Bovine Tuberculosis (TB): A Review Of Cattle-To-Cattle Transmission, Risk Factors And Susceptibility

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REVIEW TITLE AND TERMS OF REFERENCE

Cattle-cattle transmission, risk factors and susceptibility: a review of transmission, within and between herds, of bovine TB with particular reference to cattle-to-cattle spread (a) in cattle housing, (b) at pasture, and (c) any other significant circumstance or location such as during transport or at markets. This review should also seek from published work or work nearing completion to identify, summarise and rank those factors that influence susceptibility to bovine TB. The review should similarly seek to identify, summarise and rank those practical management actions that could best mitigate the risk of transmission in housing and at pasture and identify any other factors likely to commend further beneficial study.
[DARD Animal Health and Welfare Branch 2010]

Running Title: Cattle-cattle transmission, risk factors and susceptibility

To stamp out *"...so terrible a malady."*

Waddington K (2004). Bovine tuberculosis and tuberculin testing in Britain, 1890-1939. Med Hist. 2004 Jan; 48(1):29-48.

SUMMARY

Tuberculosis (TB) is a chronic disease of animals and man caused by pathogenic bacteria, which are particularly well-adapted to fighting long wars as opposed to short battles. The knowledge base that exists on TB in humans (infection with *Mycobacterium tuberculosis*) provides important insights into the life history of TB in animals; the likely transmission of, and risk factors for, bovine TB (infection with *Mycobacterium bovis*) and the factors which influence susceptibility. Relevant aspects have necessarily been discussed in this review.

Human TB

- Whilst treatable, human TB is the leading cause of avoidable deaths due to infection, although only ~30% of exposed individuals become infected. Between a third and a half of the global human population (>2 billion) have a positive tuberculin skin test and are thought to harbour the bacterium. Current diagnostic tests have limited sensitivity and specificity.
- Not all individuals exposed to *M. tuberculosis* become chronically infected. Multiple genetic and non-genetic (environmental) risk factors interact to influence susceptibility to infection and disease, infectiousness and transmission.
- Importantly, only a small proportion of infected people progress rapidly to active TB. The remainder (>90%) are considered innately immune to disease, but not to infection, and enter a latent stage from which *M. tuberculosis* may reactivate years or even decades later. Although central to human TB epidemiology, because of their potential to reactivate if immune-suppressed, they (acid-fast bacilli (AFB) sputum smear-negatives) are considered to be non-infectious, or at least significantly less infectious than AFB sputum smear-positive cases.
- The two major clinical forms of human TB correspond to two age-dependent peaks of incidence; disseminated disease in young children and lung infection in adults (reactivation of latent TB from a silent primary infection).
- The literature supports the view that human TB is exclusively an airborne respiratory disease transmitted via aerosol, comprised of small 'droplet nuclei', which mostly requires prolonged close, direct contact with an infectious case. We were unable to find any

convincing evidence of indirect transmission, such as via a contaminated environment etc.

- Human TB is still a 'social' disease; a disease of minorities, inextricably linked to poverty, overcrowding, poor ventilation and malnutrition. It affects mostly young people in their otherwise most productive years.

Bovine TB

- In countries with advanced test and control programmes (a comprehensive set of surveillance and control measures to address cattle-cattle transmission) bovine TB is a low incidence infectious disease with an apparently low transmission rate. Infection would appear to be relatively poorly transmitted between cattle in most, but not all, circumstances. However, there is clear evidence of ongoing cattle-cattle transmission in several recent studies.

- Pathogenesis studies indicate that bovine TB is principally a respiratory infection and the majority of infections are thought to occur via 'direct' aerosol transmission between animals in close proximity. Pathogenesis research indicates that the minimum infectious dose via the respiratory route is very low. In contrast, the infective dose required to establish infection via the oral route is many times higher and other than milk-borne infection in young calves, infection via the oral route appears to be relatively uncommon.

- Although 'indirect' routes of infection (via a potentially contaminated environment, housing or feeding stuffs etc) may be possible, evidence suggests that these routes are likely to be of relatively minor significance. However, recent US data support a role for indirect transmission in the epidemiology of bovine TB in their cattle-deer system. Also, the possibility that the local environment is directly contaminated with potentially viable *M. bovis* cannot be excluded currently.

CATTLE-CATTLE TRANSMISSION

What is the general evidence base (not exhaustive) supporting cattle-cattle transmission?

- Cattle are susceptible to *M. bovis* infection and are the preferred host for *M. bovis*. Cattle mount a demonstrable immune response to *M. bovis* infection.
- *M. bovis* can be isolated from infected cattle. The pathogenesis of *M. bovis* in cattle has been described and supports the natural history of *M. bovis*.
- Cattle can be experimentally-infected with *M. bovis* by several routes. Naïve cattle placed in direct contact and shared airspace with experimentally-infected cattle can be infected with *M. bovis* (several studies). Naïve cattle placed in direct contact and shared airspace with naturally-infected cattle can be infected with *M. bovis* (one study).
- Historically, before the introduction of control measures and regardless of the source, *M. bovis* infection was widespread in the national cattle herd. However, it is important to review the evidence for current cattle-cattle transmission in regions operating extensive cattle-based controls.
- Molecular typing data support the dispersal of *M. bovis* from the British Isles in infected cattle in the 1800s to former British colonies. In the absence of effective control programmes, further cattle-cattle transmission, including to other imported or native breeds and to wildlife, clearly ensued.
- The introduction of targeted, exclusively cattle-based control measures dramatically reduced the prevalence of bovine TB in cattle in the mid-1900s in many countries. In the 1960s, the disease was almost eliminated from GB through rigorous testing of cattle herds and strict quarantine.
- The enhancement of solely cattle-based measures has contributed to a significant reduction in bovine TB prevalence in Northern Ireland (NI) from 2002 – present.
- Results from the Randomised Badger Culling Trial (RBCT) led Professor John Bourne and the ISG (2007) to conclude for GB at least, that:
    - *"...weaknesses in cattle testing regimes mean that cattle themselves contribute significantly to the persistence and spread of disease in all areas where TB occurs, and in some parts of Britain are likely to be the*

main source of infection. Scientific findings indicate that the rising incidence of disease can be reversed and geographical spread contained by the rigid application of cattle-based measures alone."

- During the GB foot and mouth disease (FMD) outbreak in 2001 cattle movements and bovine TB testing were suspended. This was associated with a consistent increase in prevalence of bovine TB in cattle in RBCT areas, which suggested increased cattle-cattle transmission. In addition, bovine TB was detected in herds in N England that had restocked post-FMD. Molecular typing of confirmed cases supported the conclusion that, in most herds, infection had been introduced by the unwitting purchase of animals with undisclosed infection from TB hotspot areas. Identification of tuberculin skin test reactors in home-bred and purchased animals suggested further within-herd cattle-cattle transmission.

- *M. bovis* 'strains' (genotypes) have recently been surveyed systematically in GB, NI and the ROI. Genotypes that are virtually unheard of in GB, but common in either NI or ROI, turn up occasionally in GB. Although frequently associated with NI and ROI ear-tags, sometimes these genotypes are isolated from GB cattle that are 'home-grown', leading to the conclusion that they have been imported and transmitted to home-grown GB cattle. The converse has also been demonstrated.

- Sporadic bovine TB breakdowns still occur in other countries, including TB-free countries (the Netherlands, Belgium, Germany, Scotland, the Isle of Man etc) linked to the import of infected (undetected) cattle. There is clear evidence of onward transmission in several cases, especially where infections were not detected rapidly. Some such breakdowns have been sufficiently large to merit whole herd depopulation. However, it is accepted that onward cattle-cattle transmission is not normally very efficient.

- The distribution of reactors by herd is highly skewed; the Pareto Principle ("*the law of the vital few*") applies. Tuberculin reactors tend to cluster within herds. This also infers that a relatively small number of herds contribute disproportionately to the overall number of reactors and there are likely to be converging risk factors for those herds, which support either extensive cattle-cattle transmission or multiple exposure(s) to a point source(s), which could be a badger source or a super-shedder cow, or a mixture of all of these. Targeting appropriate investigative and control

measures to multi-reactor herds should lead to disproportional benefit. Even with a comprehensive programme of cattle controls, the tendency to identify the same, or highly similar, molecular types within multiple reactor breakdowns suggests ongoing transmission.

- The current geographic clustering of *M. bovis* molecular types at a local level in the UK is striking. One of the plausible hypotheses to explain this phenomenon is that it may reflect the opportunities for cattle-cattle transmission supported by the natural and imposed contact networks and movement restrictions imposed on cattle. Clustering would tend to suggest ongoing transmission and the emergence and spread of what are clearly new variant *M. bovis* genotypes would tend to support this, where transmission is not being interrupted sufficiently by local controls. However, it has also been hypothesized that this regional clustering simply reflects the underlying structure of infectious wildlife.

How does cattle-cattle transmission occur?

- The main methods of cattle-cattle transmission of bovine TB are summarized below (not in priority order). Methods of transmission resolve into direct cattle-cattle transmission and potentially indirect cattle-cattle transmission.

Main methods of cattle-cattle transmission of bovine TB
Within-herd transmission at housing
Within-herd transmission at pasture
Vertical (congenital) transmission
Pseudo-vertical transmission (via milk)
Spreading bovine excreta on pasture
Between-herd transmission through cattle movements
Between-herd transmission across farm boundaries
From soil and silage
Via drinking water
Via arthropod vectors

Table 1: based on Phillips and others (2003)

- In general, the routes of infection identified in pathogenesis studies strongly suggest that bovine TB is predominantly a respiratory infection. Hence, any situation of close cattle contact with an

infectious case may facilitate transmission. Review of the literature supports the view that *M. bovis* is mostly transmitted via infectious aerosol.

- 'Direct' routes of transmission require close and mostly sustained contact with an infectious case, whereas 'indirect' routes would include transmission via, for example, a contaminated external or internal environment, contaminated feed, water, equipment etc. On balance, direct ('speaking distance') contact would seem to be far more significant than transmission potentially supported by 'indirect' routes.

- 'Direct' infection from dam to calf(s) is clearly demonstrated in several recent studies. This is supported by pathogenesis data, where pathology differing from the classical respiratory tract pathology suggests ingestion of *M. bovis*.

- Recent US research tends to support a role for indirect transmission, most likely at pasture (via contaminated deer feed), in their deer-cattle bovine TB system, without clear evidence of direct contact between species.

- *M. bovis* appears to be able to survive in the environment for significant time periods, depending on prevailing weather and conditions. Whilst considered unlikely, it has not been possible to exclude that the environment is contaminated with *M. bovis*. Whether this would be predictive of bovine TB breakdowns in local cattle herds remains to be demonstrated.

Where does cattle-cattle transmission occur?
- Any situation (pasture, housing, movement, markets, shows etc) which allows close direct contact between cattle, including infectious case(s), may facilitate cattle-cattle transmission. There is still significant cattle social contact at pasture and when housed, although the proximity of such contacts is likely to be variable. Literature review supports the view that opportunities for such transmission are likely to be greater in housing than at pasture.

- Cattle housing type and condition may influence the transmission within-herd. Insufficient ventilation in cattle housing is a recognized risk factor in many studies.

- Given the known routes of transmission and the expected contact networks, we would predict that opportunities for cattle-cattle

transmission probably decrease in farm buildings in the following
order: cubicles>pens>byres.

What factors affect cattle-cattle transmission?
- Issues that affect transmission in general will influence within-
  and between-herd transmission regardless. These include the
  nature of within- and between- herd cattle movements and
  contacts, adequate separation, farm fragmentation etc.
- 'Susceptibility' and 'transmission' are intimately linked and some
  or all of the same risk factors (genetic and non-genetic) may
  influence both. Experience suggests that if susceptibility to
  infection can be reduced this would be reflected in reduced
  infectiousness and reduced onward transmission.
- Several studies identify the risk (<20%) of moving infected
  (undetected) animals into a herd.
- Contact networks support transmission. The contact networks
  established by normal cattle movement (local and long-range) will
  influence within- and between-herd spread. Cattle contact
  networks appear to be relatively stable and predictable, although
  highly skewed in that some herds have many contacts, whilst
  others have few or indeed none. The nature of cattle contact
  networks and the management structures (cattle groupings,
  feeding arrangements etc) within the herd will also influence
  transmission dynamics.
- A surprising amount of recorded, and by extrapolation 'unrecorded'
  within- and between-herd cattle movements are of relatively short
  range (<20km in GB).
- Opportunities for contiguous contact at pasture would facilitate
  spread, as would the sharing of housing (cattle B&B etc), resources
  and equipment.
- Advanced or generalized disease within the herd would be expected
  to promote cattle-cattle transmission. It is not known which
  animals transmit the most, although current epidemiological
  evidence and modelling studies are lending support to the concept
  of the 'super-shedder' (or super-spreader/super-excretor) animal.
  The phenomenon of tuberculin test anergy in advanced disease is
  well supported.

- The impact of immune-suppression (including HIV co-infection) on driving the human TB epidemic may have significant resonances in bovine TB epidemiology.
- On occasion, the unwitting import of bovine TB-infected animals to OTF countries has resulted in explosive outbreaks. The factors supporting such infection dynamics are not yet understood. The rate of within- and between-herd spread is determined largely by those factors which affect transmission and possibly susceptibility.
- Early efficient diagnosis and intervention to interrupt transmission should remain the priority for control. However, the efficacy of this measure depends on how early the infections are detected and on how sensitive the test(s), as applied, are. Also, the test interval and/or time to de-restriction may be such as to support further within- and between-herd transmission.

SUSCEPTIBILITY
- There is relatively limited knowledge about the factors which influence susceptibility/resistance to bovine TB and this is an obvious blind-spot in the knowledge and evidence base, especially locally.
- The risk factors which influence susceptibility can be divided broadly into the classical 'nature' (genetics) and 'nurture' (non-genetic or environmental) factors. If these can be identified and quantified, several are likely to be amenable to intervention.
- There are different levels at which susceptibility and resistance act; resistance to infection, resistance to disease, resistance to disease progression and resistance to onward transmission/excretion etc.
- Existing literature and reviews, whilst identifying and discussing appropriate risk factors for susceptibility, as we have attempted here, have been unable to rank such factors. Hence, as identified in our early correspondence on this issue, we have also been unable to rank such factors from the literature.
- Recent genetic studies indicate that there is a heritable genetic component (~20%) which influences bovine TB risk. To the best of our knowledge this is the only susceptibility risk factor that has been estimated and only in specific populations. This makes ranking other factors unachievable at present. By definition, the remaining ~80% is attributable to non-genetic effects, including

'environment' (nutrition, concurrent disease, husbandry, exposure to pathogen etc).

- It is biologically untenable that genetic variation in both the host and the pathogen does not influence the outcome of exposure, detection, infection, disease and infectivity. The heritability disclosed in susceptibility to disease and, indeed, to the tuberculin test itself, indicates that there is exploitable genetic variation in risk. It should be possible to improve the resistance of the national herd by selective breeding and to understand better the genetic variation that underpins susceptibility or resistance.
- Immune-suppression, due to concurrent infection, nutrition, physiological or metabolic state, appears to be a significant and underestimated risk.

RISK FACTORS

- Risk factors will vary across regions due to factors such as differing farm structures, farm management practices, bovine TB control and eradication programmes, regional TB incidences, wildlife densities and the relative importance of specific risk factors within individual areas.
- Risk factors may operate at different scales; regional-level, herd-level and animal-level. The risk of a bovine TB episode is accepted to vary between herds with some herds experiencing multiple breakdowns over time, whilst others appear to remain free of infection.
- The nature of bovine TB breakdowns is not uniform; they can be classified as 'sporadic', 'persistent', 'recurrent' etc and the literature supports the view that different risk factors are likely to apply, almost on a case-by-case basis. However, some risk factors have tended to emerge in several studies and we have discussed these.
- Previous case-control studies have identified a number of risk factors associated with bovine TB herd breakdowns, including the purchase of cattle, the occurrence of bovine TB in contiguous herds and/or the surrounding area and herd size.
- Other factors identified in some studies include farm and herd management practices such as the spreading of slurry, the use of certain housing types, farms having multiple premises and the use of silage clamps etc.

