

REVIEW ARTICLE

A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland

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SUMMARY

Bovine tuberculosis (bTB) is an important disease of cattle caused by infection with *Mycobacterium bovis*, a pathogen that may be extremely difficult to eradicate in the presence of a true wildlife reservoir. Our objective was to identify and review relevant literature and provide a succinct summary of current knowledge of risk factors for transmission of infection of cattle. Search strings were developed to identify publications from electronic databases to February 2015. Abstracts of 4255 papers identified were reviewed by three reviewers to determine whether the entire article was likely to contain relevant information. Risk factors could be broadly grouped as follows: animal (including nutrition and genetics), herd (including bTB and testing history), environment, wildlife and social factors. Many risk factors are inter-related and study designs often do not enable differentiation between cause and consequence of infection. Despite differences in study design and location, some risk factors are consistently identified, e.g. herd size, bTB history, presence of infected wildlife, whereas the evidence for others is less consistent and coherent, e.g. nutrition, local cattle movements. We have identified knowledge gaps where further research may result in an improved understanding of bTB transmission dynamics. The application of targeted, multifactorial disease control regimens that address a range of risk factors simultaneously is likely to be a key to effective, evidence-informed control strategies.

Key words: Bovine tuberculosis, cattle, *Mycobacterium bovis*, risk factor, transmission, wildlife.

INTRODUCTION

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* infection is an important infection of cattle and can also infect a wide range of domestic and wild animals as well as humans [1]. Cattle act as the

maintenance hosts in many countries and the infection has proved extremely difficult to eradicate in domestic species once it has become established in a wildlife reservoir [2]. The incidence and epidemiology of bTB varies widely both internationally and locally [3–5]. Much of Europe is officially bTB free but the infection is endemic in cattle in parts of England, Wales, Ireland and Spain and other parts of Europe, which are dissimilar in terms of environmental features and wildlife reservoirs [6, 7]. Transmission of *M. bovis* is influenced by many factors including the intrinsic

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characteristics of the bacterium and potential host, the persistence of the bacterium in different environments, the probability of exposure of infected animals, e.g. cattle or wildlife vectors and/or fomites and the effectiveness of control strategies such as removal of infected animals.

Assessment of risk is dependent on unbiased measurement of factors associated with transmission between the bacterium and host and accurate classification of the infection status of animals and herds. A risk factor is defined as a characteristic, that in its presence increases the likelihood of the detection of infection, and they may be causal or non-causal [8]. Many risk factors are inter-related, e.g. each animal production system has its own distribution of sexes, breeds and age groups, which contribute to the exposure profile of each herd. Diagnostic tests for bTB range from those used in live cattle [e.g. tuberculin skin test (TST) and blood tests for interferon-gamma (IFN- γ) or antibodies] to post-mortem inspection confirmed with culture for *M. bovis*. Intrinsic test performance varies with the animal's immunological profile, disease infection stage and many other factors. For example, increasing the frequency of skin tests, a feature of intensified surveillance and control strategies for bTB, can modulate cell-mediated immunity and depress the response to subsequent skin tests [9, 10].

The relative importance of different risk factors for *M. bovis* transmission will vary with the background prevalence and magnitude of other risks. An understanding of risks and how they may interact is therefore necessary to understanding the epidemiology of the disease and the design of bTB eradication and control policies and forms the focus of this review.

METHODS AND RESULTS

A series of search strings were developed to identify relevant papers from CAB Abstracts, Web of Science, Medline and Current Contents electronic databases (see Supplementary material for the specific search strings). Two sets of searches were performed, the first deliberately excluding *Mycobacterium avian paratuberculosis* (MAP) and the second, specifically including it as a risk factor. The results of these were then combined. The search period was not limited historically and initially ended on 14 March 2013. Abstracts to 3738 non-duplicate references identified were reviewed by three reviewers to determine whether the entire article was likely to contain relevant information. Twenty-eight abstracts were

reviewed by two reviewers to detect any perceived inconsistencies in selection. Inconsistencies were resolved through discussion, which then informed abstract selection. All publications that specifically investigated the risk of infection to cattle, or where that risk could be inferred, were included and the full text was requested. Papers in German, Spanish, Italian and French that were selected for further review were translated into English. Entire texts for selected papers were then obtained and assigned to one of ten full-text reviewers who each had a specific risk factor category as follows: animal-level, herd-level, environment and landscape features, herd history including previous testing, wildlife factors, and other risk factors. Reviewers were free to add further papers that had been omitted during the electronic search. The search was updated to 6 February 2015 using the original search criteria and a further 517 references identified, of which 81 were considered potentially relevant by one of the original reviewers.

The search was not limited geographically, but during the review emphasis was placed on references that could improve understanding of the Great Britain (GB) epidemiological situation. Methodology and reporting was consistent with PRISMA (<http://www.prisma-statement.org>) where possible with respect to the introduction, eligibility criteria, information sources, search and discussion, and as far as the qualitative assessment of the publication allowed.

ANIMAL-LEVEL RISK FACTORS

Genetics

Significant variation in heritability of susceptibility to bTB has been reported in studies of dairy cattle, mainly Holstein Friesian pure bred or crosses, in GB [11] and in Ireland [12]. In these population-based studies, around 16% and 18% of the variance in bTB resistance, measured by responsiveness to the single intradermal comparative cervical tuberculin (SICCT) test and post-mortem evidence of infection, respectively, was estimated to be heritable [13]. There has been less investigation of heritability of resistance in beef herds. However, a study in Ireland has estimated heritability of susceptibility to bTB of around 13% in beef herds and also reported variation in susceptibility across different beef breeds [14]. These large studies, using surveillance data from thousands of cattle, may underestimate the contribution of heritability due to misclassification of the resistant phenotype because of

imperfect accuracy of diagnostic tests and variation in level of exposure to *M. bovis* [15]. Greater variability in the estimates of heritability of susceptibility to bTB has been observed in high- compared to low-infection prevalence herds [14].

Resistance to bTB is likely to be multifactorial and polygenic [16]. In addition to heritability studies using phenotype data, candidate and whole genome approaches are being taken [15]. These approaches have indicated that resistance can be conferred by a single nucleotide polymorphism. Both breeding strategies and genomic selection are likely to have utility in bTB control [17–19]. Breeding for performance traits has led to large increases in milk outputs from dairy cows. Until recently the latter was accompanied with a reduction in fertility [20]. No association between susceptibility to *M. bovis* infection and economically important traits has been measured to date [21].

Breed

The earliest evidence for differences in resistance to bTB infection by breed originates from the 1920s and 1930s [15, 22]. Evidence mainly originates from Africa and strongly suggests that native cattle such as zebu, found in pastoral environments, are more resistant to bTB than introduced European cattle [22–26]. A weakness of some comparisons of bTB prevalence between breeds is the reliance on an ante-mortem test which may be less specific than evidence from post-mortem examination. There has been comparatively far less evaluation of the differences in the susceptibility to infection between European breeds. A study in India showed a threefold higher prevalence of SICCT test reactors in Jersey cattle compared to Holstein Friesian [27] but differences in exposure to *M. bovis* between breeds require exploration.

The UK Bovine HapMap Consortium has illustrated that greater diversity exists in allele frequencies between cattle breeds than within breeds [28]. Greater variability in resistance might be anticipated with beef cattle than with cattle in the modern dairy sector since the latter are more homogeneous in terms of breed.

