* in naturally contaminated cheese. In Brazil, *M. bovis* was detected using quantitative polymerase chain reaction (PCR) in cheese samples collected from neighbourhood grocery stores, bakeries, and open-air markets (Cezar et al., 2016), and also in cheeses in the baggage of incoming travellers from Spain, the Netherlands, Italy, Lebanon, Morocco, Norway, and Portugal (de Melo et al., 2014). *M. bovis* was isolated in raw milk cheese seized from travellers crossing the US-Mexico border (Harris et al., 2007) and also in soft fresh cheeses originating in Mexico (Kinde et al., 2007). Further, Hammer and Babel (1957) reported that cheddar naturally contaminated with *M. bovis* remained infective after 120 days while Kastli and Binz (1949) reported Tilsiter naturally contaminated with *M. bovis* was infective after 305 days. *M. bovis* has also been detected in Bulgarian white cheese after 120 days maturation, in Camembert after three months and in Emmental and Edam after two months (IDF, 1980). Pullinger (1935) reported the detection of *M. bovis* in UK hard cheeses at 7 days, but not after 21 days.

In Table 1, we present studies that quantify the survival of *M. bovis* and, for comparison, *M. avium* subsp. *paratuberculosis* in experimentally infected cheeses. Forgrave et al. (2016) studied the survival kinetics of three different *M. bovis* genotypes (AF2122/97 reference strain and spoligotypes SB0130 and SB0140) at high (10^3–10^5 CFU/mL milk) and low (10^2 CFU/mL milk) inoculum doses during the manufacture and ripening of raw milk cheddar and caerphilly produced on a laboratory scale. The authors reported an increase in the concentration of *M. bovis* from inoculated milk to 1-day-old cheese: *"counts of* M. bovis *from high-inoculum milk increased by 4.8-fold and 2.3-fold for Cheddar and Caerphilly, respectively, and from low-inoculum milk 5.4-fold and 5.8-fold increases were observed for Cheddar and Caerphilly, respectively"* (Forgrave et al., 2016). Decimal reduction times (D-values) at 12 °C were reported at 57 and 59 days in high-inoculum Cheddar and Caerphilly, respectively, and 41 and 24 days in low-inoculum Cheddar and Caerphilly, respectively. Starikoff et al. (2016) studied the decline of *M. bovis* (spoligotype SB1033; isolated from cattle slaughtered in the state of São Paulo, Brazil) in parmesan-type cheese made from inoculated (10^6 CFU/mL) pasteurised milk; the authors reported an average D-value of 37.5 ± 5.3 days at 18 °C (Table 1). Lafont and Lafont (1980) studied the survival of *M. bovis* in blue cheese made from pasteurised milk artificially inoculated (10^4 CFU/mL) with *M. bovis*. In this study, a rapid reduction in the number of *M. bovis* bacteria was observed in the first 14 days post-manufacture, however viable *M. bovis* persisted until 3–4 months of ripening. The authors estimated a decimal reduction time of approximately 10 days. In addition, Nasr et al. (2014) studied the survival of *M. bovis* in Kareish cheese (soft cheese type) made with pasteurised milk inoculated with 10^3CFU/mL and 10^5CFU/mL *M. bovis*; viable bacteria were detected up to 30 days at both inoculum doses.

Several types/sources of uncertainty are apparent in the available literature, primarily relating to extrapolation. To this point, studies on the survival of *M. bovis* have only been undertaken on a limited number of cheeses, including cheddar, caerphilly, parmesan-type and Kareish cheeses. Extrapolation to other cheese types would need to be undertaken with care. As noted by Forgrave et al. (2016), bacterial numbers during the manufacturing and ripening phases of cheese production can be influenced by various parameters, including temperature, pH and salt. Further, there is limited data on the influence of cheese manufacturing temperatures, particularly in relation to *M. bovis*.

In conclusion, some data are available regarding the survival of *M. bovis* throughout cheese making process and maturation period. The work of Forgrave et al. (2016) is particularly relevant to the current framework as it was undertaken with *M. bovis* strains isolated in Ireland and UK, was undertaken using raw milk, and utilised modern methods of *M. bovis* isolation and quantification from dairy matrices (cheese). This work, and each of the other studies cited, demonstrate that *M. bovis* can persist in cheeses. It is noteworthy that the studies with MAP (Table 1) provide similar (or slightly greater) D-values.

Table 1
Survival of *Mycobacterium bovis* and *M. avium* subsp. *paratuberculosis* in experimentally inoculated cheeses.