- In general, the most consistently identified risk factors are biologically plausible and consistent with known transmission routes involving cattle-cattle and badger-cattle pathways.
- Whilst many of the general risk factors for the introduction and spread of bovine TB have been identified, less is known about the practical measures that farmers could reasonably take to minimize their risk and the possible impact of common biosecurity practices.
- Although of relatively high specificity, there is increasing evidence from recent studies that the tuberculin test has substantially lower sensitivity (Se ~56%) than previously estimated (see Review 3). This moderate sensitivity is only problematic if it can be shown that cattle missed by the tuberculin test are, or become, infectious and if herds are susceptible to infection from outside the herd. It would be unwise to assume that a tuberculin test-negative animal is not infected. However, it is far from clear that such animals are infectious under field conditions. Abattoir inspection also has high specificity but relatively low sensitivity (Se ~25%) in recent latent class analyses of NI field data (submitted).
- Lower sensitivity obviously infers that bovine TB prevalence in cattle is higher than estimated by current surveillance. This implies that substantial numbers of undetected infections persist in cattle herds and could be a source of infection within- and between-herd and to local wildlife.
- The limited tuberculin test sensitivity is also likely to have contributed to incorrect extrapolation of the impact of several risk factors, such as cattle movement in some studies.
- To compound the above, multiple unreported local movements and contacts are described between farms in several studies and are recognised as a factor in underestimating the role of contact and movements, particularly over short range.

Herd-level risk factors most consistently identified*
Cattle movement (estimated to contribute <20% in some GB and ROI studies)
Occurrence of TB on contiguous premises and/or level of TB in surrounding areas
Herd size

Table 2: The most consistently identified herd-level risk factors for bovine TB.

Other herd-level risk factors identified in some studies
Contact with contiguous cattle
Indicators of badger density/activity
Sourcing cattle from herds with TB history
Providing cattle feed inside housing
Use of multiple premises
Housing type
Herd type
Farmland habitat
Fertiliser usage
Mineral deficiencies (selenium)
Use of silage clamps
Rotational grazing

Table 3: Other herd-level risks identified in some studies.

Animal-level risk factors most commonly identified*:
Concurrent disease(s)
Host genetic variation
Immune suppression
Age
Cattle behaviour

Table 4: The most consistently identified animal-level risk factors for bovine TB.

Other animal-level risk factors identified in some studies
Gender
Breed
Body Condition Score (BCS)
Physiological state
Cattle enterprise type
Nutrition
Therapeutics
Climate and weather
Pathogen variation

Table 5: Other animal-level factors identified in some studies.

- *It is important to note that epidemiological studies may differ in the variables examined, the exact measures used (in relation to the association with badgers etc) and the study size and power. Therefore, not all risk factors would be expected to be identified equally across all studies.

- Those risk factors which tend to converge from disparate studies would support the currently hypothesized sources of infection and routes of transmission. Having reviewed the literature and evidence, including their own, the Independent Scientific Group on Bovine TB (ISG) concluded that, whilst some risk factors were evident in most studies, it was not possible to prescribe those that would apply to any specific study, neither was it possible to rank such factors.
- It is important to remember that risk factors identified in herd- and animal-level studies are *associated* with the measured outcome and are not necessarily causal.

METHODS TO MINIMISE CATTLE-CATTLE TRANSMISSION

- Early diagnosis and intervention to interrupt transmission should remain the priority for control. Whilst not the main focus of this review, the effectiveness of testing and removal of infectious animals will impact on transmission.
- However, the efficacy of this measure depends on; how early the infections are detected, how sensitive the test(s) are in practice and other variables, including inter-operator characteristics. Also, the test interval and/or time to de-restriction may be such as to support ongoing transmission.
- Although local data are currently limited, and there is no absolute guarantee that suggestions would work, there are cost-effective, biologically-plausible measures which should be seriously considered to minimize the risks from direct and indirect sources.
- Hiring and sharing of bulls should be discouraged. Artificial insemination (AI) should prevent purchased animals bringing disease on farm. Replacements should only be acquired from trusted sources whose bovine TB status is known and current.
- TB incidence increases with age, so it may be wise to purchase cattle at as young an age as farm management would sustain.
- Milk from reactors and inconclusives (including those awaiting slaughter) should not be fed to calves and the timely culling of dairy cows with high somatic cell counts and which may have TB mastitis has been advocated.
- Purchased animals should be isolated for 3-4 weeks and pre- or post-movement testing should be seriously considered.
- Secure fencing (3m high hawthorn hedge, double fencing 3m apart or electric fencing) and physical barriers to between-herd contact (growing forage rather than grazing) should be encouraged and enforced.
- Improved biosecurity, approaching 'fortress farming' (Grove-White 2004), including secure perimeter fencing offers a cost-effective means of controlling numerous infectious diseases (brucellosis, IBR, salmonellosis, ringworm, lice, BVD etc) and not just bovine TB.
- Practical management measures which should be considered include; improved farm-specific outbreak investigation, risk assessment and biosecurity advice, avoidance of shared

housing/equipment etc. Optimal ventilation (mechanically forced if necessary) and possibly indoor UV irradiators and de-humidifiers could be evaluated for cattle buildings.

- Further reduce the extent of within- and between-herd movements. Pre- and post-movement testing, movement restriction duration and adequate quarantine should be reconsidered. Where test inconclusives alone are disclosed, consider their restriction. The whole herd should be restricted where reactors and inconclusives are disclosed.

- Total or partial herd depopulation may be justifiable in some circumstances.

- Potentially mitigating the risks posed at the cattle-badger interface is discussed in Review 2 (bTB B-C review).

- Direct transmission routes would appear to be more important than indirect routes. However, their relative importance depends on the local spatial and temporal circumstances and the scale being considered.

- Regionalisation, based on TB prevalence and wildlife risk, has been deployed successfully in the New Zealand and Australian programmes, allowing more effective implementation of region- and risk-based control objectives and is believed to have contributed to a reduction in prevalence in cattle herds. Argentina is also considering the merits (or otherwise) of regionalized control. The ISG also promoted the concept of restricting movement of animals (other than to slaughter) only to herds of similar disease status and subject to pre-movement testing.

- Efforts should be taken to minimize the production of potentially-infectious aerosols on farm premises.

- Once developed, evaluated and validated, deployment of an effective cattle vaccine should further limit cattle-cattle transmission.

OTHER FACTORS LIKELY TO COMMEND FURTHER BENEFICIAL STUDY

Cattle-Cattle Transmission

- The distribution of reactors by herd is highly skewed; tuberculin reactors tend to cluster within herds. This also infers that a relatively small number of herds contribute disproportionately to the overall number of reactors. It implies that there are likely to be converging risk factors for those herds, which support cattle-cattle transmission and which may include susceptibility risks. It implies that targeting of appropriate control measures should lead to a disproportional benefit. Targeted analysis and control of the relatively few herds which provide disproportionately more reactors should be cost-beneficial.

- Targeting investigation and control at those cattle that are most highly connected in contact networks should also be beneficial. In addition, there should be merit in deployment of alternative tests, such as humoral immunity tests, to investigate persistently-infected herds (see Review 3).

- There should be merit in investigating the actual number of generalized bovine TB cases disclosed at abattoir and their tuberculin test histories. These animals may constitute the burden of highly infectious cases within-herd. It would be valuable to re-examine the prevalence of generalized bovine TB recorded at abattoir in NI. Do such animals tend to come from particular herd types? Are they implicated in herds with disproportionate reactor numbers etc?

- In GB, significant progress is being made in interrogating the large, complex, national datasets available (e.g. CTS, RADAR and VetNet) to understand better the epidemiology of bovine TB in cattle. A general recommendation is to continue with such work in the local context as the epidemiological information generated is of direct relevance to the disease control programme.

- We recommend investigating transmission chains in cattle contact networks by integrating genetic and epidemiological data. Comprehensive epidemiological investigation of disease outbreaks should be summarized and evaluated. Analysis of cattle movement and test history data alone may not completely capture transmission dynamics and may lead to an underestimation of the

potential for disease spread, especially the extent of local spread via cattle. Investigating the spatial and temporal pattern of disease clusters (including *M. bovis* genotype clusters) should help to identify those local risk factors which contribute most to the ongoing transmission producing the cluster.

- Analysis of multi-reactor herds, which have received an index case via long range movement and where that case yields a bovine TB strain remote from its known home-range, should be particularly informative in evaluating onward within- and between-herd spread and the involvement of local wildlife.

- Re-evaluate the deployment of severe interpretation of the tuberculin test. It has higher sensitivity than standard interpretation but may have lower specificity. This would require an estimate of the cost/benefit of removing additional reactors. Once performance characteristics are more settled, consider the rational deployment of the interferon (IFN) blood test.

- Collect data on cattle interactions using proximity meters and transponders, especially for within- and between-herd movements and contacts.

- We were unable to locate a recent systematic review which summarized the changes in UK and Ireland farming practices and their likely impact on the bovine TB epidemic. Although not addressing this specific question, previous ROI studies found no reproducible difference in TB risk by cattle enterprise type.

- The ISG advised that enhanced cattle-based measures should be sufficient to control TB, in GB at least. The SGM (2008) were not so convinced and were concerned that TB might be self-sustaining in badgers in some areas. They recommend investigation of TB in badgers in areas where there are no cattle (or at least no cattle TB) to address this point.

Susceptibility

- Less well studied and understood is the impact of susceptibility risk factors, including genetic and non-genetic risks; nutrition, inter-current disease(s) etc. These clearly merit further study.

- Sufficiently powered local studies to investigate risks (susceptibility), including co-infections, nutrition, physiology, metabolism etc are merited. Exposure, infection, disease and

infectiousness are intimately influenced by susceptibility. It may be possible to reduce both genetic- and non-genetic susceptibility.
- Genetic studies are beginning to unravel the genetic basis for resistance or susceptibility to infection. There are further opportunities to explore.
- Setting aside the political and trade-based positions, an effective cattle vaccine should reduce cattle-cattle transmission.

Risk Factors
- Sufficiently powered local studies to investigate risk factors for transmission and clustering and to suggest appropriate mitigation should be considered. The DARD Biosecurity Study should inform such considerations.

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MATERIALS AND METHODS

We searched systematically on-line resources (PubMed, Web of Science) to find appropriate peer-reviewed literature and relied on previously identified key publications. Literature was accessed until October 2011, inclusive. We purposefully selected publications that were judged most relevant for the review, with a preference for high-quality systematic reviews. Publications in the last 10 years were favoured, but we did not exclude highly regarded older publications.

Searches were conducted using combinations of the following key words: "bovine", "tuberculosis", "transmission", "risk", "susceptibility". In addition, open-access DEFRA R&D project web-pages were searched.

PubMed (http://www.ncbi.nlm.nih.gov/pubmed) comprises >20 million citations for biomedical literature from MEDLINE, life science journals, and online books. DEFRA web-pages on bovine TB, Phillips and others (2003) and the Final Report of the Independent Scientific Group (Bourne and others 2007), '*Bovine TB: the Scientific Evidence*' were referenced throughout.

The literature on transmission, risk factors and susceptibility is immense. Consequently, this review is sizeable and is effectively 3 reviews! Even in 1935 the literature on human TB was so immense that recommendations for reading were considered *"…futile"* (Halloran 1994)!

We recognize the difficult in providing 'strong advice from weak evidence' on occasions.

The following relevant areas have been discussed:
- Bovine TB epidemiology
- Human TB epidemiology
- Bovine TB pathogenesis
- Direct and indirect transmission
- Animal movements and contacts
- Herd-level risk factors
- Susceptibility

1. INTRODUCTION

The knowledge base that exists on human TB (*Mycobacterium tuberculosis*) provides important insights into the natural history of bovine TB (*Mycobacterium bovis*).

1.1 "THE GREAT WHITE PLAGUE" - HUMAN TB (*Mycobacterium tuberculosis*)

1.1.1 The Natural History Of Human TB

"…the captain of all these men of death."
John Bunyan (1628-1688)

Human TB has co-existed with man for millennia and has proven to be difficult, if not actually impossible, to eradicate. Even today, between a third and a half of the global population (>2 billion people) have a positive tuberculin skin test for human TB and are thought to be harbouring the bacterium (Burgos and Pym 2002, Russell and others 2010). Human TB is the seventh biggest killer on earth (1.45M deaths in 2010) and is the leading fatal infectious disease, although these deaths are preventable. In 2010 there were 8.8M new active TB cases and >4,000 people died every day. However, only about 30% of exposed people become infected (Manabe and Bishai 2000).

The economic cost of TB-related deaths (including HIV co-infection) in Sub-Saharan Africa from 2006 to 2015 is ~US$ 519 billion when there is no effective TB treatment as prescribed by the WHO *Stop TB* Strategy (WHO 2007). Human TB is the leading killer of HIV+ individuals and 1.4M TB cases were co-infected with HIV (Diedrich and Flynn 2011). Human TB is still considered to be 'a social disease'; a disease of minorities, inextricably linked to poverty, overcrowding and malnutrition, affecting mostly young people in their otherwise most productive years (Anon. 2011, WHO 2011, Raviglione and Krech 2011, Lawn and Zumla 2011).

In the 'standard model' of human TB biology (Dye and Williams 2010) the pathogen (*M. tuberculosis*) is considered as a single, invariant entity and the response to lung infection is either; fast (primary progression) or slow transition (via 'latency') from infection to infectious/non-infectious disease. Although this oversimplifies human TB biology, it makes epidemiology easier and facilitates management.

Intriguingly, even in hyper-endemic regions ~20% of individuals show no signs of infection and retain negative tuberculin tests throughout their lives, despite repeated exposure to TB bacteria. Since it would be highly unlikely that all such individuals were immune impaired, a substantial proportion of these individuals are likely to be **naturally resistant to infection** (Alcais and others 2005, see later).

Population dynamics and human TB control are likely to be influenced by the following observations (Dye and Williams 2010) and exacerbated by emerging antibiotic resistance and treatment non-compliance:

- Some infected cases are at high risk of developing active TB
- Some patients transmit more infections than others
- Epidemics of some chronic diseases exacerbate human TB
- Some strains of *M. tuberculosis* are more transmissible
- Some human genetic variation is linked to TB susceptibility
- Most people live in high-density urban settings
- Young people are rejuvenating TB epidemics
- Populations are ageing
- Current diagnostic tests have limited sensitivity and specificity

The concepts and clear definition of terms such as *exposed, infected, infectious, diseased* etc are important in TB epidemiology, especially when recent research supports the view that *M. tuberculosis* infection has a wide spectrum of outcomes (Barry and others 2009), although it would be reasonable to expect some level of overlap between these phenotypes. For the avoidance of doubt we have used the following working definitions:

- **Infected** - an individual who has been colonized by *M. tuberculosis*. They may or may not be symptomatic and detectable with current tests and procedures. Most individuals are in this '*established but contained infection*' class.
- **Infectious** – an infected individual, capable of maintaining the natural history of TB by transmitting infection to other susceptibles.
- **Diseased** – an infected individual who has progressed to show detectable signs of TB disease when tested. Although data are sparse, it would seem reasonable to assume that individuals with advanced disease would be relatively more infectious. However, the literature suggests that 'diseased' does not necessarily imply 'infectious'.

Furthermore, only a small proportion of infected people progress rapidly to active TB (within 5 years) and, sooner or later, are defined as **infectious;** if they have moderate/severe pulmonary TB, or are defined as **non-infectious;** (or relatively non-infectious) if they have mild pulmonary or non-pulmonary/extra-pulmonary TB. Amongst those infected, 10% develop active TB (5% within 5 years and 5% beyond 5 years) and about half will be infectious and half relatively non-infectious. The remainder (>90%) are considered non-infectious, or at least significantly less infectious than sputum-positive cases, and innately immune to developing disease, but importantly they are susceptible to infection. To clarify, these individuals have been **infected** by *M. tuberculosis* but have not progressed to show detectable signs of **disease**.