Sex

Differences in bTB prevalence by sex are confounded by differences in the production environment and husbandry. Contact rates are higher for many dairy cattle. Additionally, milk-producing dairy cattle and suckler cows tend to have longer lives than beef cattle,

increasing the lifetime probability of exposure to *M. bovis* [29, 30]. Incidence of bTB infection in dairy cattle, aged ≥ 2 years, was 40% higher than beef cattle, although incidences in very young cattle (0–1 years) were similar [31]. Most studies report a higher prevalence or incidence of bTB in female cattle compared to males [31–37], but not all [38], and none comprehensively controlled for differences in infection pressure, production environment and husbandry. At this time, an intrinsic difference in susceptibility to bTB between male and female cattle has not been demonstrated.

Reproductive status and milk yield

Few studies have examined the effect of reproductive status on susceptibility to bTB infection, possibly because of the difficulty in separating out gestation and lactation effects. The dairy cow experiences large hormonal shifts and stresses throughout production and it seems likely that these will affect both her response to diagnostic tests that measure immunological factors and susceptibility to infection. In contrast to susceptibility to bTB infection, cows in the weeks before and after calving tend to have weaker reactions to the TST, but the IFN- γ is less affected [39]. Evidence is primarily found in studies outside of Europe and in the absence of stringent control programs where animals may experience advanced clinical disease. In Bangladesh, consumption of infected milk is also a potential (pseudo-vertical) transmission route for infection between cows and calves and a higher prevalence of bTB was observed in lactating cows compared to pregnant cows [40]. In Argentina, calves fed raw milk from a herd with endemic bTB were more likely to test positive (caudal fold test) than calves fed replacement milk substitute [41]. However, a large cohort study in Northern Ireland found no evidence of an increased risk of bTB in the progeny of TB-confirmed dams compared to those born to non-reactors in the same herd [risk ratio 1.2, 95% confidence interval (CI) 0.8–1.79] [42].

Age

Virtually all studies reviewed reported that the prevalence of bTB infection increased with age [23, 27, 34, 43–48]. The relationship between bTB infection and age is generally shown as monotonic or linear although a U-shaped relationship has also been reported [23, 49]. Modelling bTB surveillance data from GB

showed that age-specific incidence increased monotonically to 24–36 months, followed by a levelling-off, with cattle aged between 12 and 36 months experiencing the highest rates of infection [31]. The most likely explanation for a positive correlation between bTB infection risk and longevity is a higher probability of contact and/or prolonged exposure to other infected cattle, wildlife or environmental contamination; infected cattle aged >36 months are possibly more responsive to the skin test [31] and therefore more likely to be detected in surveillance tests. A review of age effects in Tanzania, Zambia, Chad and Ireland also concluded that the positive association with increasing prevalence of bTB is a result of cumulative exposure [50]. Other possibilities include decline in resistance with age [1]. The influence of latency on detection and transmission of bTB in cattle is not known, although it is recognised as an important influence on disease prevalence and transmission in human infection with *M. tuberculosis* [51].

Nutritional status and body condition

Poor nutrition can suppress the cellular immunity response and increase susceptibility to infectious diseases [52–54]. Responsiveness to the SICCT test was positively correlated with body condition score and fat production in lactating Holstein-Friesian dairy cows in GB, which implies some association between susceptibility or at least responsiveness to the diagnostic test [21]. A cross-sectional study of cattle from herds with bTB infection found that significantly lower levels of the dietary selenium-requiring enzyme glutathione peroxidase were associated with a higher prevalence of post-mortem confirmed infection [55]. However, two Irish studies comparing animals with energy-restricted and unrestricted diets did not detect significant differences in bTB infection [56, 57]. Reports from Ethiopia are similarly mixed, with one study reporting a higher prevalence of bTB in animals with lower body condition scores [47] while another found no differences [44]. Future research needs to distinguish nutritional status/dietary factors and body condition from the immunological consequences of infection and the possible confounding effects of breed and other factors.

Behavioural factors

Contact networks between and within wild and domestic animals are heterogeneous [58, 59]. Cattle are

inquisitive towards new objects and calves and some adult cattle are particularly so [60]. A study using proximity data loggers identified individual adult cattle with higher levels of contact both with other cattle in the herd and with badgers [61]. In a New Zealand study, 86% of the tuberculin test-positive cattle were among the most dominant fifth in the herd, and in four of five herds, the dominant animals investigated a sedated possum (the principal wildlife vector of bTB) most actively [62]. The differing contact behaviour of cattle towards live and inanimate objects implies that the individual risks of exposure to infection are heterogeneous.

Concurrent infection

Parasitic helminth infection status has been shown to be negatively correlated with bTB infection status in bovids [63–65]. Evidence suggests that infection with the helminth *Fasciola hepatica* may bias the immunological response towards a Th2 (eosinophilic) type at the expense of the Th1 (cell-mediated) immune response that influences the ability to control *M. bovis* [65]. Concurrent infection with or vaccination against MAP can influence the sensitivity and specificity of diagnostic tests for bTB [66, 67] (see below for further discussion). Outbreaks of bTB in two herds with a concurrent bovine viral diarrhoea (BVD) infection have been described anecdotally [68]. Compared to calves with *M. bovis* infection alone, calves co-infected with BVD and *M. bovis* show non-significantly increased bacterial shedding in nasal secretions, in experimental infections [69]. Further studies are needed, however, to evaluate whether there is an association between the two diseases.

HERD-LEVEL RISK FACTORS

Many factors describing the characteristics and management of herds have been associated with infection risk, but the direction of findings is inconsistent, even between studies conducted using a similar sampling frame [70, 71], possibly due to difficulty in characterising management factors accurately. A number of observational studies relating to herd-level risk factors within the UK and Ireland in specific circumstances have been well reviewed recently [72–75]. Rather than add to these, we have supplemented these with information from other countries that may inform the situation in GB.

Herd size

Herd size is the most frequently identified risk factor for bTB incidents (herds that are found to have at least one infected animal). Dynamic modelling has shown that, adjusting for test performance, the increased probability of between-animal contacts in larger herds could increase within-herd transmission [76]. Most studies, but not all [77–79], conclude that the risk of infection detected in a herd increases with herd size; this holds true across a broad geographical and prevalence range (Table 1). Herd size may be a proxy for other factors, such as replacement policy, farm acreage, number of premises and neighbouring herds; each of which may be independently associated with the risk of a bTB incident. Additionally, most studies have not adjusted for the imperfect specificity of diagnostic tests for bTB which may affect the strength of association since the number of animals that are false positives will also increase with herd size. However, increased herd size could also result in increased risk of disease persistence.

Herd type

Dairy herds are generally considered to be more at risk of infection than beef herds because of longer life expectancy of the former and management practices that increase the risk of contact with other cattle [31, 50, 80]. Relatively few studies have considered herd type and of those, even fewer studies differentiate between beef fattening/finishing herds and beef breeding/suckler herds. The relative risk to beef cattle may be greater in areas where there is a local wildlife reservoir because beef cattle more frequently use rough or dispersed grazing. Direct contact between cattle and badgers at pasture is relatively rare [81], although indirect contact may be more common. In Italy, enterprises consisting of both dairy and beef animals, presented a greater risk [odds ratio (OR) 4.92, 95% CI 1.26–19.19] than exclusively dairy enterprises, attributed to differences in purchasing policy [78]. Dairy herds experienced a recurrent bTB incident sooner than non-dairy herds in Northern Ireland, even after controlling for herd size [82], but a similar study in Ireland showed that dairy animals had a lower risk of a future restriction [83]. GB surveillance data indicate that the relationship between bTB incidence rate and herd type may be a result of the larger sizes of dairy herds and their location [84].

Dairy reactors to field tests for bTB appear to be less likely than non-dairy reactors to have post-mortem

evidence of infection such as culture, histological evidence or macroscopic lesions typical of bTB PMC than non-dairy [55, 84–86]. In Northern Ireland the adjusted odds ratio for visible lesions in non-dairy reactors detected during routine surveillance was reported to be twice that in dairy [86]. The reason for the differences in post-mortem evidence of infection confirmation between dairy and beef production classes exposed to bTB is currently unknown [87].