Strain	Inoculum CFU/ml milk	Milk type	Cheese Type	D-value (days)	Maturation temperature	Refs.
M. bovis						
AF2122/97 SB0130/SB0140	10^3–10^5	Raw	Cheddar	57	12 °C	Forgrave et al. (2016)
AF2122/97	10^2	Raw	Cheddar	41	12 °C	Forgrave et al., (2016)
SB0130/SB0140	10^3–10^5	Raw	Caerphilly	59	12 °C	Forgrave et al., (2016)
AF2122/97	10^2	Raw	Caerphilly	24	12 °C	Forgrave et al. 2016)
SB1033	10^6	Pasteurised bovine	Parmesan-type	37.5 (95%CI ± 5.3)	18 °C	Starikoff et al. (2016)
Not specified	10^4	Pasteurised bovine	Blue-veined	10	13–14 °C	Lafont and Lafont, (1980)
M. avium subspecies *paratuberculosis*						
ATCC 19698 Niebull	10^4–10^5	Raw bovine milk	Semi-hard Cheese (Swiss Tilsiter)	45.5	Curve 12 °C-20 °C-12 °C	Spahr and Schafroth (2001)
ATCC 19698 Niebull	10^4–10^5	Raw bovine milk	Hard cheese (Swiss Emmental)	27.8	14–15 °C	Spahr and Schafroth (2001)
Non-heat treated ATCC 19698	10^6 cells/ml	Pasteurised milk	White soft cheese (Queso Fresco)	59.9 days	4 °C	Sung and Collins (2000)
Heat-treated ATCC 19698	10^6 cells/ml	Pasteurised milk	White soft cheese (Queso Fresco)	36.5 days	4 °C	Sung and Collins (2000)
Isolate from milk (strain 806PSS)	10^4–10^5	Pasteurised bovine milk	Cheddar-type cheese	107 days	10 °C	Donaghy et al. (2004)
Isolate from milk (strain 796PSS)	10^4–10^5	Pasteurised bovine milk	Cheddar-type cheese	96 days	10 °C	Donaghy et al. (2004)
Reference strain (NCTC 8578)	10^4–10^5	Pasteurised bovine milk	Cheddar-type cheese	90 days	10 °C	Donaghy et al. (2004)

3.1.7. Estimating the average daily cheese consumption per person

The estimated daily cheese consumption amongst Irish adults and children is presented in Table 2. Our estimates are derived from several large nationally representative Irish studies, namely the Irish National Adult Nutrition Survey (2008–10) (Irish Universities Nutrition Alliance, 2011) and the National Children's Food Survey II (2017–18) (Irish Universities Nutrition Alliance, 2019).

Several types/sources of uncertainty are apparent in the available literature, primarily relating to extrapolation. These data are specific to Ireland, and would need to be extrapolated to other countries with care. In Ireland, as in the UK (Bates et al., 2014), most people consume dairy foods (Feeney et al., 2016, 2017), which is not dissimilar to the relatively high dairy intakes observed in other northern European countries (Woolhead et al., 2015).

In summary, detailed data are available on the average cheese consumption per person. These data could be fitted to a lognormal, gamma, or a truncated normal probability distribution in order to estimate the likely probability distribution of the daily cheese consumption per person in Ireland. Alternatively, a simpler approach could be used, as reflected in Fig. 1, with a focus on the estimated number of *M. bovis* CFU per 25 g, which is roughly equivalent to an average cheese serving.

In the present study, the focus is on assessment and management of risk posted by *M. bovis* following the detection of bovine TB in a herd supplying raw milk cheese. The context here is farmhouse cheese production in Ireland (that is, small-scale cheese production at the farm where milk is produced), and the safety of raw milk cheese that had been produced in such a facility prior to the detection of bovine TB. Average cheese serving is appropriate given the context of this risk assessment question. Although beyond the scope of the current study, additional risk assessment questions may arise, including risk (or exposure) estimates at a national level. This would require data on raw milk cheese production for domestic distribution, and the proportion of raw milk per type of cheese.

3.2. Hazard characterisation

3.2.1. Experimental single oral inoculation of animals with M. bovis resulting in evidence of infection

A master list of 296 results was created and saved to a list on Scopus. Bibliographies within these publications were also searched for further relevant publications; an additional 7 articles were identified and added to the master list of 303 records. Records were screened; the criteria for inclusion depended on whether the title and/or abstract included information relating to oral inoculation/infection of animals with virulent *M. bovis* (not heat-treated *M. bovis* or *M. bovis* vaccine such as *M. bovis* BCG). One hundred and ten articles were included in full text review, of which 23 were considered eligible and hence included in the literature synthesis.