The literature is somewhat ambigious on the relative infectivity of latent TB cases. Some authors conclude that such individuals are not infectious to other susceptible contacts (no detectable TB bacteria in the airways) (Colijn and others 2007, Ehlers 2009) whereas others imply that such individuals are "significantly less infectious". One of the accepted 'certainties' of life, along with death and taxes, is that individuals infected with *M. tuberculosis* carry the organism in a 'latent' state to the grave ("…o*nce infected, always infected*"), with a relatively small and diminishing risk of reactivating to active TB years or even decades later (Nardell and Wallis 2006, Dye 2006, Mack and others 2009, Barry and others 2009, Huynh and others 2011, Dye and Williams 2010). The current consensus view of chronic persistence of *M. tuberculosis* is that of *"…a lazy, occasionally recrudescent* Mycobacterium *within a dynamic granulomatous lesion"* (Ehlers 2010).

Latently-infected individuals do not show overt clinical signs; they have either a positive skin test or evidence of old pulmonary lesions on X-ray. Although such humans are considered central to the propagation of human TB in parts of the world where endemic TB disease is low, through reactivation of their latent infection as a result of old age or immune-suppression (Dye and Williams 2010), recent studies confirm the benefit of targeting preventive therapy to those most recently infected through contact with infectious cases, rather than targeting just "*anyone with latent TB*" (Borgdorff and others 2011).

Striking variation in the host response to exposure to these closely-related bacteria and striking geographical distribution (phylo-geography) of pathogen families have been identified recently (Gagneux and Small 2007). Despite sharing >99.95% nucleotide identity these bacteria resolve into a series of 'ecotypes', each with its own (non-absolute) host-

preference (Smith and others 2006a, Whelan and others 2010). This suggests that host and pathogen genetic variation influences the outcome of infection (see later). Infection is increasingly viewed as a continuum of host-pathogen interactions, rather than as the classical binary outcome of active versus latent TB (Barry and others 2009, Young and others 2009) and the understanding of latent infection is being revised constantly (Sridhar and others 2011).

Latently-infected individuals may also be re-infected with the same or a different strain, their primary infection conferring only partial immunity (<35%). Latent TB would be identified by a positive tuberculin test or, more recently, by a positive IFN test. Chest X-rays, although sensitive for pulmonary TB, are not sufficiently specific. If an acute infection, definitive diagnosis requires isolation or identification of *M. tuberculosis* in sputum or other samples and finding acid-fast bacteria (AFB) in a sputum smear equates to infectiousness (severe pulmonary disease). These are the principal, but not exclusive, sources of infection. On average, each infectious case survives ~3 years, with or without treatment (Tiemersma and others 2011), and infects ~15 others annually (WHO 2007). In a study of household and community contacts in Uganda, the 'secondary attack rate' for infection and disease, respectively, was estimated at 47.4 (95%CI 44.3-50.6) and 3.0 (95% CI 2.2-3.8) (Whalen and others 2011). So, more people become infected than ever develop overt disease. In addition, based on tuberculin test reversion data, Nardell and Wallis (2006) make the case for transient acute TB infection.

22 high-burden countries contribute >80% of active human TB cases. Large gaps still remain in our understanding of pathogen biology and there are tools missing from both the prevention and control toolboxes. For example, there is no simple test to determine whether an individual is containing infection (non-infectious) or progressing to active (infectious) disease. It is increasingly evident that progression to disease is determined locally within the lungs, at the individual lesion (granuloma) scale (Russell and others 2010) ie. TB lesions are not synchronized and appear to develop independently of each other (Ehlers 2010).

Infection with *M. tuberculosis* follows a pattern that has been well-established in various animal models (Apt 2011, Philips and Ernst 2011). Infectious bacilli are inhaled as small 'droplet nuclei' (as opposed to large droplet nuclei in influenza) that have been exhaled as an aerosol into the atmosphere from an infectious case. They are small enough to remain

airborne for short periods (see later) and the minimum infectious dose is very low, ranging from one bacterium upwards. The related and infamous mycobacterial pathogen (*Mycobacterium leprae*) that causes human leprosy is also spread via aerosol and the respiratory route, contrary to traditional belief (Alter and others 2011).

Human TB often occurs in household contacts of active TB cases, although, until the advent of TB strain typing, it was not clear whether this represented within-household transmission or exposure to shared risk factors in the environment (community). These risk factors probably differ between studies and regions. A recent molecular epidemiology study in Peru indicated that a proportion of non-index household cases were actually infected in the community rather than in the household. The average interval between household cases was ~3.5 years and they estimated that immunity acquired from prior exposure reduced the risk of infection by <35% (Brooks-Pollock and others 2011, Cohen and others 2011).

Furthermore, molecular typing studies in San Francisco, New York, Amsterdam and Wisconsin (Malakmadze and others 2005) demonstrated that ongoing and recent transmission, as opposed to reactivation of TB, was much higher than previously accepted (at ~10%, Burgos and Pym 2002). The literature supports the view that human TB is exclusively an airborne respiratory disease transmitted via aerosol, which mostly requires prolonged close, direct contact with an infectious case (van Soolingen and others 1999). However, as demonstrated clearly by molecular studies, on occasion it can be transmitted via only transient and non-intimate exposure (van Soolingen and others 1999, Golub and others 2001, Malakmadze and others 2005, Colijn and others 2007).

Therefore, in some human populations most people are infected with *M. tuberculosis*, but most do not develop obvious disease. The bacterium is inhaled and settles in the lungs, before dispersing fairly rapidly to the associated lymph nodes and possibly beyond (Neyrolles and others 2006, Bold and Ernst 2009, Ehlers 2010). Normally, the host immune response is able to prevent further spread by encapsulating the bacteria in a structure of immune cells in the lung known as a **granuloma**, the enigmatic hallmark of TB (Reece and Kaufmann 2011) and where the bacilli can persist for decades without causing disease. It is also possible that viable TB bacteria persist in only a fraction of individuals with a persisting immune response to TB antigens (Ehlers 2010). However, simple formation of the granuloma is no longer viewed as enough to control infection and it is a common misconception that granulomas are

uniformly protective and that the mycobacteria are effectively "walled-off" (Ehlers 2010). Only optimal immune function can contain or eliminate the pathogen (Diedrich and Flynn 2011).

The most common form of human TB in adults is lung (pulmonary) TB. Mycobacterial replication and the host granulomatous response both damage the lungs and can result in cavitation and necrosis, which allows the pathogen to enter the airways and be expelled to facilitate spread and secondary infection. If left untreated by chemotherapy ~70% of *diseased* individuals would die (Tiemersma and others 2011).

Active, infectious human TB is treatable with a cocktail of antibiotics which must be taken over a protracted period and do have some unpleasant side-effects. However, most active TB is rendered non-infectious within a few weeks of initiating chemotherapy. Latent TB is contained by mono-drug antibiotic (isoniazid) therapy (Ehlers 2010), although there are justifiable concerns about the development of multi-drug resistant (MDR), extremely drug resistant (XDR) and even totally drug resistant (TDR) *M. tuberculosis*, which is influenced by patient non-compliance with chemotherapy. Recent studies indicated that the bacteria in 'dormant' TB lesions were probably as metabolically-active as those in active TB (Ford and others 2011), which may help explain the emergence of resistance to isoniazid. A recent study suggests that the bacteria may even alternate between active and latent states (Cardona and Ivanyi 2011).

Human TB infection is detected by the tuberculin skin test. Studies show that tuberculin test reactivity increases with age (a proxy for cumulative exposure), up to a maximum, followed by a rapid decline. This indicates that the most susceptible individuals develop human TB relatively soon after infection and those with a stronger immune system progressively eliminate persisting bacteria (Rieder 2011).

A live, laboratory-attenuated vaccine (*M. bovis* BCG) has been in use against *M. tuberculosis* for nearly a century. It is noteworthy that it was derived from a virulent French *M. bovis* field isolate and billions of doses have safely been administered. Although BCG provides solid protection in young children, especially against the worst forms of generalized human TB (miliary TB), its efficacy against pulmonary human TB in adults is poor. Regrettably, the bulk of the global human TB burden is pulmonary TB, so the contribution of BCG to global TB control remains controversial (Orme 2010). The protection afforded by BCG vaccination varies significantly in different studies, up to a maximum of 80% protection in GB trials, which was maintained for ~10 years, before a

dramatic drop in protection within a further ~5 years (Rieder 2011).
Substantial efforts and resources are being directed towards development
of a replacement or augmented vaccine, several of which are based on
BCG, but BCG itself is currently the accepted gold standard.

Even before direct human intervention (BCG vaccination and
chemotherapy), mortality from *M. tuberculosis* was falling rapidly in
industrialized countries, a decline ascribed to improved living conditions,
improved nutrition, improved sanitation, fewer concurrent infections and
reduced overcrowding (risk factors - see later). A current hypothesis
suggests that the 1918 'Spanish' influenza pandemic accelerated the
decline in human TB by removing infected and infectious cases of human
TB (Noymer 2011).

Darwinian natural selection and/or neutral population effects
(Spurgin and Richardson 2011) favouring host resistance to infectious
disease are suggested as major drivers in shaping human genetic
diversity and evolution. Increased host genetic resistance may also have
contributed to the observed decline in human TB (Lipsitch and Sousa
2002). *M. tuberculosis* is currently facing both old (host immunity,
human demography) and new selective pressures (HIV and antibiotics,
Brites and Gagneux 2011). Whilst *M. tuberculosis* is currently described
as undergoing 'relaxed selection' there is some evidence of positive host
selection acting on TB antigens that interact with human T-cells, where
natural selection acts to maintain the amino acid sequences of such
proteins (Comas and others 2010, Hershberg and Petrov 2010). These
'signals of selection' may be easier to detect in bacterial genomes than
they are in mammalian genomes (Coop and others 2009).

The probability of developing a new infection, or of reactivating an old
dormant infection, depends on the competence of the host immune
system, which in turn depends on a range of factors including the
nutritional state of the host (see later) ie. the probability of developing
disease, if infected, increases when nutrition is poor (Halloran 1994).
This probability of developing disease, particularly pulmonary disease, is
crucial to the natural history of the tubercle bacillus because
transmission is so tightly linked to disease causation. Individuals with
open cavitations in the lung are the primary source of infection for
susceptible individuals (Wallace and others 2009).

M. tuberculosis has developed the ability to escape from the
granuloma, creating the opportunity for aerosol transmission. Increased
ability of the host to resist disease should help reduce the number of new
infections. So, in principle, it is possible for everyone to be infected, but if

only very few develop disease, there would be little or no ongoing transmission. In addition, recent data from Canada suggest that *M. tuberculosis* can persist in human populations at very low levels for very long periods of time, only developing into epidemics when host conditions are more favourable. For example, infections acquired by natives from European settlers ~1710 only became common in the 1800s and 1900s (Pepperell and others 2011a, Pepperell and others 2011b).

1.2. BOVINE TB (*Mycobacterium bovis*)

Bovine TB is a chronic disease of animals caused by infection with the slow-growing, obligate intracellular bacterium *Mycobacterium bovis* (Bourne and others 2007, OIE 2009). This highly-adapted and 'successful' pathogen has a world-wide distribution and in several countries bovine TB remains a major, costly infectious disease of cattle and other domesticated, feral and wild animal populations, including; badgers, possums, deer, goats, sheep, camelids etc (Pollock and Neill 2002, Mathews and others 2006, Carslake and others 2011).

Bovine TB is an OIE (World Organisation for Animal Health) listed (formerly List B) disease: *"…one that is considered to be of socioeconomic or public health importance within countries and of significance to the international trade of animals and animal products"* (OIE 2011, Cousins and Roberts 2001).

Bovine TB affects cattle health, impacts negatively on profitability and trade and can decimate years of genetic improvement towards desirable production traits (Boland and others 2009). It also impacts negatively on the welfare of affected farming families (Farm Crisis Network 2009). Although effectively controlled by herd testing, pasteurization, meat inspection, health surveillance and BCG vaccination, transmission to humans can occur and is still considered a public health risk (Moda and others 1996, Smith and others 2004, de la Rua-Domenech 2005), although some more recent opinion considers this risk to be negligible (Torgerson and Torgerson 2010). Hence, bovine TB control is currently more concerned with trade implications.

Despite sustained and costly implementation of eradication programmes since the 1950s bovine TB has not been eradicated from either the UK or ROI. Indeed, there has been a sustained and largely unexplained increase over the last 20 years in parts of the UK (Gilbert and others 2005). Consequently, bovine TB is the most complex and difficult multi-species endemic disease currently facing government, the

veterinary profession and the farming industry in the UK and ROI (Reynolds 2006, More and Good 2006).

Whilst it is important to view bovine TB as an infectious disease which requires preventive as well as control measures, *M. bovis* infection in cattle now rarely appears to present as clinical disease. More commonly it appears as apparently healthy animals responding to an immunological test based on tuberculin, an entirely different scenario to that which existed when control programmes were first introduced (Collins 2006).

The problem of bovine TB in the UK is exceptionally complicated and the relationship between evidence, uncertainty and risk is difficult to communicate (Krebs 2011). It is recognised as a very significant policy challenge and continues to be, almost inevitably, highly politicized (Spencer 2011). Key to understanding bovine TB epidemiology is the relationship between infection and disease (TB) and the relationship between disease and transmission. Risk factors (biological, behavioural, environmental or genetic; see later) are known to influence both transmission and susceptibility.

1.2.1. Bovine TB – A Global View

Bovine TB is distributed throughout the world and has been reported on every continent (except Antarctica). Basically, where there are cattle there is, or has been, bovine TB (Smith and others 2006b), with the notable exception of those countries that have eradicated bovine TB using a test-and-slaughter policy to remove infected cattle, such as Australia, some Caribbean islands (including Cuba) and parts of South America. In other countries, notably the USA, Canada, South Africa and most of the European Union (EU), with the exception of the UK and Republic of Ireland (ROI), bovine TB has been reduced to negligible levels, although sporadic, and sometimes persistent and damaging outbreaks associated with the import of infected cattle or the existence of wildlife reservoirs have been reported (Smith and others 2006b, Reviriego Gordejo and Vermeersch 2006).

In New Zealand (NZ), the control of bovine TB in cattle and farmed deer has proven difficult, and a wildlife reservoir in brush-tailed possums has been implicated. Their control programme targets bovine TB in cattle and in local wildlife. Recent evidence indicates that NZ is now making significant progress towards eradication, although the epidemiology is different to that in the UK and ROI. The combination of cattle surveillance and controlling bovine TB in local wildlife has also limited a

persistent outbreak associated with white-tailed deer in Michigan (USA). The suspected wildlife reservoir in Michigan, Canada and the Kruger National Park in South Africa is itself of economic/social value. Social issues and public approval could also be important factors in eliminating the disease, and has strong resonance for the UK, where the badger, a protected species, has been implicated as a reservoir host (Smith and others 2006b).

1.2.2. Bovine TB In The British Isles

In GB and other industrialized countries, initial attempts to control bovine TB began in the late 1800s, following the discovery of a link between some human TB cases and consumption of unpasteurized cows' milk (Smith and others 2006b, Woods 2011). Control measures (clinical examination of cattle herds, bacteriological examination of milk and voluntary slaughter of tuberculous dairy cows), were designed to protect the public rather than eliminate the disease, the latter started in 1935 with a government-introduced voluntary test-attestation scheme to cull cattle reacting to the tuberculin test. This became a compulsory area eradication scheme in GB in the 1950s and successfully reduced the annual number and incidence of test reactor cattle from nearly 15,000 in 1961 to 569 in 1982 (based on Smith and others 2006b). By the mid-1960s less than ten clinical cases of bovine TB were being detected and culled every year. Nowadays, it is almost unheard of for bovine TB to be detected in cattle in GB by clinical examination.