Total farm area, fragmentation and neighbouring herds

Many authors identified the importance of contact between adjacent herds as a risk factor. This can be expressed in terms of lengths of common boundary, fragmentation of holdings, or observed contact with cattle from contiguous farms [70, 88, 89]. Farm size (acreage) has been associated with a higher probability of a bTB incident [90, 91], even after controlling for herd size [92] and it may be a proxy for increased risk of infection from contiguous premises or for increased wildlife contact. Operating over multiple premises has been associated with infection in GB [71, 90] although not in all studies [91, 93].

Many studies have shown an increased risk of bTB spread associated with bTB in neighbouring or contiguous herds. In Ireland, 23–25% of incidents were attributed to spread from herds on adjacent farms [94] and herds with a neighbouring infected herd were almost four times as likely to sustain a bTB incident themselves [95]. In Northern Ireland, ~40% of breakdowns were attributed to the presence of a neighbouring herd with a breakdown [89]. Contact with an infected neighbouring cattle herd was associated with persistent incidents (lasting more than 5 years) in Spain [92] and was associated with a more than twofold increase in the odds of a bTB incident in high incidence areas of England and Wales [70]. In Ireland, it was estimated that being within 1 km of higher animal incidence in the previous 2 years accounted for 35% of bTB incidents [96]. However, distinguishing between spread from one herd to another and sharing a common source of infection is difficult where there is a significant reservoir of infection in wildlife.

Cattle movements

A large number of studies have considered the risk of introducing stock in general and in specific scenarios,

Table 1. Risk factors identified in the literature according to theme, country in which the study was conducted and case definition

		Animal			Herd							Environment			Wildlife		
				Nutrition/ feeding	Age	Farm area/ fragmentation	Size	Type	Management	Cattle purchasing	Bio- security	TB history	Local TB	Land use	Weather	Badger	Ref.
Country	Diag.	Genetic	Breed														
GB	SICCT	x															[12]
Ireland	SICCT	x															[11]
Ireland	SICCT	x															[297]
GB	SICCT						x										[283]
GB	SICCT				x			x									[31]
GB	PMC			x													[55]
Ireland	PMC			x													[21]
GB	SICCT						x										[76]
Italy	PMC							x		x							[78]
N. Ireland	Rct TB						x	x		x			x				[82]
Ireland	Rct TB						x	x				x	x				[83]
Spain	PMC				x			x									[298]
N. Ireland	PMC		x		x		x	x									[86]
Ireland	PMC				x		x	x					x				[88]
N. Ireland	PMC												x			x	[89]
England	PMC					x			x			x	x	x		x	[70]
Ireland	PMC			x					x				x				[95]
Spain	Pst TB			x		x			x				x				[92]
Ireland	SICCT+						x			x		x	x				[96]
England	PMC					x										x	[91]
England	PMC					x	x										[90]
England	SICCT+					x			x	x							[71]
England	Pst TB			x			x	x	x	x				x		x	[93]
England	SICCT+						x		x	x							[93]
Scotland	SICCT+									x							[106]
Belgium	PMC						x			x			x		x		[103]
England	PMC						x	x	x	x							[6]
England	PMC			x					x		x						[114]
Spain	SICCT+			x							x			x			[115]
England	PMC						x		x					x			[116]
Spain	SICCT+								x								[117]
GB	Pst TB								x								[123]
Ireland	SICCT+						x					x	x				[125]
GB	Rct TB											x					[124]
Ireland	Rct TB						x					x					[130]
GB	SICCT+													x	x		[198]

Table 1 (cont.)

Country	Diag.	Animal		Herd			Environment						Wildlife		Ref.		
		Genetic	Breed	Nutrition/ feeding	Age	Farm area/ fragmentation	Size	Type	Management	Cattle purchasing	Bio- security	TB history	Local TB	Land use		Weather	Badger
Ireland	SICCT+														x		[299]
GB	PMC						x									x	[205]
GB	SICCT+						x			x			x				[97]

SICCT, Single intradermal comparative cervical tuberculin test; PMC, post-mortem confirmed; Ret, recurrent TB incident; Pst, persistent TB incident.

such as restocking after foot-and-mouth disease in 2001 in UK [6, 97, 98] (Table 2) and the influence of trading in England and Ireland has been well reviewed [72, 73, 75]. All report an association between restocking and subsequent increased risk of infection [71, 78, 99–101]. Molecular epidemiology is used increasingly to confirm sources of incidents [102, 103]. The reported percentage of bTB incidents attributed to cattle movements in Ireland, where disease is spread throughout the country, varies from 6% to 15% [103–105]. In GB, where disease is clustered in the West and South-West Wales, the proportion of infection attributable to purchased infection is much higher in lower incidence areas. An investigation of 31 incidents in a low bTB incidence area of England found that all but one were most likely due to purchased animals [102]. Prior to legislation requiring the pre- and post-movement testing of cattle imported into Scotland from regions with a high bTB incidence, Scottish farms importing cattle from these regions were almost four times as likely to experience a breakdown than farms that did not. Following the legislation change (2006–2009), this risk, while still significant, had reduced substantially (OR 2.5, 95% CI 1.03–6.27) [106].

Markets and agricultural shows

The evidence for a role of markets in bTB disease transmission is inconsistent. Moving animals into herds from markets has been identified as a significant risk factor in GB [6, 71]. One analysis found that herd owners buying cattle from markets had a lower risk of bTB infection than those that did not but this only applied to one region and it was not significant when herds that had been under restrictions, and so could not purchase, prior to the study were excluded [70]. The largest investigation found no relationship between movement or purchasing via markets and bTB in 1544 lesioned, TST reactors compared to matched controls in Northern Ireland [107]. The evidence for a role for agricultural shows in transmission is anecdotal. A descriptive account of an outbreak in the 1970s found that 51 of 56 cattle from 42 herds reacted to the skin test 80 days after being at the same agricultural show [108].

Farm management

There is evidence that ‘good’ management and hygiene practices may reduce the risk of *M. bovis*

Table 2. *Studies investigation an association between introduction of stock and bTB incident*