Within the 23 full text-articles, a total of 46 experiments were reported where *M. bovis* was inoculated into the following species: buffalo calves, bovine calves, deer (adults and fawns), ducks, ferrets, house mice, meadow voles, Norway rats, opossums, pigeons, young pigs (3–4 months), young wild boar (3–4 months), racoons and turkeys. Inoculation routes included oral/oropharyngeal and intra-tonsillar inoculation.

Thirty-one experiments were excluded for the following reasons:

- Experiments which inoculated animals via the intratonsillar route ($n = 12$) (Michel et al., 2007; Nol et al., 2008, 2009; Palmer et al., 1999, 2001, 2002a, 2004, 2014)
- Experiments which inoculated animals over the course of a number of consecutive days ($n = 9$) (as opposed to a single exposure event) (Basso et al., 2018; Palmer et al., 2002a, 2002b, 2004).
- Experiments which did not result in evidence of *M. bovis* infection or disease in the inoculated animals ($n = 7$) (Clarke et al., 2006, 2007; Fitzgerald et al., 2005; Palmer et al., 2002b)
- Experiments using animals that had received an *M. bovis* vaccine prior to *M. bovis* infection/inoculation ($n = 3$) (Beltrán-Beck et al., 2014b)

A summary of all 15 eligible studies, from 10 articles, are presented in Table 3. In monogastric mammals (ferrets, house mouse, meadow vole, pigs, opossums and wild boar), the experimental single oral inoculation which resulted in evidence of infection varied between 100 and 1000,000 CFU. In pigeons, infection was established following inoculation with 160,000 CFU (Fitzgerald et al., 2003).

As yet, no dose-response relationship has been established, based on data from experimental single oral inoculation of *M. bovis* in animals. Extrapolation uncertainty will be substantial, in seeking to interpret surrogate data from animals.

3.2.2. A review of EU and Irish legislation

According to Regulation 853/2004 and SI 58/2015, milk from reactor (and inconclusive) animals is prohibited from entering the food chain from the time tuberculosis has been diagnosed. With respect to the *"control of product from restricted holdings or holdings where tuberculosis is suspected"*, SI 58/2015 states that *"a person shall not deliver milk produced by a reactor or a bovine giving an inconclusive reactor result to a test for onward sale or processing"*. Further, Regulation 853/2004 states that *"raw milk from any animal [..] showing individually a positive reaction to the prophylactic tests vis-à-vis tuberculosis or brucellosis as laid down in* Directive 64/432/EEC *and* Directive 91/68/EEC *– must not be used for human consumption"*. Consequently, it is assumed that there is zero-tolerance for *M. bovis* in foods of animal origin destined for human consumption in the national and European legislation.

An assessment of the types/sources of uncertainty was not relevant.

In conclusion, zero-tolerance is assumed for *M. bovis* in foods of animal origin destined for human consumption, and a dose-response model in future risk assessment would not be applicable.

3.3. Past risk assessments

To our knowledge, two risk assessments have previously been conducted, relevant to this question, using either semi-quantitative (Ministry for Primary Industries, 2014) or qualitative (Advisory Committee on the Microbial Safety of Food, 2011) methods.

In New Zealand, the Ministry for Primary Industries (2014) assessed the microbiological risks associated with the consumption of raw milk, including a quantitative assessment of *Campylobacter* spp., *Listeria monocytogenes*, Shiga toxin producing *Escherichia coli* with an emphasis on *E. coli* O157 and *Salmonella* spp, and a semi-quantitative assessment of *M. bovis*. With respect to *M. bovis*, the report concluded *"Given the current low level of* M. bovis *infection in dairy herds in New Zealand, the*

Table 2
Mean, standard deviation (SD), median and percentile values of cheese intakes (g/day) in Ireland, based on results from the National Adult Nutrition Survey (2008–10) and the National Children's Food Survey II (2017–18).

	Mean	SD	Median	Percentiles 5th	95th
National Adult Nutrition Survey (2008–10)					
Total study population aged 18–64y (n = 1274)	14	18	9	0	48
Total Population aged 18–64y (cheese consumers only) (n = 839)	21	18	16	5	57
Aged ≥ 65y (n = 226)	11	17	5	0	40
Aged ≥ 65y (cheese consumers only) (n = 124)	20	18	14	3	54
National Children's Food Survey II (2017–18)					
Total study population aged 5–12y (n = 600)	11	13	6	0	38
Total population aged 5–12y (consumers only) (n = 378)	17	13	14	3	43

Table 3
Studies reporting experimental single oral inoculation of animals with *Mycobacterium bovis* which resulted in evidence of infection.