The success of the test and slaughter scheme in GB was replicated in most EU countries, particularly those with no significant wildlife reservoir of *M. bovis*. Sadly, this dramatic progress stalled in the mid-1980s and GB now has one of the highest animal and herd incidences of bovine TB in the EU, despite an intensive test-and-slaughter programme to minimise cattle-cattle transmission. Since the mid-1980s the number of bovine TB breakdowns in GB has risen at an annual rate of ~16%. In 2005 almost 30,000 cattle were slaughtered and 7.8% of herds tested disclosed reactors.

In NI, bovine TB control has largely followed the GB experience. Disease control commenced in 1935 with the slaughter of clinically-affected cattle, but it was only after the introduction of a compulsory tuberculin-testing scheme in 1959 that a significant reduction in the incidence of bovine TB was observed. In the 1970s and mid-1980s the incidence of the disease fluctuated and was on an increasing trajectory in the late 1990s, which was then confounded by the 2001 FMD outbreak.

The highest recent prevalence was recorded in 2002. This was followed by a ~50% reduction associated with the enhanced application of purely cattle-based measures.

In the ROI, a bovine TB eradication programme commenced in 1950, becoming compulsory by 1962 (Good 2006). As in GB, progress was made initially; in the late 1950s and early 1960s the annual number of reactors was >100,000 but by the mid-1960s the reactor number was only ~20,000. Unlike in the UK, since the mid-1960s the annual number of reactors has remained fairly static at 20,000-40,000 reactors *per annum* despite an exhaustive cattle-based programme and other control measures aimed at reducing cattle-cattle and badger-cattle transmission.

Despite the failure of the test and slaughter scheme to eradicate bovine TB from the British Isles, regular herd testing and pasteurization of milk has significantly reduced the risk of *M. bovis* spillover to humans (Torgerson and Torgerson 2010). Routine herd testing ensures that tubercular lesions of the udder are now rarely seen in milking cows (Liebana and others 2008). Compared to *M. tuberculosis*, the number of *M. bovis* isolates from humans in GB has been gradually falling for the past thirty years from ~100 cases in the 1970s to ~30 cases *per* year for the past five years, most of which are either acquired abroad or are reactivations of latent infection acquired pre-pasteurization. The epidemic of bovine TB in British cattle is currently having little impact on human health (Smith and others 2006b).

1.2.3. Bovine TB in Northern Ireland

NI is subject to annual tuberculin testing using the approved single intra-dermal comparative tuberculin test (SICTT); the bovine TB tuberculin 'skin-test' (NIAO 2009). The annual (2010) herd and animal incidence were approximately 5.07% and 0.40%, respectively (Department of Agriculture and Rural Development, DARD 2011) and the number of <u>a</u>nimals positive, *per* <u>t</u>housand tuberculin tests (APT), was 2.74, significantly lower than the recent 2002 peak when herd and animal incidence were approximately 9.94% and 0.91%, respectively, with APT of 6.19.

The historic existence of small, fragmented farms, the strong reliance on rented pasture (conacre), the high level of cattle movement between and within herds and an infectious reservoir in wildlife are believed to contribute to the maintenance and spread of bovine TB in NI (Abernethy and others 2006) where control presents a significant burden to the NI

taxpayer, with testing and compensation costing £199M (1996-2006, NIAO 2009).

1.3   What Do Pathogenesis Studies Reveal About Bovine TB Biology?

Pathogenesis is the process or mechanism of disease (Neill and others 1994, Cassidy 2006). A major problem for bovine TB control is that, unlike many infectious diseases, infected cases frequently show no outward clinical signs. Having a better understanding of bovine TB pathogenesis, and being able to compare and contrast with human TB, informs the debate on the most probable route(s) of infection and on the likely infectiousness of infected cattle.

A general impression from the literature strongly supports a widespread assumption that the basic pathogenic mechanisms in bovine TB and human TB are essentially the same (de la Rua-Domenech and others 2006, Pollock and others 2006). This is supported by unusually highly conserved genome sequence identity between the bacteria that cause TB in a range of animals (>99.95%, Smith and others 2006b). However, the structural and expressed genomic differences that separate *M. tuberculosis* and *M. bovis* lineages (Garnier and others 2003, Hewinson and others 2006) presumably contribute to the observed differences in host range and pathogenesis (Whelan and others 2010). Our understanding of pathogenesis and immunity is constantly being revised and updated due to technology developments. However, pathogenesis research has rather tended to focus on the differences rather than on the similarities.

The distribution of lesions in TB-affected cattle, humans and laboratory animals indicates the dominance of the respiratory tract as the major route of infection, raising questions about the role of the upper respiratory tract, tonsils and dorsal lung in pathogenesis and transmission (Cassidy 2006). The predominant distribution of TB lesions in the respiratory tract and associated lymph nodes of naturally-infected cattle infers that infection occurs following inhalation of airborne bacteria. In calves, the minimum dose required to establish infection by the respiratory tract is ~1,000-fold less than that required to establish infection via the oral route (Collins and Grange 1983). More recently, direct inoculation and 'in-contact' infection resulted in lesions largely confined to this location (Neill and others 1989, Buddle and others 1994, Cassidy and others 1999, Villarreal-Ramos and others 2003, Liebana and others 2008).

Four hundred animals (200 reactors and 200 in-contacts) from 242 farms in 14 counties in western England and Wales were examined (Liebana and others 2008). The mean number of lymph nodes (LNs) with TB-like lesions *per* TB-confirmed case was 1.7 for reactors and 1.5 for in-contacts. Lesions in both were mostly observed in the LNs of the thorax, followed by the head and abdomen, particularly the mediastinal, retropharyngeal and tracheobronchial LNs. Twenty-five reactors had macroscopic lesions in the palatine tonsils. Among TB-confirmed cattle, 27% of reactors and 9% of in-contacts had gross TB-like lesions in the lungs, particularly in the caudal lobes and caseous necrosis and calcification were common features, synonymous with infectiousness, although granuloma typically contained few AFB. However, there is often only one lymph node with macroscopic lesions in reactors and 16% of those lesions were not in thoracic or medial retropharyngeal lymph nodes (Whipple and others 1996, Liebana and others 2008). Indeed, the UK Food Standards Agency (FSA) isolated viable *M. bovis* from 21% of 135 cattle with only one or no lesions detected (FSA 2003).

The main aspects of the pathogenesis of TB in cattle are fairly settled, including the main route of infection, infective dose and incubation period before infectivity. The typical TB-infected animal in the UK is now a 4–5 year-old cow, grazed on summer pasture, probably exposed to intermittent challenge with *M. bovis*, unlike the experimentally-infected calves retained in-house with a controlled environment, ventilation etc (Liebana and others 2008). However, the role that TB-positive cattle with minimal or no observed lesions play in the transmission of infection is far from clear. A recent literature review concluded that inhalation is the most likely and important route of infection in cattle with TB, since lesions in field cases predominantly involved the upper and lower respiratory tract and associated LNs (Neill and others 2005).

1.3.1 Accurate Diagnosis And Surveillance

The epidemiology of bovine TB is highly complex and many of the processes driving the current epidemic are not fully identified and/or observed. Hence, bovine TB is largely *"an unobserved epidemic"*. An important challenge in understanding how, when and where bovine TB transmits to, from and between cattle is that infections are not immediately apparent (DEFRA SE3242). A reduction in testing frequency results in increased prevalence and a reduced ability to detect disease. The ability to control infection is constrained by test sensitivity, the

effectiveness of clearance of infected herds and the effectiveness of movement restrictions. To compound this, a substantial proportion of GB cattle were never tested for bovine TB (Mitchell and others 2006) and there is also a period of reduced reactivity in infected animals following an initial tuberculin test, the precise duration of which has not been determined.

The suggestion to lengthen the inter-test interval and to allow more reactors to accumulate is not well supported in the literature. It is possible that once one cow has been infected with TB, a longer testing interval would allow more time for cattle-cattle transmission within the herd. However, there was no association between a herd having multiple reactors and the testing interval in the GB TB99 data (Bourne and others 2007). It may be argued that opportunities for the disease to be transmitted cattle-cattle are actually greater than those for the transfer of infection badgers-cattle; this implies that transmission between cattle and dissemination through cattle movements could be relatively important (Bourne and others 2007).

1.3.2 Latent Infection

The concept of 'latent' infection (where the pathogen resides long-term in the host and may or may not be detectable) is well supported in human TB epidemiology (Manabe and Bishai 2000), where infection is relatively common and productive (transmitting) infection is rare. Latent TB is now seen as a spectrum of pathogen burden and host immune control (Sridhar and others 2011) and is considered to occur when the host response forces the pathogen into a state of allegedly 'non-replicating persistence' (Flynn and Chan 2001). However, more recent evidence suggests that these interactions are largely pathogen-driven and to its own ends; survival and transmission (Bold and Ernst 2009, Ehlers 2010, Sasindran and Torrelles 2011).

It has been proposed that transmission of TB would be enhanced by the ability to establish latent infections (Brites and Gagneux 2011), an ability which may have evolved as an adaptation for persistence when population densities were low. Reactivation after a long latency period might allow the pathogen to jump whole generations to access new susceptibles. Also, a 'persister' phenotype has recently been described for *M. tuberculosis*, which could allow it to resist the anti-bacterial defences of the host and to adapt to long-term survival within the host (Keren and others 2011) and possibly even the environment.

To what extent latency and reactivation apply to cattle, or other animal populations, is unclear (van Rhijn and others 2008), but these processes have previously been suggested to operate in cattle (Pollock and Neill 2002). The relevance of a latent infection is that it can, given the right circumstances, reactivate to full-blown TB and the physical nature of the pathogen during latency is important because it determines what strategies might contain (including post-exposure vaccination) and more effectively detect it (diagnosis). For example, if it was truly dormant and hidden from the immune system; it might not be detectable by current diagnostics.

From analysis of the size and distribution of lesions in cattle, Francis (1946) claimed that disease exacerbation could occur following periods of lesion dormancy, although relatively poor test sensitivity might explain such observations. The existence of skin or IFN test positive cattle in which no lesions can be detected (Neill and others 1994, Monaghan and others 1994) or that are culture positive in the absence of lesions (Cassidy and others 1999) would support the concept of latency in cattle.

In addition, the evidence base on the longevity of memory immune responses to *M. bovis* exposure in cattle does not appear to be settled and has implications for the interpretation of tuberculin tests and the definition of latency (Gupta and others 2011), such as; does the immune response to exposure fade over time? T-cell exhaustion, due to persistent exposure to antigen, is well-recognised in human TB in which studies suggest that latently-infected individuals often have evidence of lesions on X-ray and at autopsy.

Data suggest that IFN positive, skin test negative animals can subsequently convert to tuberculin positivity with lesions at abattoir (Monaghan and others 1994). Similarly, in the final stages of eradication in Australia, undetected infected animals were identified several years later in a number of herds (Cousins and Roberts 2001). The existence of such animals, should they be infectious or reactivate to active bovine TB, would pose a significant challenge to control and eradication.

1.3.3 Shedding Of Bacteria

The factors which influence the spread of TB between cattle are not well understood. DEFRA SE3033 failed to culture *M. bovis* from 500 nasal mucus samples from 21 infected cattle, indicating that shedding in nasal mucus was an extremely rare occurrence, consistent with the findings from the DEFRA-funded cattle pathogenesis study (DEFRA

SE3013). The stress of handling, testing, movement, calving etc may also play a role, but data are sparse (Verbrugghe and others 2011). Cattle inoculated with a range of doses by the intra-tracheal route developed lesions on the same timescale; there was no reduction at smaller doses. Hence, it is likely that diagnostic tests would be relatively effective for early detection, even for animals infected with a low dose (DEFRA SE3024).

In naturally-infected cattle (field reactors) housed at VLA (2004–2007) there was again a very low level of nasal shedding detectable (DEFRA SE3033). A detailed pathological investigation was undertaken of 200 reactor and 200 non-reactor (exposed) cattle (DEFRA SE3033). 52.5% of the reactor cattle had infection confirmed, as had 11% of the exposed in-contacts (tuberculin negative). Surprisingly, having controlled for likely confounders (herd size and skin thickness), dairy cattle were more likely to be non-visibly lesioned (NVL reactors) or uninfected than other classes or breeds. In reactor cattle, lesions were again predominately found in the chest lymph nodes and rarely in the tonsils.

Lesion distribution was more complex in in-contact animals, indicating that they might have a different pathogenesis which influences their reduced immune response. *M. bovis* was not isolated from any of the nasal mucus samples, and subsequent studies indicated that detectable nasal mucus excretion in naturally-infected animals was very low or sporadic, leading the authors to suggest that the importance of shedding of *M. bovis* in nasal mucus might have been overstated in earlier experimental infection studies. Reactors and infected in-contact cattle tended to yield the same *M. bovis* genotype, suggesting that multiple sources were unlikely in the sampled herds and animals. This study emphasised the need for repeated testing of infected herds to clear all infections. Failure to do so may explain why some herds remain persistently infected for years. The conditions under which an infected animal becomes an effective disseminator of infection are currently not well defined.

Laboratory-based experiments with infection models of bovine TB have confirmed the shedding of organisms in the early stages of the disease process, with a potential for disease transmission, and the failure of the tuberculin test to detect all infected animals, including some with well-developed pathological lesions (Neill and others 1998). Field studies have shown that tuberculin test-negative animals, in contact with tuberculin reactors from multi-reactor breakdown herds, were infected and missed by the disclosing tuberculin test (DEFRA SE3033). Although

shedding has not been detected in these animals there is clear potential for disease transmission.

The tuberculin test has been used very effectively as a herd test, but has serious limitations in identifying individual infected animals in a herd and it is unable to differentiate between infected animals showing varying degrees of infection and pathology. Undisclosed infection in cattle is likely to contribute to local persistence as well as having the potential to initiate new breakdowns via within- and between-herd animal movements and contacts. The tuberculin test will fail to detect some diseased animals that are potential transmitters of disease to other cattle and possibly to local wildlife.

1.4   The Bacteria That Cause TB In Animals

There are ~120 known 'species' of mycobacteria, most of which are harmless saprophytes living in the environment (Rastogi and others 2001). However, a small number are major pathogens; including the highly-related *Mycobacterium tuberculosis* (MTB) complex mycobacteria, the most infamous of which is *M. tuberculosis*; the most important bacterial pathogen of man. Genetic analysis implies that the most recent common ancestor (MRCA, Smith and others 2009) of the MTB complex existed relatively recently, although it cannot be assumed necessarily that tuberculosis is a recent disease.

M. bovis is a member of this closely-related MTB complex, which has been shown recently to have shared ancestry (Brosch and others 2002). On a global scale the MTB complex can now be sub-divided into discrete lineages which show strong phylo-geographical localization to regions (Gagneux and Small 2007, Hershberg and others 2008, Wirth and others 2008, Muller and others 2009). This has significant implications for control.

The MTB complex has identical 16S rRNA sequences and shares a staggering >99.95% sequence identity (Smith and others 2009). Despite significant differences in host range and other features the MTB complex members are too close to be considered as separate species (Garcia-Betancur and others 2011), although it is now relatively easy to distinguish them using fixed molecular markers. The MTB Complex has diverged from a common ancestor, which was probably already a human pathogen, into a series of host-adapted or host-associated bacteria or 'ecotypes' (Smith and others 2006a). The degree of host adaptation is not quite as strict as that for some other bacteria, for example *Salmonella typhi*. Although it is possible on occasion to isolate *Mycobacteria* from

hosts other than their preferred host, it is proposed that strains of one *Mycobacteria* adapted to one host will not be able to sustain their population in an alternate host with its own host-adapted strain (Smith and others 2006a, Wirth and others 2008, Smith and others 2009).