Study type	Study size (herds)	Country/year	Measure of risk	Comment, e.g. market/shows/dispersal	Ref.
Source of stock: type					
Case-control	268	England	Logistic regression: OR 3.26 (95% CI 1.71–6.21) OR 1.93 (95% CI 1.03–3.60)	RBCT triplets after initial pro-active badger cull and prior to Jan. 2001 Markets Farm sales	[71]
Retrospective cohort	148	England	Logistic regression: OR 1.95 (95% CI 1.05–3.63)	RBCT from Oct. 2001 to Nov. 2004 Response rate 40% Incidence of 50% over 3 years 24% restocked Markets	[6]
Matched case-control	218	England and Wales	Conditional logistic regression: OR 0.18 (95% CI 0.03–0.91)	Only confirmed cases. Analysis by region Market (Taunton)	[70]
Source of stock: bovine bTB history					
Retrospective cohort	4742	Ireland	Multivariate logistic regression (GEE): OR 1.78 (95% CI 1.06–1.55)	Slaughterhouse cases excluded Source herds had incident with SICCT. ≥ 8 reactors in previous 7 months compared to source herd not being under restrictions in last 7 months	[49]
Matched case-control	218	England and Wales	Conditional logistic regression: OR 0.93 (95% CI 0.96–0.99) per additional 10% OR 1.90 (95% CI 1.0–3.62) OR 8.48 (95% CI 2.23–32.2)	Case used confirmed and unconfirmed Proportion sourced from 4yrly testing interval Sourcing from herd with incident within previous 2 years Sourcing form herd with incident in previous 2 years (Stafford data only)	[70]
Case-control	18 670	GB	OR 1.35 (95% CI 1.22–1.49) per every log increase in number of cattle introduced	Number of cattle brought form yearly testing interval compared to not purchasing	[97]
Type of stock introduced					
Case-control	160	Ireland, 1986–1990	Conditional logistic regression: OR 3.9 (95% CI 1.2–12.4)	Matched on herd size. Cases under restrictions for 12 months Purchasing a bull in previous 5 years	[95]
Comparative case-control	229	UK, 1995–1999	OR 4.9 (95% CI 1.1–22.8)	Risk of persistent incident (>6 months) when introducing adult cows compared to store, calves and no introductions	[93]
Case-control	102 781	Ireland, 2006	OR 1.04 (1.03–1.05)	Incident defined as the presence of one reactor to the SICCT. Introduction of stock >1 year in the previous year	[96]
Number of stock introduced					
Comparative case-control	229	UK, 1995–1999	Multivariate logistic regression: OR 4.0 (95% CI 1.0–16.0)	Risk of transient incident (<6 months) Comparing introduction of >50 cows to <50 cows	[93]
Case-control	62	Argentina, 2005–2007	Logistic regression OR 3.3 (95% CI 1.1–10.2)	Pastoral systems, herd size 50–500. Using caudal fold test. Cases >4 reactors, Controls no reactors in 24 months >19 heifers introduced into the herd in 3 years	[79]

OR, Odds ratio; CI, confidence interval; RBCT, Randomized Badger Culling Trial; SICCT, single intradermal comparative cervical tuberculin test.

transmission, particularly in developing countries [109–113] but also in Europe [114, 115]. In Spain, the communal use of equipment (cattle race) between herds was positively associated with a positive TB test [115]. In GB, poor dairy herd management such as; farm buildings and feed stores being accessible to badgers, allowing grazing at field margins and grazing in fields with badger activity were associated with an increased risk of bTB [114].

Intensive management practices that increase cattle to cattle contact within a herd are associated with an increased risk of bTB incidents [116]. Case-control studies have found dairy herds kept in cubicle housing [95] and covered yards [71] have a substantially increased risk of bTB. This type of housing may be a proxy for the effects of greater stress or reflect a more intensive management system that increases cattle contact rates. This is supported by studies in GB that found the presence of a loafing yard or paddock adjoining cattle housing [114] and not providing housing for cattle [75] reduced the risk of bTB. The use of barns in which cattle can move freely was associated with a positive SICCT test in Spain; the aggregation of cattle within the barn increasing both direct and indirect contacts [117].

Rough grazing in Ireland [95], set stocking in England [70] [114] and grazing cattle on sown or ley pasture [114] have all been associated with an increased risk for bTB in multivariate analyses controlling for herd size. Feeding from the ground either at pasture or during housing [115], storing silage in clamps [93] and feeding green foods/kale or barley have been associated with an increased risk of a bTB incident [114]. These findings could be potentially attributed to increased contact with wildlife. Silage, in particular maize silage [118] is attractive to badgers, and was found to be associated with badgers entering farm buildings in one study [119], but visits to silage clamps were rare in another larger scale study [120]. The storage and feeding of silage from clamps is associated with persistent breakdowns [93] potentially related to the clamps being more accessible to badgers. Feeding hay rather than silage/concentrates has been reported as protective [93, 95, 121], and a case-control study found herds fed silage had a greater risk of a breakdown [116]. In one study, all farms that experienced a persistent breakdown were fed grass silage, although 85% of the TB-free farms also did so [93].

Three studies have found that nutritional supplementation lowers the herd-level risk of bTB. Farms

with areas of rough grazing that did not supply mineral licks were up to 30 times as likely to be infected with bTB, although with wide 95% CIs (1.46–594.4) suggesting it should be interpreted with caution [95]. Two further studies in England support the finding, however, as the provision of protein supplement blocks [114] and vitamin and mineral licks [6] were protective.

There is some evidence, although none from the UK and Ireland, of an increased risk of transmission of *M. bovis* infection to calves fed raw milk or colostrum. However the evidence as to its contribution to bTB risk within the context of an ongoing control program is weak. A case-control study in Argentina found no increased risk in herds feeding raw milk, but did find a higher risk in herds where weaning had been delayed for 4 days, which could be attributed to either drinking infected colostrum or increased exposure to an infected dam [79]. In Spain, bTB incidents in herds where calves were fed replacement milk from test-positive dams were more likely to be persistent but this verged on significance [92].

Other domestic species

While *M. bovis* has been isolated from a range of non-bovine domestic species in GB [122], they are not thought to represent a substantial risk to cattle. Few studies have directly examined the risk they pose and no clear trend has been identified [92, 123, 124]. In Spain, bTB incidents were almost four times more likely to be persistent if goats were present on the farm, although only a small number of farms were affected ($N=11$) while incidents in herds that also farmed pigs were more likely to be resolved quickly [92]. Conversely, contact with any domestic species from a non-contiguous farm was associated with an increased risk of a prolonged incident in GB [123].

bTB HISTORY AND TESTING

bTB history in the region and herd

Local geographic areas with a history of bTB have been consistently identified as being at significantly higher risk of future incidents than other areas [100, 103, 125–127]. The origins of recurrent infection are difficult to separate. There may be a failure to detect and remove all infected cattle or there may be repeated reinfection of the herd through exposure to one of several risk factors which might include infected cattle in

contiguous herds, local movements and exposure to other wildlife or environmental sources of *M. bovis*. Evidence that reinfection can cause recurrent bTB in GB has come from studies of bTB incidents in herds after complete depopulation and restocking, ruling out infection persistence within the herd [97, 128]. In Ireland, herds depopulated for bTB control were no more likely to have a further bTB incident than herds that had been depopulated because of BSE control, but localized badger culling prior to depopulation had occurred in the bTB herds [129].

The number of reactors or the duration of previous bTB incidents is also associated with future risk at a herd level [82, 83, 123, 125–127, 130] and also at an animal level [131, 132]. Post-mortem evidence of infection in cattle in the original incident has been positively associated with a higher future incident risk in at least one study in Ireland [125], although in others no such relationship has been found [82, 123, 126, 130]. Mathematical modelling of recurrence of bTB within a herd estimated that around 24–50% of recurrent incidents are attributable to persistence of infection in the herd, depending on the length of the assumed latent period [76].

In some countries including GB, inconclusive reactors (IRs) are re-tested and, depending on the result, either remain in the herd or are culled. Follow up of IRs has found that they are more likely to have post-mortem evidence of infection than cattle that had a negative response to the skin test [133]. Furthermore, IRs that re-tested negative and remained in the herd were almost four times more likely to be diagnosed with bTB at a later date [134].

Persistence and test performance

Recurrence and infection persistence in a herd may indicate failure of testing, thereby allowing infected animals not only to remain in the population, but potentially to continue to spread infection. Several factors have been identified that influence test performance and hence possible failure to detect infection:

- (1) *Intrinsic test sensitivity.* Diagnostic accuracy varies between different types of tests and tests currently approved for bTB in Europe are considered to have fairly moderate sensitivity and variable specificity [135]. Potency of the tuberculin (purified protein derivative; PPD) may also affect the sensitivity and specificity of tests that rely on this

biological product such as TST and the IFN- γ blood test [136–138].