Animal species inoculated	Strain of *M. bovis* inoculated	No. CFU[a] inoculated	Inoculum medium	Refs.
Monogastric mammals				
Ferrets	Isolated from tuberculous wild ferret (strain 92–3452)	500,000	Deer lung tissue	Cross et al. (2000a)
Ferrets	Isolated from tuberculous wild ferret (strain 92–3452)	500,000	Deer lung tissue	Cross et al. (2000b)
Ferrets	Isolated from tuberculous wild ferret (strain 92–3452)	500,000	Deer lung tissue	Qureshi et al. (1999)
House mouse	Field strain (Michigan strain) isolated from white tail deer	100	0.25 mL	Clarke et al. 2007)
House mouse	Field strain (Michigan strain) isolated from white tail deer	10,000	0.25 mL	Clarke et al. (2007)
Meadow vole	Field strain (Michigan strain) isolated from white tail deer	5000	0.5 mL	Clarke et al. (2007)
Meadow vole	Field strain (Michigan strain) isolated from white tail deer	100	0.5 mL	Clarke et al. (2007)
Pigs (3–4 mo)	Field strain (spoligotype SB0295)	100,000	2 mL suspension	Beltrań-Beck et al. (2014a)
Opossums	Field strain isolated from white tail deer	100,000	Strawberry preserve on bread	Diegel et al. (2002)
Wild boar (3–4 mo)	Field strain (spoligotype SB0339)	1000,000	5 mL suspension	Garrido et al. (2011)
Wild boar (3–4 mo)	Field strain (spoligotype SB0339)	100	5 mL suspension	Ballesteros et al. (2009)
Wild boar (3–4 mo)	Field strain (spoligotype SB0339)	10,000	Not reported	Ballesteros et al. (2009)
Wild boar (3–4 mo)	Field strain (spoligotype SB0339)	1000,000	Not reported	Ballesteros et al. (2009)
Wild boar (3–4 mo)	*M. bovis* (spoligotype SB0339)	100,000	5 mL suspension	Gortazar et al. (2014)
Birds				
Pigeon	Field strain isolated from white tail deer	160,000	1 mL sterile water	Fitzgerald et al (2003)

[a] Colony forming units.

likelihood of unpasteurised cows' milk being contaminated with M. bovis *at the farm gate is very low"* (Ministry for Primary Industries, 2014). A number of recommendations regarding herds supplying raw milk were made, including restricting these to those located in vector free areas (a geographic area where absence of *M. bovis* from all non-bovine maintenance hosts has been declared, based on scientific proof of freedom, OSPRI, 2018), to herds with a minimum status of Clear 5 (herds considered free of bTB for at least 5 years), confirmation of the bTB free status of all introduced animals, setting a maximum period of 12 months between herd tests, immediately removing all positive and test-suspect cows, and sourcing solely from herds with bTB free status.

In the UK, the Advisory Committee on the Microbiological Safety of Food (2011) conducted a qualitative assessment of the possible health risks to consumers associated with *M. bovis* and unpasteurised milk and milk products. The Committee concluded that risk to human health from *M. bovis* in unpasteurised cows' milk and milk products remains very low, but did acknowledge an increased risk of human TB infection acquired from unpasteurised milk and milk products due to the increase in *M. bovis* incidence in cattle that was observed in the years prior to 2011, when this report was released.

4. Conclusions and perspectives

In this study, we have determined parameter estimates to support the future risk assessment of *M. bovis4* in raw milk cheese. To our knowledge, this is the first time that these parameter estimates are available, noting that earlier assessments relied on either semi-quantitative (Ministry for Primary Industries, 2014) or qualitative (Advisory Committee on the Microbial Safety of Food, 2011) methods. Our work with exposure assessment was guided by a conceptual model, which describes the steps through which *M. bovis* may be transferred from an infected animal(s) through to manufacture of the final cheese product. Most of these parameter estimates are supported by systematic literature reviews, and where relevant meta-analyses, except those relating to the fecal contamination of bulk tank milk and the average daily cheese consumption per person. With respect to the latter, we accessed detailed population survey data (the Irish National Adult Nutrition Survey (2008–10) and the National Children's Food Survey II (2017–18)). This work has highlighted important gaps in knowledge, and areas for further research, particularly in relation to the number of *M. bovis* bacteria excreted in milk and present in the faeces of SICTT positive and/or bTB infected animals. A systematic literature review is also suggested, to quantify the fecal contamination of bulk tank milk. For each of the parameters for which estimates are available, we outline the types/sources of uncertainty as reflected in relevant published papers. In any future application of these parameter estimates, care will be needed to reflect the uncertainties associated with these elements of exposure assessment.

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CRediT authorship contribution statement

Áine B. Collins: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Simon J. More:** Methodology, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

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