A phylogenetic (family tree) study of the MTB complex has recently shown that the animal-adapted strains are found in a single lineage marked by the deletion of chromosomal region of difference 9 (RD9-deleted, Brosch and others 2002). This important and elegant work has since been extended (Smith and others 2006a) to predict the most likely genotype in a series of inferred ancestors for modern animal-adapted MTB complex bacteria. These mutations can now be used to define these various organisms. This definition (RD9-deleted) is used by international databases such as www.mbovis.org to define the branch, including *M. bovis* (Smith and Upton 2011).

MTB complex bacteria have co-evolved with their hosts over millennia and consequently their interactions are highly complex (Huynh and others 2011). They effectively behave as one species with evolving 'sub-species' (ecotypes) which are constrained by adaptation to specific hosts (Smith and others 2006a). Although transmission between mammalian species remains possible, the frequency of this is largely determined by the density of contact (Djelouadji and others 2011). Although they can all actually be considered as variants of *M. tuberculosis* (Garcia-Betancur and others 2011), the bacteria are referred to as human-adapted (*M. tuberculosis*), bovine-adapted (*M. bovis*), marine mammal-adapted (*M. pinnipedii*), vole-adapted (*M. microti*), goat-adapted (*M. bovis ssp. caprae*) etc.

M. bovis itself at a global level shows striking phylo-geographical localization of genotypes to regions (Smith and others 2011). A number of distinct lineages (clonal complexes) have been identified through population sampling worldwide and the British Isles, in contrast to Iberia, France and Italy (Rodriguez-Campos and others 2011), is dominated by one such clonal complex, known as European 1 (EU1, Smith and others 2011).

The vast majority of MTB complex isolates recovered from cattle worldwide and particularly in the UK and Ireland are *M. bovis* (>99.99% in NI, R Skuce unpublished). It seems reasonable to assume that *M. bovis* has experienced significant selection pressures, including host immunity, host demography and more recently rigourous "test-and-slaughter" policies. It is not yet clear how these have driven and shaped the current epidemic (Smith and others 2006).

2. CATTLE-CATTLE TRANSMISSION

Phillips and others (2003) and Bourne and others (2007) provide excellent reviews of cattle-cattle transmission.

2.1. Evidence Supporting Cattle-Cattle Transmission?

A recent publication from Germany makes the case for more effective surveillance and a re-introduction of tuberculin testing to support abattoir inspection, at least in dairy cattle. Germany has officially bovine TB free (OTF) status and no known wildlife reservoir(s). However, 118 bovine TB outbreaks were recorded between 1997 and 2009, with 23 in 2008 alone. Gross lesions were disclosed in a cow at home slaughter and tuberculin testing identified 56% reactors in that herd. Primary and secondary contact tracings (contact within 8 years) and tuberculin testing yielded a further 11 linked and affected herds comprising 135 reactors. The same genotype of *M. bovis* was isolated in all herds, indicating a common source of infection. This study illustrates that bovine TB can be readily transmitted and dispersed via contact and movement and cattle-cattle transmission in normal cattle trading and in this case remained undetected for several years, in the absence of regular and effective tuberculin testing (Probst and others 2011).

As observed on restocking post-FMD in GB (Biggs 2006), the German result above supports the view that, normally, within-herd spread is relatively inefficient. That few reactors were found in most contact farms may have been influenced by the herd- and animal-level risk factors on those farms that affect susceptibility and infectiousness (see later). On one farm, only the purchased batch of 20 cattle were reactors and only 1.3% of cattle on a further farm were reactors, despite having purchased and managed infected cattle from 6 years previously. Although no direct evidence for transmission via contaminated transport vehicles was found (Probst and others 2011) the extensive cattle contact network illustrates the danger of unperceived spread via cattle movements.

Belgium has retained OTF status since 2003 and uses abattoir surveillance and targeted tuberculin testing. However, as in Germany and the Netherlands, sporadic outbreaks do still occur (Welby and others 2011). It was assumed that the main route of (re)infection, or spread, was by trade in undetected infected animals. France gained OTF status in 2000. However, there has been a recrudescence of damaging outbreaks in some regions (Dommergues and others 2011).

Taken together, these studies in OTF countries illustrate that cattle movement can translocate infection and that further within-herd spread is possible, although it is normally relatively inefficient. Cattle movement is likely to contribute to local and long-distance spread of infection. Although contiguous spread was also demonstrated in the German study, probably via direct contact, it was concluded that cattle-cattle transmission was probably insufficient to sustain infection in these OTF cattle population networks. Infectious wildlife was not implicated.

M. bovis 'strains' (genotypes) have now been surveyed systematically in GB, NI and the ROI (NH Smith, RA Skuce, E Costello, unpublished). Genotypes that are virtually unheard of in GB but common in either NI or ROI turn up ocassionally in different parts of GB. Although frequently associated with NI and ROI eartags, sometimes these genotypes are isolated from cattle that are 'home-grown,' leading to the conclusion that they have been imported and transmitted to home-grown GB cattle (NH Smith, pers comm). The likelihood of onward transmission will probably be influenced by the enterprise type and its management and the tuberculin testing frequency of the receiving herd.

Onward spread from sporadic and recurring breakdowns can potentially initiate further breakdowns in other cattle herds, creating a wider knock-on impact. This is well illustrated by damaging breakdowns in the Netherlands (OTF) in 2008 traced to movement of undetected infected cattle (veal calves) from a GB herd (declared bovine TB free) (EC 2008, Karolemeas and others 2011).

On the basis of the East Offaly proactive badger cull and the Four Area Project (Griffin and others 2005), cattle-cattle transmission was believed to be of relatively less importance in the ROI (More 2009). Whilst purchased animals are acknowledged as a significant cause (0.8-6.9%) of herd breakdowns (O'Keeffe and O'Driscoll 1996), little evidence to support onward transmission has been published in ROI studies and, despite very close contact in winter housing, large outbreaks are reportedly rare. However, it has been difficult to determine the cause of such breakdowns, particularly locally, and to distinguish contiguous spread from shared wildlife spread etc.

Bovine TB is primarily a respiratory infection. Infectious aerosols may originate from sputum (the respiratory tract) or from contaminated fine dust particles, a potential route by which environmental contamination could be rendered infectious (Menzies and Neill 2000). Up to 20% of infected cattle excrete *M. bovis* at any point in time (Menzies and Neill 2000). Onward transmission appears to require lesions in the

lungs and associated lymph nodes and, contrary to dogma, most (40-73%) confirmed reactors have lung lesions, although many are too small to be detected routinely at abattoir meat inspection (McIlroy and others 1986).

A mathematical modelling study in Canada estimated the incidence of within-herd spread as 2.0-9.2 cases *per* 100 cow years contact and 2.9-20.4 for dairy and beef cattle, respectively (Munroe and others 2000). Field studies suggest a wide spectrum of transmission rates, which might be a feature of the host and/or the pathogen. A more recent modelling study in Argentina investigated trends and spatial clustering (spatial scan statistics) of bovine TB herds (Perez and others 2011). They identified 6 significant clusters, all of which overlapped areas of dairy cattle production, supporting the view that there may be value in regionalization of control. They estimated the latency period and transmission rate for bovine TB in these dairy herds to be 24 months and 2.2 infective contacts *per* year, respectively.

Economically, it would appear prudent to prevent introduction rather than to have to deal with the consequences of subsequent within- or between-herd spread (Menzies and Neill 2000). Operation of a truly closed herd and artificial insemination should prevent purchased animals bringing disease on farm (Grove-White 2004). Replacements should only be sourced from trusted sources whose bovine TB status is known and current. TB incidence increases with age (cumulative exposure), so it may be wise to purchase cattle at as young an age as farm management allows. Purchased animals should be isolated for 3-4 weeks and pre- or post-movement testing should be seriously considered. Secure fencing (3m high hawthorn hedge, double fencing 3m apart or electric fencing) and physical barriers to between-herd contact (growing forage rather than grazing) should be encouraged and enforced (Menzies and Neill 2000). Improved biosecurity, including secure perimeter fencing offers a cost-effective means of controlling numerous infectious diseases (IBR, salmonellosis, ringworm, lice, BVD etc) and not just bovine TB.

A well-documented infectious wildlife reservoir has also been identified in the brushtail possum in New Zealand. Systematic removal of 88% of infected possums in some regions led to an equivalent reduction in bovine TB in local herds. However, the herd-level decline was gradual due to continuing within-herd cattle-cattle transmission of residual infection and the accepted imperfect detection of infected animals (Barlow and others 1997). Disease mathematical modelling suggested that within-herd spread contributed 20-32% of infections prior to wildlife

intervention, but was considered to be significantly below that required to maintain bovine TB in these cattle herds in the absence of infectious wildlife (Barlow and others 1998) and/or anergic cattle.

There will be instances where infection persists in cattle due to within- and between-herd transmission, but in the absence of another source of infection (wildlife), an effective test-and-slaughter cattle campaign should eventually remove infection (Pfeiffer 2005). In a multi-host epidemic, such as bovine TB, it is vital that management and potential interventions are applied at the spatial and temporal scale on which the inter- and intra-species interactions occur, in this case at a geographical scale well below the county level (Pfeiffer 2005). It is important to consider the local ecological and epidemiological variables which interact in a particular system, rather than extrapolating from studies elsewhere. As has been discussed in Review 2 (bTB B-C review2011), the extent to which TB is self-sustaining in either cattle or wildlife (badger) populations alone is of crucial importance to disease control. Whilst it is widely accepted that infectious badgers contribute significantly to the epidemiology of bovine TB in the British Isles (Donnelly 2010), the question remains; *"What can reasonably and cost-effectively be done to minimize or mitigate this particular risk, without exacerbating it?"*

2.1.1. The Distribution Of Reactors By Herd

Transmission of infection within populations is influenced by many sources of variability, including biological, genetic, behavioural and geographical factors. Aggregation (clustering) is a function of this variability, such that a few hosts are heavily-infected and/or highly-infectious, whilst the majority evades infection (Woolhouse and others 1997). Transmission potential within a contact network can be evaluated in terms of the basic reproduction number (R_0, Diekmann and others 1990), in this case; the expected average number of secondary cases *per* primary case in a naïve population (Volkova and others 2010). R_0 must be greater than one for epidemic (or endemic) infection to be sustained, although the actual value of R_0 will vary with location and over time.

More than one reactor was identified in most (>66%) confirmed bovine TB incidents in GB (Goodchild and Clifton-Hadley 2001). Cattle-cattle transmission could introduce infection into herds, through purchased infection or contiguous spread (straying cattle, poor fencing, hiring bulls etc) from affected neighbours (Goodchild and Clifton-Hadley 2001), although these authors considered it of less significance than

introduction via local wildlife. They considered the sub-optimal identification of infected animals by the tuberculin test as of less importance than the failure to prevent (re)infection from sources external to the herd. However, they do argue that some of the initial introduction, and most of the within-herd spread, may well be cattle-cattle transmission and they propose that enhanced biosecurity could reduce between-herd spread, and that farm management (ventilation, reduced crowding and improved hygiene) could reduce within-herd transmission. More recently, ~30% of GB herd breakdowns investigated were found to extend for >8 months (Karolemeas and others 2010) and to consume disproportional resources and could act as potential persistent sources of infection for other cattle and wildlife.

Preliminary data exploration for the BBSRC-funded Roslin-AFBI(QUB) bovine TB Genetic Susceptibility Study disclosed the striking distribution (clustering) of reactors across Holstein-Friesian herds in Northern Ireland. Although 43% of herds had only one reactor, with an overall median of 2, 27% of herds accounted for over 75% of reactors, and 12% of herds accounted for 57% of reactors. A similar analysis for the DARD TB Biosecurity Study of data for the period 2005-2009 indicated that 14% of reactor herds in the study area had 20 or more reactors, equivalent to 61% of the total number of reactors in the area (DARD Bovine TB Biosecurity Study, SWJ McDowell, pers comm).

NI province-wide animal-level sampling for *M. bovis* genotyping (2009-present) indicates similar trends. A pivot table of herd by *M. bovis* genotype identified that most of the largest herd breakdowns yielded only one pathogen genotype. Explanations for observing the same (or very similar) genotypes in large multi-reactor herds include; extensive within-herd cattle-cattle transmission, repeat exposure to point source(s) possibly including contact with infectious badger(s) or super-shedder cow(s) or some combination of these. There appeared to be extensive susceptibility, within-herd exposure and transmission occurring.

It will be important to investigate, and mitigate where possible, the risk factors associated with such herds and it will be important to separate those herds where the locally-fixed genotype(s) appear from those which have clearly received genotype(s) via purchase or import, the latter providing a unique opportunity to index within-herd spread, should it occur. A number of smaller herds with multiple reactors tended to contain multiple bovine TB genotypes, indicating multiple introductions from several external sources.

Taken together, these various studies indicate a pattern of substantial clustering of reactors within herds against a background of sporadic cases of single or low numbers of confirmed and unconfirmed reactors. However, a relatively small number of herds provide a disproportional number of reactors. Consequently, if risk factors were identifiable in those herds and mitigated where possible, this should have a disproportionate impact on the current epidemic (Woolhouse and others 1997).

2.2. How Does Cattle-Cattle Transmission Occur?

The distribution of pathological lesions, concentrated in the respiratory system and the very low infectious dose required to establish respiratory infection suggest that transmission of *M. bovis* between hosts is most likely through the airborne route via inhalation of aerosol droplets.

Previous studies showed that low numbers of bacilli were needed to infect animals experimentally via the lung (Dean and others 2005), whereas much larger doses were required to infect animals via ingestion. This is supported by the distribution of lesions found in naturally-infected cattle. Oral infection of humans and calves was much more important when there was still clinical bovine TB in the national dairy herd and milk from cows with bovine TB mastitis was readily available. Thankfully, this is now much rarer due to routine tuberculin testing and prompt removal of reactors.

In theory, transmission can be either **direct**, through close contact, or **indirect** from exposure to viable bacteria in a contaminated environment (for example pasture, feed, housing etc). The relative contribution, if any, of each of these routes has not been quantified. However, we know that *M. bovis* only lives and multiplies in mammalian hosts (or in laboratory incubators on specific media) and only small numbers of viable bacteria are likely to persist in the environment for any length of time. Survival of *M. bovis* outside the host is thought to be usually short (weeks) as bacteria deteriorate quickly, especially in dry, sunny and warm conditions, as they are killed readily by desiccation and germicidal UV light. However, there is published evidence of survival for up to 11 months in ideal conditions (moist and dark areas).

Direct aerosol contact is thought to be the primary route of infection of *M. bovis.* Whilst indirect transmission via a contaminated environment cannot currently be excluded (Courtenay and others 2006), it is generally considered not to be a significant source of infection. To

estimate the frequency of respiratory excretion of *M. bovis* and its relationship with the immune response the presence of MTB complex bacteria in nasal exudates was analyzed using nested PCR during a period of six months in a herd of dairy cattle in Mexico with a high risk of bovine TB transmission. Results confirmed respiratory excretion of *M. bovis* and within-herd transmission (Romero Tejeda and others 2006).

Although it is still one of the most important questions in bovine TB epidemiology, the exact nature and mechanism for transmission of infection, between and within cattle and badger populations, remains largely unanswered. The ISG concluded, and CSA Sir David King agreed (King and others 2008), that inhalation of infected droplets from the lungs of other infected animals, or oral ingestion of mycobacteria from farm environments, were the most likely means of transmission (Bourne and others 2007), as suggested previously (Francis 1958).

Cattle movements are the most significant factor in translocation of bovine TB outside its traditional hotspots in GB (Gilbert and others 2005, Gopal and others 2006). Cattle movements must also play a role within hotspot areas, although, as yet unidentified local factors or processes may account for up to 75% of the variation in incidence. It is noteworthy that in this study the model fit was improved by assuming low cattle-cattle transmission (Green and others 2008). In western England and Wales, 43% of cattle movements occurred over a distance of less than 20km (Mitchell and others 2005). Hence, cattle movement must contribute to local as well as long-distance spread of infection.