- (2) *Choice of diagnostic cut-off.* The interpretation of the SICCT test relies on comparing the difference in size between the reaction to avian tuberculin and bovine tuberculin. Altering the cut off for a reactor from >4 mm size difference (standard interpretation) to >2 mm (severe interpretation) increases the sensitivity of the test and decreases specificity [139].
- (3) *Rigorous test procedure.* Qualitative research has identified several key issues that may influence the quality of testing, including on-farm conditions, tester training and experience, and the conflict of interest in the relationship between private veterinarians and the farmer [140]. Some breeds of cattle may pose testing difficulties that can cause decreased sensitivity, for example bullfighting cattle in Spain [85]. Considerable variation has been found between veterinarians in their application of the comparative test [141], although this study was in Belgium, which has had ‘officially tuberculosis free’ status since 2003 and so TB testing would be relatively infrequent. Differences in test protocol have also been shown to influence the IFN- γ test [142]. In Ethiopia, higher throughputs in abattoirs have been associated with lower lesion detection in routinely slaughtered cattle [143].
- (4) *Disease stage in the animal.* bTB is a chronic, slowly progressing disease. The sensitivity of abattoir surveillance based on detection of visible lesions increases with disease advancement [143]. While the TST detects early immunological changes associated with infection, there is still a delay, with earliest detection being estimated at between 21 and 50 days post-infection [10, 136]. During the late stages of the infection, particularly in severe and generalised disease, responsiveness to the skin test is reduced (anergy) [10, 143, 144]. During anergy, the infection is likely to be systemic and severe, cattle are potentially more infectious thus increasing the significance of inadequate detection.
- (5) *Desensitization to the test.* The majority of studies reviewed, but not all [10], have shown that the responsiveness to tuberculin is reduced for up to 60 days after the skin test [136, 145]. Some evidence suggests that a TST may increase the sensitivity of subsequent IFN- γ tests [146], although findings tend to vary [142, 147]. Immunosuppression due

to stress, comorbidity, advanced tuberculosis or calving may also lead to variation in reactivity to tests [51, 145, 148, 149].

- (6) *Host or pathogen genetic variation.* There is some evidence that some cattle may be genetically predisposed to pass the standard TST used in GB [150]. However, in an extensive study (21 000 isolates) in Northern Ireland, no association between skin test response and *M. bovis* genotype was detected, after controlling for confounding factors [151].
- (7) *Cross-reactivity.* A diverse range of saprophytic mycobacteria exist in the environment, which although they rarely cause disease in cattle, are significant in immunological diagnosis. For example, *M. avium* [152–154], *M. fortuitum* [155] and *M. kansasii* [156–158], may all interfere with the interpretation of diagnostic tests for *M. bovis*. Such bacteria can cause animals to react to bovine tuberculin and hence be interpreted as a false-positive bTB diagnosis, or otherwise interfere with test interpretation [144, 155, 156, 159]. Conversely, it has been shown experimentally that a genuine positive response to bovine PPD can be masked by a larger or commensurate response to avian PPD [153, 157, 160, 161], suggesting that *M. bovis* infection may be concealed for some time in cattle sensitized by mycobacteria of the *avium/intracellulare* complex, thus preventing detection and removal.
- (8) *Concurrent infection.* There is accumulating evidence that concurrent infection with *F. hepatica* and with MAP may affect the performance of immunological tests for bTB infection. Evidence suggests the immunomodulatory effects of the helminth *F. hepatica* may reduce the sensitivity of immunological diagnostic tests for tuberculosis [63, 65, 109, 162]. Stronger skin responses to tuberculin tests have been reported in cattle that had been dewormed compared to animals that had not, although bTB infection status was not determined [163]. Cross-reactivity of some proteins shared between *M. bovis* and MAP, can impede diagnosis of bTB in concurrent infections [164–168]. False-positive bTB diagnoses, by the caudal fold test, have been recorded in animals that tested positive for MAP either by the detection of MAP in their faeces or a positive ELISA [166]. Conversely, infection with MAP has been known to obscure the detection of *M. bovis* in concurrent infections in Spanish cattle [167]. Vaccination against MAP can interfere

with immunological methods of bTB diagnosis in cattle [169–172] although, in one study, animals vaccinated with an inactivated MAP vaccine in field trials showed cross-reactivity to the single intradermal tuberculin test (SIT) but not to the SICCT [173]. Despite its limitations, serology has been used to distinguish TB-free cattle that have been vaccinated against Johne's disease [174].

LANDSCAPE, CLIMATE AND OTHER ENVIRONMENTAL RISK FACTORS

Survival and persistence of *M. bovis* in the environment

Estimates of environmental survival of *M. bovis*, evaluated for artificial and natural contamination of several sites and substrates are extremely variable, ranging from a few days to 2 years [175] (Table 3). Much of the data derives from early studies in GB and other countries where levels of natural environmental contamination may have been high, given the abundance of clinically advanced bTB in cattle. In general, tubercle bacilli survive best in cool, moist environments shaded from direct sunlight and survival depends on temperature, sunlight and relative humidity (Table 3). Longer survival times in winter compared to summer months have been recorded for *M. bovis* in experimental studies conducted in GB [176], New Zealand [177], Michigan [178] and Australia [179] (Table 3). *M. bovis* remained viable up to twice as long in shady conditions compared to sunny conditions [180].

Experimental studies demonstrate that *M. bovis* can be isolated >5 months after inoculation when mixed with faeces [176] and after one month in spiked possum carcasses [181]. Viable bacteria were recovered from hay at 7 days after inoculation and could still be isolated from samples of apples, corn and potatoes after 112 days [182]. In silage, the survival of *M. bovis* is likely to be shortened by the acidity (pH reduction) and temperature during fermentation. When silage containing large quantities of naturally infected faeces was fed to guinea pigs, it was infective for up to 10 weeks post-inoculation (Ulli Reuss, 1955 quoted in [80]).

Farm practices relating to both the storage and spreading of slurry can increase the risk of a herd bTB incident [6, 93, 95, 183] and the role of slurry in the transmission of bTB has recently been evaluated [184]. *M. bovis* can persist in slurry for up to 6 months [183, 185] and spreading of slurry after storage for <2 months has been associated with an increased

Table 3. *The effect of environmental variables on the survival of M. bovis*

Conditions	Exposure	Survival	Detection by	Ref.
Temperature				
Mixed with faeces in open air (Berks)	Winter from November	≥ 5 months	Guinea pig infectivity	[176]
	Autumn from September	4 months		
	Summer from June	<2 months		
Mixed with faeces or soil (Berks)	Summer from June or May	5 months		[300, 301]
Absorbed onto cotton ribbon (New Zealand)	Winter and spring	>14, <28 days		[177]
	Summer	<7 days		
In the laboratory	0 °C	50% at 36 days	Culture	[302]
	16 °C	50% for <1 day		
In soil, water, hay or maize (Michigan, USA)	Autumn and winter	30 ± 13 (s.d.) days	Culture	[178]
	Winter and spring	29 ± 26 days		
	Spring and summer	6·9 ± 12·5 days		
In possum carcasses on pasture (New Zealand)	Winter	20–27 days		[181]
	Summer	<3 days		
Moisture				
In the laboratory	100% relative humidity	50% at 43 days	Culture	[302]
	57% relative humidity	50% at 8 days		
	5% relative humidity	50% for <1 day		
In faeces, dry soil and moist soil in Tropical Australia	Shaded conditions	Favourable for survival		[179]
Ultraviolet radiation (including solar radiation)				
Mixed with faeces in open air (Berks) (September)	Exposed	4 months	Guinea pig infectivity	[176]
	Shaded and sheltered from rain	6 months		
In the laboratory	UVA radiation (375 nm)	80% for 20 min	Culture	[302]

risk of bTB [105]. Experimental studies have demonstrated that *M. bovis* can remain viable for up to 12 h after aerosolization and the bacteria is resistant to stresses of being airborne [186]. Methods that reduce the distance over which slurry is dispersed and injecting slurry into soil, rather than spraying under pressure, reduces the risk of drift [183].