The results of molecular typing reveal strong geographical clustering of *M. bovis* genotypes to particular regions. This has been taken as evidence of local transmission of infection (cattle-cattle and/or wildlife-cattle). Pathogen genotyping and animal movement databases are continually being used to trace origins of infection in herd breakdowns arising from cattle movements (Gopal and others 2006).

Cattle movement is probably responsible for most transmission in areas without infectious wildlife in NZ (Barlow and others 1998). In the UK, ~15% of breakdowns have been attributed to cattle movement. The figure is higher outside traditional bovine TB hotspots. This statistic will alter with geography and over time and there have been localised episodes in remote regions, suggestive of cattle movement (or re-emergence from latent sources), rather than gradual spread through a wildlife vector (Bourne and others 2007). Proximity to badgers was not a prominent predictor of risk in that study (Gilbert and others 2005).

In GB there are ~1·63 million farm-farm movements *per* month, equivalent to 19·6 million *per annum*. The geographical distribution of such movements appears to be relatively stable from year to year with most cattle moving less than 100 km *per* journey, although many tens of thousands move distances up to 1,000 km (Wint and others 2004, DEFRA SE3034). The pattern of bovine TB spread in GB (1984-2003) shows an expanding core and outlying areas, interpreted as combining short-range spread and longer-range dispersal (translocation) (Gilbert and others 2005). Hotspots represent isolated clusters of infection and appear to have expanding fronts of infection. In non-hotspot (sporadic) areas isolated clusters tended to remain isolated, resembling hotspots which failed to establish (Goodchild and Clifton-Hadley 2006). In ROI studies, cattle movements appeared to explain ~7% of breakdowns (Clegg and others 2008). Purchase of bulls introduced a high risk of a breakdown, maybe indicating increased susceptibility due to social behaviour or the stress of transport.

NZ research suggested that some horizontal transmission occurs, but insufficient to sustain disease in cattle alone (Kean and others 1999). In contrast, a small Italian study concluded that importing cattle was a major risk, rather than any wildlife vector (Marangon and others 1998). Importation of cattle, some from infected herds, was sufficient to sustain TB in the absence of infectious wildlife (Pillai and others 2000).

Commenting on an extensive analysis of GB data it was concluded that cattle movement around the country and the presence of badgers were both implicated in the high incidence of bovine TB, in GB at least (Woolhouse 2005). Using statistics (multiple logistic regressions) they showed that the geographical distribution of bovine TB was closely linked to the number of recent cattle imports into herds from high risk areas. Their movement-based model predicted the presence or absence of bovine TB *per* 5km^2 grid square with 80% accuracy and also predicted the spread to new cells (Gilbert and others 2005). Other predictors included the recent presence of bovine TB locally as well as indicators of land usage, ground cover and climate. The role of movements was more obvious outside traditional hotspots, which implied that movements were more important for spread than for persistence.

Regions of GB were identified, such as Scotland and the Isle of Man (IOM), which only experienced sporadic breakdowns, despite regularly importing cattle, including from high risk areas (White and others 2008). Chalmers and others (1996) describe an outbreak of bovine TB in 2 herds in SW Scotland. Lesions were seen in a 5 month old calf at

routine abattoir inspection, triggering backward tracing and further testing. Although the calf was not born at the time, this herd was negative when last tested 8 months previously. Testing disclosed 4 lesioned reactors in 185 cattle. Subsequent tests disclosed a further 7 reactors, 3 of which were lesioned. Most of these cattle were bought as calves the previous year from a dairy farm in Dumfries and Galloway and testing there disclosed a further 84 reactors and 6 inconclusives in 261 cattle. This case study illustrates that, in the absence of risk-based tuberculin testing and infectious badgers, bovine TB can still be maintained within, and spread between, cattle herds.

The IOM is interesting in that it has no native badgers. To maintain low prevalence status, the IOM insists on pre-movement tuberculin testing, post-movement tuberculin testing and movement restrictions (CVO IOM pers comm). Despite this, the IOM still reports sporadic breakdowns, some of which have been sufficiently large to merit whole herd depopulation. The source of these breakdowns has been ascribed most likely to importation in cattle, but substantial cattle-cattle transmission has been reported on occasion, possibly associated with susceptibility issues within-herd. In addition, pathogen genotyping indicates that most of the sporadic breakdowns appeared to be independent and not epidemiologically-linked.

In 2000, 35% of the NI herd moved at least once between herds or to market. On average each animal moved 1.4 times, which would appear relatively high by national standards. Antrim and Tyrone recorded substantial farm-farm and farm-market moves. For Tyrone, at least, this appears to be linked to the large number of premises rather than to individual herd size. In summary, 66% of herds undertook farm-farm or farm-market movements 6 or fewer times in 2000 and accounted for 25% of all movements. Only 4% of herds contributed 31% of all movements and there was a trend whereby almost 30% of herds sold animals shortly before routine tests, a practice which would exacerbate disease spread (Abernethy 2004, internal).

Age, environment and local farming practices are likely to influence the routes by which cattle become infected; infection via the alimentary (ingestion) route would not be unexpected in young calves ingesting milk from tuberculous dams, although mesenteric (intestinal) lesions are relatively rare in countries with advanced control programmes. Direct transmission via the respiratory route is also supported by natural cattle behaviour, especially with high stocking density and substantial cattle movement. The most probable transmission route can be inferred from

the pattern of lesions observed in slaughtered cattle. Most bovine TB reactors in the UK have lesions involving the lymph nodes and associated organs of the head and chest cavity, suggesting that the route of infection is predominantly the respiratory route.

The consensus is that intestinal (mesenteric) lesions are more likely to be due to swallowing of the animal's own sputum. Transfer of organisms the other way, from the rumen to the respiratory tract, is theoretically possible due to regurgitation or eructation (Mullenax and others 1964), although the expected accompanying intestinal lesions are not observed normally, although, in experiments, significant numbers of non-pathogenic 'indicator' bacteria were conveyed to the lungs during eructation. In famous early transmission studies, comprising oral challenge of cattle with *M. bovis,* many cattle developed lesions in the alimentary tract and abdomen (McFadyean 1910), a very different lesion pattern to that observed today in UK cattle (Liebana and others 2008).

2.2.1. Vertical (Congenital) Transmission

Transmission of *M. bovis* via the umbilical vessels, due to uterine infection of the dam has been reported (O'Reilly and Daborn 1995). Calves are believed to be congenitally-infected if they present lesions in the liver and portal system only. However, few cows in the UK present with uterine bovine TB. Approximately 1% of calves born to tuberculous dams are likely to be infected congenitally. No confirmed isolations of *M. bovis* were reported from uterine tissue submitted to VLA Weybridge (1986 – 1994). This route is probably insignificant in bovine TB epidemiology in the UK and ROI and no specific control measures are indicated currently (Phillips and others 2003 and references cited therein).

2.2.2. Pseudo-Vertical Transmission (Via Milk)

Pseudo-vertical transmission from sub-clinically infected dams is therefore possible via the ingestion of tuberculous milk, although TB mastitis is also rare in the UK and ROI. However, it is possible for an affected dam to infect large numbers of calves. Her own calf is principally at risk in a suckler herd, whilst in a dairy herd several calves can be at risk by the pooling of colostrum and milk in calf pens (Serrano-Moreno and others 2008). Presumptive TB was reported in only 0.5% of tissue from supra-mammary lymph nodes received by VLA Weybridge (1986 – 1994) (Phillips and others 2003 and references cited within). While there

may be a significant risk in individual cases the overall importance of milk-borne transmission in the epidemic is probably low to negligible.

An assessment of vertical/pseudo-vertical transmission of *M.bovis* infection in NI cattle (*ie.* are the progeny of TB-infected dams at higher risk of bovine TB than progeny from non-infected dams?) has been made (Menzies 2007, internal). Using >1,000 matched affected and unaffected cohorts; a small effect (not statistically-significant) was detected between the two groups, suggesting that the progeny of bovine TB-affected dams were not at significantly increased risk of becoming infected. If the study had shown a significantly increased risk it may have been necessary to revisit the disease control policy on progeny of reactors. This study would also suggest that dam genetic background was not a strong risk factor. However, the study sample size may have been too small to detect such an effect (see later). Milk collected from reactors and inconclusives (including those awaiting slaughter) should not be fed to calves and the timely culling of dairy cows with high somatic cell counts and which may have TB mastitis has been advocated (Phillips and others 2003 and references therein).

2.3. What Exactly Is Transmitted?

When considering how people or animals become infected, and what can cost-effectively be done to mitigate or minimize risks, answers are sought from many specialisms; epidemiology, biology, engineering and physics (Morawska 2006). Microorganisms may become airborne when droplets are generated during speech, coughing, sneezing, spitting, vomiting, or atomization of faeces during sewage/slurry treatment (mixing/spreading). Although some of these mechanisms have the potential to generate more droplets (coughing), it is important to consider the frequency of lower potential mechanisms (breathing). Respiratory tract pathogens are not actually expelled as readily, nor in as great numbers, as would be estimated from the mechanisms of droplet generation.

M. tuberculosis is carried on small airborne droplet nuclei produced by aerosolisation. Respiratory droplets of 0.5-2.0um are the most significant and are rapidly dispersed. They can remain suspended for an extended period, although once they settle on surfaces, they do not aerosolise again and are no longer considered infectious (Segal-Maurer and Kalkut 1994). Larger droplets tend to settle more quickly. Recent analytical developments, including air sampling instruments,

allow these phenomena to be investigated systematically (Mastorides and others 1999, Fennelly and others 2004).

For human TB treatment, the promotion of sanitoria in the late 1800s and early 1900s was in response to an increased recognition of the importance of ventilation, UV irradiation and nutrition (Mitchell-Heggs 2002-2003). The physical properties of indoor space and ventilation then become of critical importance (Wilson 2007). Among the most important building-related factors are rate of ventilation, rate of air circulation, disinfection of re-circulated air and occupation density (Morawska 2006). Infection is more likely to be transmitted in confined spaces where poor ventilation and/or recirculation allow infectious particles to accumulate (Li and others 2007). The risk of infection with *M. tuberculosis* is strongly associated with exposure to air exhausted from an infectious case. Each air change will reduce the level of airborne contaminants by 67% (Segal-Maurer and Kalkut 1994).

2.3.1. Aerosols And Droplet Nuclei

Droplet nuclei are the dried remains of larger, potentially-infectious, respiratory droplets (Nardell 2004), but it has not been possible to catch and culture those produced indoors by an infectious human TB patient, principally due to the insensitivity of detection methods and the very low levels actually present. Although the results (survival and dispersal) of several of these studies differ, the principles are generally supported.

Previous work has placed caged guinea pigs, which are highly susceptible to TB, inside the exhaust ventilation from human TB hospital wards. Guinea pigs were tested by tuberculin test and *post-mortem* examination indicated that they tended to be infected by only one inhaled bacillus. Significantly, large variation was disclosed between patients in their infectiousness. Relatively little is known about the aero-biology of MTB complex bacteria, apart from transmission factors such as cough frequency, lung cavitation, sputum smear positivity etc. It is not understood why some patients are considered 'super-shedders' or 'super-spreaders' whereas some infect few, if any. Recent whole-genome sequencing and social-network analyses of a human TB outbreak support the existence of a super-shedder phenotype (Gardy and others 2011).

The generation and behaviour of potentially-infectious aerosols (influenza, TB etc) and droplet nuclei has been studied experimentally and using simulations and mathematical modelling by biomedical

scientists on behalf of the air-handling industry, which advises on air circulation in hospitals, laboratories, aircraft etc. In a simulated aircraft cabin, airflow played the most important role in droplet transport (Gupta and others 2011), with droplets contained in the same row as (and the row in front and behind) the infectious index case within 30s and then dispersed to the whole cabin within 4 minutes. The fraction of droplets airborne reduced to 12% within 4 minutes dependent on the air handling system operating. Human studies found that some individuals expire many more bioaerosol particles than others even during quiet breathing. Relatively simple nebulizer treatments (saline and surfactants) reduced excretion in these 'high-producers' by 64-80% (Edwards and others 2004). *M. bovis* needs to be able to survive the stresses of airborne transmission and Gannon and others (2007) concluded that airborne *M. bovis* was robust, with 94% surviving the initial 10 minutes of aerosolisation. Once airborne, their viability decreased with a half-life of ~1.5 hours, supporting the hypothesis that airborne transmission is the main route of infection for bovine TB (Gannon and others 2007).

2.3.2. Ventilation And UV Irradiation

While mechanical and filtered ventilation (Knibbs and others 2011) and germicidal irradiation (254nm UV) are advised for indoor hospital wards (Nardell and others 2008), personalized ventilation is also highly effective at reducing airborne infectious disease transmission (Pantelic and others 2009). In human TB epidemiology, masks and respirators are effective at preventing infection in healthcare workers. Surgical masks, HEPA filter masks, cartridge respirator masks and powered respirators decrease the risk of contracting human TB 2.4-fold, 17.5-fold, 45.5-fold and 238-fold, respectively (Harrop and others 2011).

Research in resource-poor settings indicated clearly the benefits of natural ventilation (Escombe and others 2007). It is interesting to note that current USA Centres for Disease Control (CDC 2005) guidelines for prevention of transmission of human TB in healthcare facilities specify ventilation, supplemented by air filtration and UV irradiation. Continuous germicidal (254nm) UV irradiation has broad spectrum activity against airborne bacteria and viruses and kills *M. tuberculosis*. Germicidal activity is affected adversely by relative humidity and is a relatively poor surface disinfectant due to poor penetration. UV irradiation of air, exhausted from a human TB ward and passed over guinea pigs, prevented their infection (Riley and others 1962).

The guinea pig model has been recreated and used with *M. tuberculosis* genotyping to illustrate that a small number of inadequately treated human TB patients (co-infected with HIV) were responsible for almost all TB transmission in a hospital ward (Escombe and others 2008). Some individuals were disproportionately highly infectious. Upper-room UV lights and negative ionization prevented most airborne TB transmission detectable in the guinea pig sampling model (Escombe and others 2009) and provided a cost-effective intervention for high-risk clinical settings, where de-humidifiers may also be useful.

2.4. Direct (<2m) Cattle-Cattle Contact

Cattle contact patterns are highly variable, both between individuals and over time. This can be influenced by their relative position in the herd social hierarchy. Cattle do form dominance hierarchies and various groupings within herds (Sauter and Morris 1995), which become even more important in low prevalence disease scenarios. Studies of between-farm cattle movements have indicated the variability (heterogeneity) between and within such networks (Brennan and others 2008). For direct contact diseases of cattle the Pareto Principle, or 80:20 rule, applies (the law of the vital few); 20% of holdings contribute 80% of new cases (Woolhouse and others 2005). Some cattle are highly connected within the herd contact network and have the potential to act as hubs in the spread of disease within these complex contact networks. Targeting prevention or control measures to high-contact individuals (or groups) should further enhance disease management.

Proximity data logging collars, fitted to a random 10% of a GB dairy herd, have recently assessed the connectedness of social networks within and between cattle herds and indeed with local wildlife (Bohm and others 2009) in an attempt to identify high risk individuals for transmission. Unsurprisingly, direct cattle-cattle contacts were much more frequent than cattle-badger contacts, although there was considerable variation (7-26 direct daily intra-group contacts). Although no significant hubs were identified in cattle contacts in this study, cattle with higher intra-herd contacts also featured prominently in cattle-badger contacts.

This concurs with data from New Zealand, where bovine TB reactors tended to be from the top half of the herd hierarchy (Sauter and Morris 1995). Given the heterogeneity in cattle contact between farms, there should be merit in further targeting cattle-based control measures

at between- and within-farm scales. More frequent testing of highly-connected farms and markets, combined with targeted testing (or vaccination?) of dominant cattle should contribute to improved control (Bohm and others 2009). A study of possums in New Zealand (Ji and others 2005) showed that there was a non-linear relationship between contact rates and population density, in contrast to the assumed density dependence.