M. bovis has been recovered from soil sampled from badger setts and fields on a farm in Ireland that had a recent TB incident [187] and was isolated from one water sample from yards frequented by infected badgers in England [188]. In the United States, despite extensive sampling, *M. bovis* was not detected in soil, water, livestock bedding, feed, faeces, hay, pasture and grass samples collected from farms in Michigan and Texas recently positive for bTB [189–191]. However, prevalence in cattle and in local wildlife, including white-tailed deer (*Odocoileus virginianus*) (<2%), was much lower than apparent *M. bovis* prevalences in badgers that have been recorded in GB and Ireland (14–16%) and infection in cattle populations was also lower [192–195].

Estimating the extent of environmental contamination with *M. bovis* may be confounded by the

practical difficulties inherent to its isolation and culture from environmental samples [175, 190]. Procedures for concentrating bacteria from larger samples are required to compensate for the patchy distribution of bacteria in environmental samples [189]. Molecular methods specific to *M. bovis* have been developed to detect and quantify mycobacterial DNA in environmental samples [196, 197] although they have limited sensitivity and positive results do not necessarily indicate viability or infectivity. On the other hand, DNA in dead cells did not survive beyond 10 days, suggesting that the DNA extracted from environmental samples may have come from intact cells [187].

Weather and climate

Wint *et al.* [198] were able to predict the geographical distribution of bTB in England and Wales with a high level of precision ($\kappa = 0.68$) using monthly remote-sensing data for weather-related variables (resolution 1–5 km) and cattle density. These variables were more important predictors than land use or vegetation. Areas with the greatest risk of bTB were more moist, having a lower water vapour pressure deficit

(VPD). They also had their peak VPD later in the year and had lower variability of VPD and temperature. Recently, Jin *et al.* [299] were able to predict bTB incidence from rainfall over a 5-year period in an area of Wicklow, Ireland; the strongest correlations being with rainfall one or two quarters before the breakdowns. However, a previous study from Ireland failed to consistently predict the incidence of bTB reactors over 15 years using a range of weather variables [299]. The North Atlantic Oscillation (NAO), a major determinant of weather conditions in Western Europe, has been shown to be a significant correlate of culled badger *M. bovis* prevalence, but its inclusion in statistical models left a great deal of unexplained inter-annual variation which was better explained by the effect of the 2001 foot-and-mouth disease outbreak [200].

Flood and drought could affect the risk of infection with bTB by influencing the survival of *M. bovis* (Table 3) or by affecting contact patterns between cattle and other cattle or infected wildlife but there is little published evidence for any relationship between flooding in temperate climates. It has been hypothesized that, in times of drought, badgers may resort to woodlands for prey in moist leaf litter and their contact with cattle would be reduced [201]. In Africa, associations between increased bTB prevalence and flooding have been attributed to enforced contact between herds [202], and with drought as it forces cattle to use communal water sources shared by infected and naïve cattle [203] and encourages large-scale movements [204].

Landscape

A number of landscape and environmental factors have been found to be associated with increased or decreased herd bTB incidents, although the degree of association varies [93, 100, 116, 205–207].

Many landscape factors identified determine the suitability (or otherwise) of an area for badgers or act as barriers for the geographical spread of bTB [208]. Landscape characteristics such as altitude and habitat composition are correlated with badger abundance [209, 210]. Soil type has also been associated with badger abundance [211, 212] with badgers preferring sandy loamy soils for sett construction. Interestingly, soil characteristics also correlate with bTB risk, with bTB-affected farms being less likely to have deep clay soils or seasonally wet soil [70] and *M. bovis* was more likely to be isolated from sandy soil in cattle farms in Michigan [213]. This soil type may provide a moist, well drained

microclimate that could maintain suitable pH and moisture levels that favour bacterial survival.

The distribution of badger latrines in a high density population in South-West England varied according to habitat types and landscape features [214]. Latrines are generally more commonly found in woodland and are less common in arable or grassland [214, 215]. A high proportion of latrines are located within 5 m of a linear feature (especially hedges and stone walls) [214] or are associated with runs crossing such linear features [209].

Higher densities of hedgerows [205] and a higher percentage of boundaries composed of hedgerows [116] on a farm were associated with a reduced risk of a bTB incident in the resident herd. The authors suggested that this was because longer forage (preferred by grazing cattle) is associated with higher hedgerow density and thicker hedgerows may mean that cattle cannot access areas that may be more contaminated with badger faeces and urine.

Water sources

Aggregation at communal water sources encourages closer contact between cattle and may increase the likelihood of contact with wildlife, although there is little evidence for a risk pathway in GB. Evidence from Spain found farms with high density of streams had a lower risk of TB, thought to arise because dispersed water resources reduced aggregation of cattle and wildlife [115]. The presence of water sources (ponds and creeks) with uncontrolled access has been associated with an increased risk of bTB in Michigan, USA [207] and in Western Uganda [216].

Transmission through birds, invertebrates and protozoa

Mechanical transfer of *M. bovis* bacteria on the feet of birds [189] or via invertebrates [217] is considered a theoretical risk although has yet to be demonstrated. *M. bovis* has been isolated from various species of birds in Europe, although they were predominantly birds of prey [218]. A report from the Soviet Union describes *M. bovis* and *M. avium* in several species of tick [219]. *M. bovis* has also been detected in horn flies (*Haematobia irritans*) [220].

Experimental studies have demonstrated that *M. bovis* can survive in protozoa (*Acanthamoeba castellanii*), potentially facilitating transmission by extending the survival of the bacteria in the soil [221]. However, co-incubation of both organisms under laboratory

conditions substantially reduced levels of bacteria, suggesting that while there is the theoretical potential for environmental amoebae to act as a reservoir of *M. bovis*, they may also reduce environmental contamination [222]. Acid-fast microorganisms have been observed inside amoebae isolated from infected badger setts, although attempts to confirm them as *M. bovis* by culture or PCR were unsuccessful [222].

WILDLIFE

Badgers

In the UK and Ireland the European badger (*Meles meles*) is the main wildlife reservoir for bTB infection in cattle. A reservoir is defined as a epidemiologically connected population in which infection is permanently maintained and transmitted to a target population [223]. Furthermore, this reservoir of infection in badgers is often cited as the main barrier to eradicating infection in domestic livestock in these countries. The most compelling evidence of the infection risk that badgers pose to cattle comes from large-scale trials in the UK and Ireland, where badger culling had significant impacts on the incidence of bTB in cattle [224–227]. Within the boundaries of proactively culled areas during the Randomized Badger Culling Trial (RBCT) cattle bTB incidence was reduced by 33% after four annual rounds of proactive culling [227]. Given that not all badgers were culled [228], this is likely to represent a minimum number of incidents that are attributable to badgers in these areas. More recent analyses of the RBCT data suggests that in an endemic area, badgers are directly responsible for 6% of bTB infections in cattle and the overall contribution from badgers, through onward cattle to cattle transmission, is ~50% [229]. How badgers transmit bTB to cattle is not known, although several likely routes are described below.

Proximity and abundance of badgers

Presence and abundance of badgers near cattle farms have been included in a range of analyses of potential bTB risk factors. Analyses of risk factors associated with cattle herd incidents that include the distance from farm buildings to badger setts have produced conflicting results. Johnston *et al.* [71] found that including presence of occupied badger setts within 1 km of farm boundaries did not improve their bTB risk prediction models, and Griffin *et al.* [88] likewise observed that the distance to badger setts did not vary relative to bTB incidents in herds. Conversely, Martin

et al. [230] found a significant, though weak, reduction in the risk of an incident with increasing distance to badger setts and [89] found positive associations between the presence of badgers and herd incidents in Northern Ireland. In South-West England, finding dead badgers on a farm was associated with a three-fold increase in the risk of a bTB incident [70].

Studies investigating cattle bTB risks in relation to local badger density have also produced mixed results. For example, Reilly & Courtenay [93] reported an association between high densities of badger setts and persistent cattle herd incidents, but only after adjusting for farm management-related variables. Similarly, another study reported a correlation between the number of active badger setts within 1500 m of a farm and the probability of cattle herd incidents [91]. However, a study by Mathews *et al.* [205] found little evidence of an association between badger density and bTB risk, as farms with management practices that favoured wildlife had a lower risk of bTB.

M. bovis transmission routes

Badger-to-cattle transmission has been experimentally demonstrated [188], but the principal routes of transmission in the field can only be inferred at present. Infected badgers may excrete *M. bovis* bacilli in urine, faeces, sputum and exudate from open abscesses [231]. Studies have identified *M. bovis* in soil and latrines [232], and there is evidence for bacilli remaining infectious for some time after being deposited, at least in certain environmental conditions [233].

Potential routes of transmission include direct transfer via very close contact between badgers and cattle, and indirect contact where cattle encounter infectious material from badgers. Both mechanisms could theoretically occur at pasture or in farm buildings, but the relative importance of these transmission routes is unknown. As smaller numbers of bacilli are needed to infect cattle via the respiratory system than via the digestive system [50], inhalation of bacteria is likely to be the main route of infection [234]. This could occur during nose-to-nose contact with badgers, but also while grazing, as cattle aerosolise and inhale bacilli on contaminated pasture or forage.

Direct transmission risks

Previous observational studies suggested that close contact between badgers and cattle at pasture is unlikely [235] and other research suggests that badgers

may actively avoid pasture with grazing cattle [236]. Studies using proximity loggers have shown that close contact between badgers and cattle does occur at pasture although it was reported as relatively infrequent [61] or very rare [81, 237]. The available evidence suggests that direct contact at pasture is unusual, although contact rates could vary across different landscapes. Badgers may frequently visit farm buildings, where they may readily come into close contact with cattle [119, 238–240]. The evidence from several studies suggests that direct contact may be more frequent in buildings and it has been suggested that it may be easier to reduce risks by modifying buildings to exclude badgers [238, 241].

Indirect transmission risks

Badgers habitually defecate and urinate at latrines [242]. This may concentrate potentially infectious material and give rise to enhanced infection risk to cattle at certain locations, although it may conversely reduce risks elsewhere [243]. Early research suggested that cattle avoided grazing on pasture contaminated by badger faeces [235, 244]. However, subsequent studies showed that cattle would explore contaminated pasture, and a small proportion would graze at latrines [245]. Visits by cattle to badger latrines recorded by proximity loggers found that 85% of the cattle in one herd actively investigated badger latrines and 15% of them visited latrines over 100 times in a 6-month period [237]. The extent to which this occurs may be affected by farm management practices such as grazing rotation patterns [244] which in turn influence factors such as sward height [245].

Badger urine may present a significant bTB transmission risk to cattle as it can contain up to 300 000 bacilli/ml [209, 246]. Grazing cattle do not appear to avoid badger urine deposited away from latrines [209, 247], which often occurs on runs crossing linear features such as hedgerows [209]. Cattle have been shown to graze readily at such crossing points, and elsewhere on pasture contaminated with badger urine [209, 245].

Badgers have been observed both defecating and urinating onto stored cattle feed in farm yards, buildings and cattle troughs [119, 240, 248]. Cattle have been recorded showing little or no avoidance of feed contaminated with faeces from rodents and wild birds [249]. It has been suggested that this non-selective feeding behaviour may represent a bTB transmission risk especially if the contaminated feed is well-mixed.

One questionnaire-based study provided some support for this hypothesis, finding a significant positive association between the perceived reported presence of badgers in feed stores or cattle housing and an increased risk of cattle herd incidents [227].

Wild boar

M. bovis infection has been isolated from wild boar in several European countries [250–257], including in a feral wild boar (*Sus scrofa*) in the UK [258]. Research on the Iberian Peninsula has reported bTB prevalence of up to 52.4% [259] and positive associations between the density of wild boar populations and cattle bTB incidence [260, 261]. The presence of wild boar on cattle farms in Spain, as reported in farmer questionnaires, was associated with TB breakdowns [115]. Furthermore, the *M. tuberculosis* complex genotypes found in wild boar are of the same origin as the bovine and caprine genotypes found in domestic swine, deer and humans [259, 262, 263]. Hence, there is evidence to support the role of the wild boar as a reservoir host of *M. bovis* in Mediterranean areas (reviewed in [264]). In the UK, wild boar have a limited geographical distribution, mainly confined to small areas in Kent/Sussex and Gloucestershire/Herefordshire. Therefore, the current risk to livestock from this species in the UK is considered to be low, although this has the potential to change should wild boar numbers and geographical distribution change. There is currently no empirical data on the growth trajectory of wild boar populations in the UK. This species has a very high reproductive rate for an ungulate and hence the potential to increase population size rapidly [265], although hunting and restricted woodland cover may constrain their numbers and distribution in the UK [266].

Deer

Several wild deer species in Europe have been found to be susceptible to bovine bTB infection, including fallow (*Dama dama*), roe (*Capreolus capreolus*), red (*Cervus elaphus*), sika (*Cervus nippon*), Reeves' muntjac (*Muntiacus reevesi*), reindeer (*Rangifer tarandus*) and elk (*Alces alces*) [218]. Spatial associations between strain types in deer and cattle have been demonstrated by restriction fragment-length polymorphisms in Ireland [267] and by spoligotyping in Spain [268], suggesting that bTB is transmitted between these species. Furthermore, experimental studies have

demonstrated that indirect transmission of bTB could occur between white-tailed deer (*Odocoileus virginianus*) and cattle through sharing of feed [269]. Spatially explicit modelling of density and distribution of white-tailed deer and cattle herds with bTB breakdowns in Michigan, USA, demonstrated that infected deer play an important role in the maintenance of bTB in that area [213].

In a case control study in Michigan, USA, Kaneene *et al.* [207] found that the prevalence of bTB in the local deer population was an important risk factor for bTB in cattle. They also found that bTB risk was increased when water was provided to cattle outdoors and large quantities of hay bales were stored in fields or on pasture fence lines. Conversely, the risk was reduced if feed was stored indoors or properly protected by bagging or wrapping. A semi-quantitative assessment of the risk of transmission from deer to cattle in the UK, based on prevalence of infection, extent of potential bacterial excretion, likelihood of contact with cattle and approximate biomass suggested that red and particularly fallow deer represented the greatest potential risk. However, any risk is likely to be localised given the geographical variation in deer densities [270].

Other species

M. bovis has a wide host range and is the most common cause of tuberculosis in mammal species [271]. *M. bovis* infection has been identified in numerous wild mammal species in the UK (see [271]), although a semi-quantitative risk assessment suggested that species other than deer and badgers are unlikely to pose a significant risk of bTB transmission to cattle [270].

HUMAN-TO-CATTLE TRANSMISSION

There are a large number of documented cases of human to cattle transmission from the first part of the 20th century in Europe, in Denmark [272], the UK [273], Germany [274–277], The Netherlands [148]. However a case was reported relatively recently in Switzerland [278]. More recently, associations between tuberculosis in humans and cattle have been predominantly documented in Africa [202, 279–282].