A cluster and network analysis in NW England cattle herds (N=56 within a 10km^2 area) concluded that significant variation existed between farms in the type and frequency of contacts (Brennan and others 2008). Some contact networks comprised ~90% of farms and were highly connected, whereas others were highly fragmented. Contiguous farms were more likely to be connected by 'other' contacts, such as direct farm-farm animal movements and shared equipment.

To illustrate, exposure time was less important than social interaction in influencing TB (*M. bovis*) risk in a study of a meerkat social group (Drewe 2010). So why don't all group members become infected once infection enters the social group? Further analysis of this system (social network analysis and diagnosis of TB) indicated that social structure was stable over time and that infection might spread locally within clusters of interacting individuals. Due to this social contact structure, infection was unable to infect all members of a large group (Drewe and others 2011) and some behaviour was more risky than others.

A New Zealand study investigated intra-species transmission of *M. bovis* between uninfected and wild-caught, infected (42% and 94% prevalence) feral pigs. Infected pigs were kept in contact with susceptibles for 7 years in total, but only one transmission event was detected and R_0 (pig-pig) was estimated at 0.25. The authors concluded that intra-species transmission, by the route simulated, was probably insufficient to sustain infection in the wild and they propose that high prevalence TB in feral pigs is more likely to result from transmission from another route or host (Nugent and others 2011). However, it is not clear how relevant these studies are to cattle-cattle transmission, since *M. bovis* is not particularly host-adapted to pigs or meerkats and this is known to influence the efficiency of within-species transmission (Smith and others 2006a).

Infectiousness appears to vary with time post-infection (Goodchild and Clifton-Hadley 2001) and is thought to be followed by a test unresponsive period, estimated at 30-50 days (Francis 1946). This period

may last up to 7 years, although 6-20 months is proposed for regularly tested cattle (Barlow and others 1997), but may be as little as 87 days in calves. More recent VLA work suggests an incubation period as low as ~35 days for experimentally-infected calves (Abernethy pers comm).

At the other ends of the infection spectrum (Barry and others 2009, Young and others 2009), a state of anergy can be identified where the animal is no longer responsive to cell-mediated immunological tests (tuberculin and IFN tests) and the bacterial load is high. Anergy is allegedly relatively rare in the UK, although there are anecdotal field reports of super-shedders and some advanced mathematical models fit the observed field data better when a super-shedder is invoked (Medley, pers comm). The rational deployment of alternative tests, such as serology, to detect and remove such anergic cattle has probably been neglected in recent years and merits further investigation (Clegg and others 2011).

It has been assumed that all infected animals excrete, sporadically, at some stage post-infection, although the original citation is quite old (Francis 1946) and how the evidence base relates to current field cases in regions operating comprehensive cattle-based controls is not clear (Goodchild and Clifton-Hadley 2001). Shedding is probably intermittent or episodic and different patterns are evident, even in calves given the same dose (Kao and others 2007). *M. bovis* has been isolated from respiratory tract secretions of 7-20% of naturally-infected cattle in various studies.

Cattle that are higher in the herd social heirarchy also show greater inquisitiveness and have a higher risk of acquiring infection from cattle introduced to the herd, as well as potentially from direct contact with infectious wildlife (Bohm and others 2009). Lower social status may also increase the potential risk of indirect transmission from pasture contaminated with badger excretions. Scantlebury and others (2004) quantified the levels of investigation and grazing of GB dairy cows (N=150) at badger latrines and crossing points. They concluded that if cattle investigative behaviour was a major route of bovine TB transmission the risk to cattle was greater in extensive rotation-grazing systems than on strip-grazing.

Even if not actually penned or herded together cattle may still share the same airspace, especially indoors and the number of reactors *per* herd tends to increase as a function of herd size, rather than in direct proportion or exponentially. Dairy herds have a higher transmission coefficient than beef herds, probably due to their longevity

and their more intensive management system, which often results in their closer confinement. Cattle-cattle transmission can be a factor in establishing and spreading infection, with close cow-cow contact, including shared feeding, occurring in cow cubicles.

In DEFRA SE3015, intra-nasal inoculation, which produces pathology similar to that of ~30% of GB field cases, resulted in more nasal shedding of *M. bovis* than via intra-tracheal inoculation. Shedding occurred in IFN+ and tuberculin+ animals with lesions of bovine TB, but also occurred in animals that were only IFN+, with no lesions visible *post-mortem*. Although tuberculin-negative, some infected animals may remain in the herd as a reservoir capable of shedding the organism. Cattle were either persistent or intermittent shedders of *M. bovis* and shedding was noted in two phases, one at <30 days and one at ~80–100 days post-inoculation (DEFRA SE3015). However, evidence on nasal shedding is somewhat conflicting. In a study of naturally-infected reactor cattle housed at VLA, *M. bovis* excretion via nasal secretions was considered to be extremely rare. It was proposed that stress might stimulate nasal shedding (DEFRA SE3033). A study of bovine TB in field cattle confirmed that nasal shedding of *M. bovis* was not a common feature of natural infection (DEFRA SE3013). Although much has been learned from experimental infection studies in housed cattle it is unlikely that they truly simulate the dose or route experienced in most field situations (Liebana and others 2008).

60% of cattle with bovine TB in GB have pathology restricted to the lower respiratory tract. In an experimental study (Johnson and others 2007) even high doses of *M. bovis* delivered via aerosol produced bovine TB confined to the lower respiratory tract and half the animals infected with only 1cfu of *M. bovis* developed pulmonary pathology. There was also evidence of latent infection. No significant differences were observed in pathology at different doses and all animals which developed pathology were tuberculin+ and IFN+ post-infection, regardless of dose, and responses to the IFN test developed earlier than to the tuberculin test.

2.5. Cattle Movement And Contact Networks

Animal movement is a major route of spread of infectious diseases through populations of livestock (Fevre and others 2006, Volkova and others 2010). It is important for disease prevention and control to understand how the features of livestock movement networks impact on

transmission potential (Kao and others 2006, Green and others 2011). Comprehensive, computerized systems, which record all locations, between-premises cattle movements and tests, have been in place in NI since 1988 (Houston 2001) and in GB since 1998 (Mitchell and others 2005). It is noteworthy that individual cattle identification was introduced in the 1950s to help control bovine TB (Mitchell and others 2005). The exposure of susceptible animals to infectious animals is essential for efficient transmission of pathogens. Depending on the pathogen, exposure can be direct and/or indirect, resulting from cattle movements, contact with people, or the use of shared resources. An understanding of the underlying contact and temporal structure is an essential prerequisite in the development of effective control measures (Heath and others 2008, Danon and others 2011).

Effective disease control is based on understanding transmission and spread. To understand disease transmission dynamics we need to understand what happens amongst animals, within farms and markets etc (Kao and others 2006, Vernon 2011). The farmed animal industries are highly structured and animal movement is an important route for disease spread. Based on recorded animal identity and movement histories, this structure can be captured as a network, with farm sites represented by 'nodes', and potentially infectious contacts by directed 'arcs' or undirected 'edges'. This is a powerful tool for studying the potential for disease spread and control and, despite the fact that many features change, a large part of the intermediate structure is conserved over time, particularly the core network (Green and others 2011).

This is illustrated by a recent analysis of cattle and pig movements in Sweden (Noremark and others 2011) which showed that the distribution of contacts between farms was highly skewed; many farms had few or no contacts with other farms, whereas several farms had extensive contacts; some farms had limited direct contacts, but many indirect contacts. The cattle network pattern was also seasonal. These results are similar to a recent UK study (Vernon 2011) which showed that most cattle movements occurred between agricultural holdings, markets and abattoirs. Movements between GB holding types were not random and most cattle moved only short distances and rarely in their life. Most movements between holding pairs occurred only once in the last 10 years, with about a third of such moves occurring 2-10 times. Hence, GB cattle movement patterns showed no real trend since 2002 (Vernon 2011).

It is important to understand the variation in risk by location and over time. The risk factors associated with cattle, badgers and their interactions will vary in space and time and in response to local management interventions (Mill and others 2011). Disease dynamics are driven by the patterns of interactions between infectious and susceptible individuals within a population, where links between nodes in a contact or movement network can transmit infection, provided that there is a pathogen in the network, as well as host, pathogen and environmental factors (Garcia Alvarez and others 2011).

Using disease modelling of GB data, most (75%) bovine TB infections on farms in high-risk areas were attributed to local factors other than cattle movement (Green and others 2008), including local persistence in cattle, an infectious wildlife source and, theoretically at least, a contaminated environment. The lack of structured pathogen surveillance data has hampered attempts to test disease dynamic models in the context of contact networks (Welch and others 2011, Welch 2011). The most common *M. bovis* genotypes in the UK show a highly clustered (aggregated) distribution that is largely stable over time (Smith and others 2006b, Skuce and others 2010). In GB this is taken as strong empirical evidence that, in high incidence areas at least, stably-located wildlife reservoirs are involved in the persistence of infection in a relatively mobile cattle host.

Having considered the evidence base, Phillips and others (2003) made the following recommendations for herd-keepers to control importation of bovine TB:

- Herd-keepers should maintain truly closed herds, where replacements are bred on farm, so that the risk of cattle-cattle transmission would then be restricted to lateral spread from contiguous herds.
- Where a closed herd is not possible, cattle purchase should be minimized to reduce the risk of introducing infection.
- Hiring and sharing of bulls should be discouraged. Pre- and post-movement tuberculin testing should be considered where cattle purchase is necessary.
- The herd-keeper should reduce the risk of introducing *M. bovis* via pre-movement testing of cattle at the vendor's premises, or post-movement isolation of purchased cattle and testing at the purchaser's premises. Post-movement testing may be more practical since many cattle are purchased through markets and

this precaution won't eliminate risk totally, since a recently-infected animal is refractory to tuberculin testing for 3-6 weeks.

- It would be better if bovine TB status was available to the purchaser.

Mitchell and others (2005) report the potential for substantial local spread of bovine TB through cattle movement. Given the relatively low sensitivity of the tuberculin test, we suggest that there might be merit in re-evaluating deployment of severe interpretation of the SICTT, including for pre- and post-movement testing. This would depend on the estimated specificity of severe interpretation and on the cost/benefit analysis of removing additional infected animals at the possible expense of additional false-positives.

Cattle movements, particularly those from endemic bovine TB areas, consistently outperform environmental, topographic and other anthropogenic variables as the main predictor of bovine TB occurrence (Gilbert and others 2005). Gopal and others (2006) investigated the source of bovine TB in 31 herds in NE England that experienced confirmed breakdowns (January 2002-June 2004). 9 herds had been restocked post-FMD in 2001. In all but one of the breakdowns the most likely source of infection was one or more purchased animals. In 17 breakdowns, reactors were traced to herds from which the same *M. bovis* genotype was isolated and in 5 breakdowns a different genotype was isolated. The most likely sources were located in Wales and west and north England, including a Cheshire herd that was the most likely source of nine of the breakdowns. Three breakdowns were traced to imports. Reactors in five breakdowns included homebred and purchased animals, providing further evidence for cattle-cattle spread within-herd. The lack of clustering of genotypes suggested that the overwhelming source of infection was bought-in cattle.

Strong spatial patterning of bovine TB makes GIS-based visualization an ideal tool for exploring disease management (DEFRA SE3001, Durr and Froggatt 2002). Cattle herd breakdowns were investigated in four counties outside SW England (1986-2000). Factors influencing herd breakdowns included calendar time, herd size, number of cattle tested, test type, inter-test interval and spatial grouping of farms. There was no evidence of spatial clustering of breakdowns in W Glamorgan and only weak evidence of spatial clustering in Shropshire (7-15 km) and Sussex (5-10 km). In Staffordshire, there was evidence of spatial (2-4 km) and temporal (3-4 years) clustering of breakdowns. The main conclusion is

that there were both local and distant components of bovine TB spread (Green and Cornell 2005).

A striking finding of molecular typing studies has been the degree of local clustering (geographical localization) of subtypes of *M. bovis* (Smith and others 2006b, Skuce and others 2010). However, there is also clear evidence of dispersal (translocation) of *M. bovis* molecular types from their expected hotspots (Skuce and others 2010), most likely due to longer range cattle movements. We suggest that this level of dispersal is also likely to occur due to local recorded and unrecorded between- and within-herd cattle moves. Most cattle moves are over relatively short range and there would be value in investigating the movement and contact networks, including for confirmed cases. Molecular typing data suggest that a small but significant component may be due to cattle movement, presenting a serious risk of transmission to other cattle and to wildlife (Woodroffe and others 2005).

Non-random distribution of cattle movements could also theoretically generate geographical localisation of *M. bovis* genotypes. However, preliminary mathematical modelling studies in GB indicate that cattle movement patterns were not compatible with extensive geographical localisation of genotypes (NH Smith pers comm), despite the extent of local cattle movements to farms and markets in GB (Mitchell and others 2005). The GB epidemic was seen as a series of local epidemics caused by different strains emerging in different regions. Each genotype has occasionally been isolated outside its traditional core area, but to date there has been little evidence of new hotspots arising. Other analysis of GB cattle movement data suggest that 26-85% of cattle herd breakdowns were not caused by cattle moving into the index herd, a percentage that could have been caused for example by infectious wildlife (DEFRA SE3117). For NI it will be important to investigate, by modelling, the extent to which cattle contacts and movements explain the striking clustering of genotypes. For modelling purposes, each genotype cluster may be considered as a separate mini-epidemic, although the clusters should eventually all connect. In human TB epidemiology, belonging to a genotype cluster suggests ongoing and recent transmission. There might be merit in considering those risk factors which place a case within a cluster, as opposed to those which exclude it.

Connected farm networks are consistent with the observed distribution of transmission rates between hosts. The Pareto Principle implies that 20% of individuals in the population contribute 80% of the

net transmission potential or 80% of the size of R_0 (Woolhouse and others 1997). Mathematical modelling predicted that targeting appropriate control, such as movement restrictions, vaccination or pre-movement testing, to this 20% would result in a reduction of 97-99% in R_0 for a low-prevalence disease such as bovine TB. There is some stability in the contribution of individual herds; the same top contributor herds tend to occur from year to year, but not necessarily. Most GB cattle movements occur within the major geographical regions rather than between them.

Although bovine TB control programmes tend to treat all breakdowns equally, it is clear that some are more serious and persistent than others. Including all breakdowns in risk factor studies might help explain the complexity and inconsistency of the results of such studies (SGM 2008). A renewed search for risk factors in such herds would be worthwhile.

Transmission is strongly influenced by *"who contacts whom?"* in the contact network within and between farms and markets etc. As a proxy for contact in the spread of airborne respiratory droplet infections, electronic recording devices were used to estimate proximity between cattle (Bohm and others 2010). Similarly, pathogen genetic sequences (genotypes) are also potentially informative about transmission networks (Skuce and others 2010, Welch and others 2011, Baker and others 2011, Schürch and van Soolingen 2011).

Multiple unreported local movements and contacts are described between farms in several studies and are recognised as a factor in underestimating the role of contact and movements, particularly over short range. Analysis of cattle movement and test history data alone is also unlikely to completely capture transmission dynamics and may lead to an underestimation of the potential for disease spread, especially the extent of local spread (Garcia Alvarez and others 2011). Investigating the spatial and temporal pattern of disease clusters (including *M. bovis* genotype clusters) should help to identify those local risk factors which contribute most to the ongoing transmission producing the cluster.

Whilst epidemiological data may record which animals were infected and may potentially estimate when and for how long, it cannot yet inform on *"who likely acquired infection from whom"* (Welch and others 2011). However, newer approaches, such as phylodynamics or genome epidemiology, which integrate epidemiology and phylogenetics (pathogen genetic family history, Falush 2009) make it possible to model and infer the most likely infector for a given infectee. This has the potential to indicate the most likely sequence of transmission events in a given

cluster (Schürch and others 2010a, Schürch and others 2010b, Laing and others 2011), even with missing data and unobserved events.

2.5.1. Pre-Movement Testing

Pre-movement testing was a required feature of the ROI programme until it was abandoned in 1996 as not being cost-effective and not contributing significantly to the programme (Good 2006). It was estimated that 0.8%-6.9% of breakdowns were due to purchased cattle and the ROI had limited evidence of onward spread to the receiving herd and beyond (Flanagan and others 1998).