DISCUSSION

We present an overview of risk factors that have been identified for bTB in cattle, with a primary focus on the UK and Ireland, but where gaps exist drawing

on evidence from further afield. However, there are difficulties in extrapolating from many studies and in weighting the relative importance of the various risk factors identified in the literature owing to differences in study design, circumstances, follow-up time, selection criteria and case definitions. Variation in herd composition, management, history of infection and local circumstances, including exposure to potential sources of infection from wildlife, will all vary in space and time creating serious challenges for between study comparisons. The case definition for a bTB-infected herd in Europe is detection of one or more reactors to the SIT or SICCT test (64/432/EEC) at standard interpretation but definitions in the literature range from other interpretations of the skin test [44, 48, 93] to laboratory-confirmed *M. bovis* infection [207], or to the detection of reactors with evidence of infection [89]. The principal outcomes examined also varied from detection of infection to disease characteristics such as duration [123], chronic persistence [92] and recurrence [82, 83, 124] (Table 1). This range supports the use of a review of evidence from several studies looking at similar research questions over a relatively broad geographical range.

Nevertheless, despite differences in study design some clear and consistent patterns have emerged across a broad range of infection prevalence and environments. Prominent are the increased risks associated with animal age, contact with a wildlife reservoir and the size of the herd. Purchasing strategies and management practices that favour intensive production, also promote increased contact among cattle (housing) and with wildlife (fragmentation, farm size) at the expense of hygiene and biosecurity. Herds with a history of bTB are consistently identified as being at higher risk of a future bTB incident. The relative importance of different risk factors also varies according to incidence. In low-incidence areas, infection is predominantly related to cattle movements [103, 106]. In higher risk areas, risk factors reflect increasing contact rates, as is unsurprising for a contagious disease, e.g. herd size, farm size, movements, housing, and exposure to wildlife (Table 1).

Causality between markers for infection or disease and many risk factors cannot be assumed. Many risk factors may act as proxies for contact and transmission opportunities or other unmeasured variables. Landscape characteristics will influence the distribution and abundance of wildlife and reflect different cattle management practices, and so may be proxies for contact rates among cattle and between cattle

and wildlife. Also, the quality of animal husbandry may affect the level of *M. bovis* contamination and attitudes to biosecurity. Hence, the significance of the reported protective association between vitamin and mineral supplementation and bTB [6, 55, 95, 114] may be a proxy for poor management and hygiene practices on infected farms. More research is needed to determine the impact and relative contribution of management factors, such as nutrition and housing on infection transmission and pathogenesis within the host animal.

The risk that bacilli released into the environment by infected cattle or wildlife will infect other animals depends on the rate at which they lose their viability and virulence. Survival of *M. bovis* in soil and other environmental substrates depends on temperature, moisture, pH, exposure to sunlight, oxygenation, and interactions with other microflora, and so will be expected to vary widely in space and time. Such environmental factors have been associated with variation in the risk of bTB in cattle [183].

Knowledge gaps and the way forward

The relative importance of the risk factors identified to date are likely to vary among farms and environments, and over time making it difficult to identify generic patterns. Many risk factors have been identified in relation to different types of herd management such as sharing of equipment [115] and types of housing [71, 95], but their impact is unknown. In many studies once location, bTB history and herd size are accounted for, the degree to which management factors may influence the risk of an incident is low. The calculation of the population attributable fraction in bTB risk factor studies is relatively rare, but can provide an assessment of the absolute contribution of a risk factor to infection incidence or prevalence [71, 96, 138]. It can be used to provide a measure of how many bTB cases might be prevented by modifying or removing a risk factor or range of factors. If prevalence of the exposure is low, then the relative importance of the risk factor will be low, even if the association with disease is quite strong.

Few risk factor analyses have matched cases and controls on herd size [70] but the dominance of herd size in many risk factor studies (Table 1) [73, 75, 283], supports a case for conducting studies matched on herd size, to attempt to tease out other management characteristics that may be a feature of large herds and may promote infection transmission.

In the UK and Ireland there is a large body of evidence to support transmission from badgers to cattle [224–227, 230, 284] and transmission is likely to be reciprocal [285] suggesting that in at least some circumstances the two populations may constitute a host community in which *M. bovis* circulates. Hence there is the potential for interactions and interdependency among cattle management and wildlife-related risk factors. The potential impact of transmission from badgers to cattle has been estimated from the RBCT at ~33% [227] although identification of the most important routes of transmission remains unresolved, thus hampering the development of targeted control measures.

In GB, mathematical modelling supports other work showing cattle movements, re-infection from wildlife and the performance of the main diagnostic test have a substantial influence on infection incidence [286]. Currently approved diagnostic tests for bTB are known to be variable in their performance and have only moderate sensitivity [135]. Significant improvements in diagnostic test accuracy and hence case-definition could improve the estimation of the impact of risk factors, transmission rates and hence the development of appropriate control strategies. It has been estimated through modelling that up to 24% of British herds had residual infection in at least one animal when restrictions were lifted [76]. This is congruent with estimates from a descriptive analysis in Spain, where 22% of incidents were determined to have been caused by residual cattle infection [287]. There is evidence that the TST is influenced in the presence of Johne's disease [29] and liver fluke [63, 162] but the extent to which this might impede diagnosis is unknown. Potential impacts could be significant as the herd-level seroprevalence of Johne's disease in South-West England is high [288]. The geographical distributions of liver fluke and bTB infections are negatively correlated in England and Wales and it has been suggested that this is a result of masking of infection [289]. However the highest prevalence areas for liver fluke, such as Cumbria in Northern England [290] are not associated with higher than average detection of bTB infection by meat inspection, and bTB incidence remains low [84].

Accumulating evidence suggests that the susceptibility of cattle to *M. bovis* infection can be affected by breed and genotype but further work is required. About 20% of variation in resistance to bTB in British and Irish dairy cattle can be attributed to sire lineage, and there is evidence of similar variation in

resistance in beef breeds. However, these may be under-estimates of heritability because of misclassification of estimated exposure to infection and imperfect diagnostic tests [291]. This approach of breeding resistance is being taken forward by the dairy industry in GB [292].

The risk from within farm movements of animals and herds, although hypothesised, has thus far not been investigated. It is rare for this data to be captured centrally and so investigation would require a bespoke study.

The identification of tractable risk factors for bTB is critical to a better understanding of infection dynamics and the development of cost-effective approaches to disease control. While a cattle farmer can alter their behaviour or farm management strategies including purchasing choices, it is less likely or more challenging to exert substantial control over extrinsic factors related to the environment or wildlife, or the level of infection in the surrounding area. In recognition of this there is a need to account for farmer behaviour and the drivers of their decisions in the development of infection control strategies [293, 294]. Few studies have related farmer perceptions to bTB incidence, and those that have shown conflicting results [115, 117, 295].

Knowledge of the importance of risk factors could be deployed to facilitate integrated control in two ways. First, certain risk factors may be shared among a range of pathogens of cattle although disease control in livestock usually focusses on individual infections. The identification of common risk factors should be used to develop more efficient, cost-effective holistic infection control programs. The identification of shared risk factors for brucellosis and bTB has indicated that such an approach is possible [117]. Second, the combination of country- and region-specific risk factors could be used to focus on multiple transmission pathways simultaneously to continuously improve a more effective, integrated and evidence-based control strategy. Such an approach would need to be carefully validated and its effects would need to be measured.

If R_0 is just >1 , changes in factors with a moderate effect on transmission will have a big impact [296]. Modelling approaches suggest that focusing on a single route of transmission will not reverse the increasing trend in incidence [286]. This suggests a tailored package of control measures, addressing many transmission pathways, made possible by the identification of relevant risk factors is likely to be the most effective approach to bTB control in cattle.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S095026881600131X>.

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DECLARATION OF INTEREST

None.

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