More recently the ROI (Clegg and others 2008) re-examined the potential infection-control benefit from pre-movement testing (tuberculin test standard interpretation). Their study used national data for 6,252 herds with a new bovine TB restriction in the 12 months from 1 April 2003 and 3,947 herds declared bovine TB-free in the 12 months from 1 October 2001. Results attributed 6–7% of current herd restrictions to the recent introduction of an infected animal. Following movement from a de-restricted herd, the odds of an animal being positive at the next test increased with increasing time in the source herd prior to movement, increased time between de-restriction and the next full herd test and increased severity of the source herd restriction. The odds decreased with increasing size of the source herd and they estimated that 15.9 destination herd restrictions *per* year could be prevented for every 10,000 pre-movement tests and that 3.3 destination herd restrictions *per* year could be prevented for every 100 source herds tested pre-movement. They concluded that cattle movements played only a limited role in bovine TB spread in the ROI. Following de-restriction they estimate that the infection risk is highest amongst animals moving from small herds, animals spending longer in the source herd, animals coming from herds with a severe breakdown and animals spending longer in the source herd between de-restriction and testing. Therefore, they propose that the yield for a post-movement test would be greater if deployed as a part-herd test as opposed to a full-herd test (Clegg and others 2008).

Rational deployment of pre-movement testing in England and Wales was designed to reduce the risk of spreading bovine TB through the movement of undetected infected cattle. In contrast to the ROI, a recent review indicates that pre-movement testing may have reduced the number of TB breakdowns in low incidence areas almost 50% up to 2008. The impact in high incidence areas was more difficult to estimate

due to inherent annual variation in breakdowns identified through routine testing (DEFRA 2010). Modelling indicated that between 2010 and 2015 pre-movement testing would prevent up to 1,500 confirmed new incidents and the number of undisclosed infected herds was predicted to be around three times lower than without pre-movement testing.

2.5.2. Cattle Purchase

Buying animals may carry a considerable risk. Not only does the herd-keeper bring additional susceptible animals on farm, but also risks unwitting introduction of infection. There is a certain dynamic to this, where the herd-keeper can also reduce risk by moving susceptibles and undisclosed infection off his/her own farm. A recent mathematical modelling study in Canada illustrated the impact of the introduction of a long-incubation disease (bovine TB) on the known movement network between farms over a 3 year period, assuming no wildlife reservoir (Dube and others 2011). If introduction of disease went undetected for 12 months, the worst-case scenario estimated that 5% of farms would be infected via cattle movements alone. The estimate increased to 26% of farms if infection remained undetected for 3 years. This illustrates the interaction between effective detection (including test sensitivity) and the role of cattle movements in translocating the disease and could help explain aspects of the UK epidemic, especially if substantial underestimation of disease burden in cattle exists, as predicted by moderate tuberculin test sensitivity.

2.5.3. Regionalisation

Regionalisation, which comprised zoning and compartmentalization of the cattle population based on TB prevalence and wildlife risk, has been deployed in the New Zealand programme (Livingstone and others 2006). This has allowed NZ to more effectively implement region- and risk-based control objectives and is believed to have contributed to a reduction in annual period prevalence in cattle herds from 18% to 3% between 1993/4 and 2004/5. It was hoped that this approach would be further endorsed by the OIE (Anon. 2004).

In the 1980s Australia also effectively used zoning to control the movement of potentially infected cattle from high prevalence Western Australia, the Northern Territories and Queensland (Tweddle and Livingstone 1994, Neumann 1999). In the latter stages of eradication

Australia deployed risk-based management policies to minimize the exposure of their cattle population (Radunz 2006).

2.6. Other Significant Circumstances Or Locations

Human TB epidemiology would recognise particularly high-risk settings for transmission to the wider community, such as prisons, mines, hospitals (known as 'institutional amplifiers'), which have a significant role in disease dynamics and should be the focus for control. Movement in and out of these institutional amplifiers fundamentally alters human TB transmission dynamics and is not adequately controlled by detection or treatment (Stuckler and others 2008, Basu and McKee 2011). Whilst most human TB transmission appears to require repeated exposure over a period of time, molecular typing studies clearly indicate that, more frequently than expected (Garcia de Viedma and others 2011), transmission can occur after brief, non-intimate contact and despite only minimal exposure (Golub and others 2001, Cronin and others 2002). DNA fingerprinting data are important in identifying unusual transmission in unexpected settings not normally pursued in traditional contact investigations, for example via public transport (Feske and others 2011).

2.6.1. During Transport

For diseases with low animal-level prevalence on farm and long periods of infectivity in infected cattle, such as bovine TB, the contribution of transient contacts of animals in transit or at markets was considered negligible, relative to the transmission potential on farm (Vernon and Keeling 2009, Volkova and others 2010). In another study, contacts via livestock transporters were only considered significant if the potential contact was longer than 24h and the final destination was other than an abattoir. Although no direct evidence for transmission via contaminated transport vehicles was found (Probst and others 2011) the extensive cattle contact network illustrates the danger of unperceived spread via cattle movements. Whilst direct contact between cattle, stressed by handling and transport may constitute a risk, this has been considered to be negligible (Probst and others 2011).

2.6.2. At Markets

Contacts at market, where cattle segregation is probably sub-optimal, are considered a risk for several animal diseases (Robinson and Christley 2007). Due to the traditional trading of cattle through markets,

cattle movement networks are highly connected in the UK and ROI. The role of markets in disease transmission depends on the disease characteristics (transmissibility) and the contact between groups facilitated by such markets (Garcia Alvarez and others 2011). In GB, livestock should be moved from markets within 48h and the premises should be disinfected daily, so it is unlikely that significant transmission of *M. bovis* occurs. However, one GB study did identify purchase of cattle from markets as a risk for herds restocking post-FMD (Ramirez-Villaescusa and others 2010) although this may relate to the bovine TB history of the source herd(s) rather than the markets themselves.

Regional markets are a key feature of traditional farming and cattle trading in the UK, as evidenced by the 2001 FMD outbreak. They continue to operate as hubs connecting large numbers and networks of farms. A GB study used modelling, such as contact networks and social network analysis (Martinez-Lopez and others 2009) to investigate the contact network established by normal cattle trading practices (Robinson and Christley 2007). Most GB markets received animals from less than 50 farm premises and the vast majority of moves, to and from market, were local (within 50km). In addition, in western England and Wales 43% of cattle movements occurred over a distance of less than 20km (Mitchell and others 2005). Hence, cattle movement is likely to contribute to local as well as long-distance spread of infection.

Some markets and some farms were highly connected within the contact network, suggesting important differences in risk. Whilst there is obvious potential to transmit infectious diseases via movements through markets (the most extreme example in the GB study predicted that 62 herds would be affected by movements of infected animals from one such farm) this potential is influenced by the infectiousness of the pathogen and the susceptibility of the host. For diseases with low transmissibility *per* contact (bovine TB), one infectious animal entering a market may only be a risk to cattle with which it comes into very close and frequent contact, which is actually more likely with cattle in the destination herd. The differences in sale management of calves, dairy and beef cattle may also affect the role of markets (and farms) in disease transmission.

The role of livestock markets, whether there was any significant difference in movement frequency and extent of contact between bovine TB cases and matched controls in the transmission of bovine TB in NI, has been investigated (Abernethy and others 2004, internal). The mean number of recorded lifetime moves was 3.86, although 1% had 12 or more moves. The study demonstrated that substantial movement of

cattle via markets was a traditional feature of local farm business (62% of all moves were via markets). No significant association was detected between disease risk and movement frequency between cases and controls (1989-1997). Most (65%) reactors in the NI study passed through a market at some time (11% within 2 months of slaughter), so it is likely that infected cattle do encounter susceptible cattle at markets.

Movements via market tended to occur either within the first 12 months or again at 18-30 months. Although stress is likely to exacerbate the potential to become infected, diseased or infectious, little published data exist (Verbrugghe and others 2011). The NI study concluded that the transient contact between cattle in a market was, on average, probably insufficient to allow significant disease spread (Abernethy and others 2004, internal) although if cattle-cattle transmission was significant in the NI system, traditional farming practices (frequent within-herd and between herd movements, fragmented grazing, conacre etc) would support disease transmission. Furthermore, most (79%) boundary fences surveyed would not have prevented direct animal contact (Denny and Wilesmith 1999).

A large number of explanatory factors from the DEFRA TB99 questionnaire were screened for association with the risk of a herd breakdown in 268 farms in RBCT areas of SW England prior to the 2001 FMD outbreak (Johnston and others 2005). The strongest factors associated with an increased TB risk were movement of cattle onto the farm from markets or farm sales, operating a farm over multiple premises and the use of either covered yard or 'other' housing types. The spreading of artificial fertilizers or farmyard manure on grazing land was associated with decreased risk in this study.

2.6.3. Agricultural Shows

Although cattle-cattle transmission is reported to be of low frequency in the field, *M. bovis* can be highly infectious on occasion. Fifty-one of 56 cattle from 42 TB-free herds were disclosed as tuberculin reactors ~80 days after attending a 4-day agricultural show in which they were housed in rows 3m apart within a 1050 m^2 tent (Steger 1970). Forty-six of 47 reactors had lesions of pulmonary TB when slaughtered. However, there appeared to be limited spread to the other animals within the associated herds, with only one reactor removed from a total of 1,000 cattle tested over the course of the outbreak investigation (Steger 1970).

At present in GB, pre-movement testing is not required for animals to be transported to agricultural shows, provided that the animal is

returned to the source premises or moved directly to slaughter (DEFRA 2010). DEFRA TBAG recognized that mixing animals at agricultural shows presented a potential TB risk. The exemption for cattle movements to shows was permitted as these movements were considered to present a lower risk of disease spread due to the high level of biosecurity at shows and the short period of time that cattle spend there. The data showed that most cattle actually moved to a showground and back to the source holding, rather than moving from the show to another holding. Therefore most of the cattle movements were exempt from pre-movement testing.

2.7. Indirect Transmission - Via An Environmental Reservoir And Fomites

Whilst experimental studies carried out in the UK, ROI, New Zealand, Australia and South Africa have shown that *M. bovis* can persist in the environment for varying lengths of time, it had been concluded that environmental persistence does not play a significant role in the epidemiology of bovine TB through indirect transmission, between or among susceptible species (Fine and others 2011).

However, recent research in the US supports real potential and a significant role for indirect transmission in their cattle-deer bovine TB system, importantly in the absence of recorded close direct contact between species. Large piles of winter feed are thought to become contaminated by infected deer shedding *M. bovis* in nasal discharges or saliva (Fine and others 2011). In US white-tailed deer, supplementary feeding during the winter months may inadvertently contribute to transmission between deer. *M. bovis* survived on all feed at all temperatures tested for at least 7 days and at 23ºC. Viable *M. bovis* was isolated from apples, corn and potatoes at 112 days, suggesting that contamination of feedstuffs by *M. bovis*-infected deer could be a source of indirect transmission, at least between deer (Palmer and Whipple 2006).

M. bovis could be recovered from inoculated substrates up to 88 days in soil, 58 days in water, 58 days in hay and 43 days on corn (Fine and others 2011). Hence, *M. bovis* may persist long enough to be a risk to deer and cattle. The following risk factors had already been identified in the Michigan cattle-deer system – the presence of ponds or open water in cattle areas, keeping cattle outdoors >50% of the time, feeding and watering cattle outdoors and not protecting cattle feed from deer (Kaneene and others 2002).

A study involving Warwick University and ROI researchers indicated that *M. bovis* DNA could persist in environmental samples, indicating further potential routes of transmission between cattle and between badgers and cattle (Young and others 2005, Courtenay and others 2007). However, molecular detection of *M. bovis* DNA in the environment does not necessarily imply the survival of viable bacteria since the study did not originally attempt to distinguish live from dead bacteria. PCR detected *M. bovis* BCG DNA in spiked soil microcosms and *M. bovis* in environmental samples taken from a bovine TB-affected farm. *M. bovis* DNA was detected in soil at 4 and 21 months post-contamination in the range 1×10^3-3.6×10^3 copies per gram of soil, depending on the sampling area. Areas around badger setts had the highest levels of DNA persistence. This study provides evidence that *M. bovis* DNA can persist in the farm environment and that climatic factors influence survival rates.

However, it is not clear whether *M. bovis* can be rendered into an infectious aerosol from this matrix and no evidence was provided to indicate that environmental detection by PCR had any predictive value for bovine TB in local cattle herds (Courtenay and others 2006). Positive sample rates were extremely high, and specificity, as well as relevance to bovine TB transmission, remains unknown (Woodroffe and others 2006). Whether cattle would mount an immune response following the inhalation or ingestion of dead *M. bovis* is also not clear. There is some evidence that *M. bovis* can remain viable in the environment, but it remains to be established that cattle-cattle transmission by indirect contact, mediated by fomites, is important in bovine TB epidemiology (Wilsmore and Taylor 2008). Molecular methods have been used to detect mycobacteria in the environment (Courtenay and others 2006). Indeed, DEFRA has since ruled that PCR is currently not fit for this purpose (Defra DPAG 2010). Whilst there may be a theoretical risk from indirect contact with contaminated, shared farm resources, we were unable to source supporting publications, other than the recent US studies (Fine and others 2011).

Transmission of *M. bovis* via contaminated cattle slurry and manure was considered (de la Rua Domenech 2007 cited by Wilsmore and Taylor 2008). It was proposed that bovine TB-infected slurry or manure could potentially spread bovine TB by the respiratory and ingestion routes. This would require that at least one bovine in the herd was infected, infectious and shedding bacteria in faeces, urine (unlikely), or milk that was disposed in slurry. Meat inspection and *post mortem*

examination indicate that only a few infected cattle exhibit the pathology necessary for this. Lesions in the mesenteric lymph nodes (ingestion) or udder (TB mastitis) are rare in naturally-infected cattle in GB (Liebana and others 2008). Contaminated slurry/faeces must then contain an infectious dose of viable *M. bovis* that must come into contact with at least one susceptible cow via inhalation or ingestion. The bacteria must survive storage and the aerial or ground environment for long enough to contact a susceptible host and must be in the right physiological condition. There is also an important dilution effect (of air, uninfected soil and uninfected slurry/manure) that reduces the likelihood of infection, but there are risks of creating aerosols by spreading slurry.

Survival of *M. bovis* is enhanced in moist, cool conditions and neutral-to-acidic substrates rich in organic matter, especially when the bacilli are protected from direct sunlight. Storage of slurry for at least six months may be necessary before all *M. bovis* organisms in contaminated slurry are naturally-inactivated. Phillips and others (2003) recommend a minimum six month storage period for slurry or manure. Further inactivation by UV light or pasteurization would be desirable. Cattle manure with low moisture levels and high straw content should be composted for at least 30 days before disposal on farm land, otherwise manure should be treated as slurry (treated with alkalis such as thick lime milk) or approved disinfectants. Cattle excreta should be injected or ploughed into arable land to minimize aerosols or disposed onto land not intended for grazing, or that is to be cut for silage, for 2 months at least and longer if spread during autumn-winter. Cattle excreta should be disposed on farm land not accessible to other herds. Slurry and manure should only be spread when conditions minimize aerosol production and herd-keepers should avoid sharing spreading equipment.

2.7.1. Transmission From Soil And Silage

M. bovis may be excreted or secreted into soil from cattle or wildlife saliva, nasal secretions, urine or faeces. The bacteria probably remain viable and pathogenic in soil for about 6 months. Soil can be ingested by cattle, comprising ~5-10% of the fresh-weight intake and 10-15% of dry-weight intake of grazing cattle. The movement of soil-contaminated fodder between farms may also be risky (Phillips and others 2003). Cattle tend to consume soil to offset mineral deficiencies and for behavioural head rubbing, during which they create dust and potentially infectious aerosols. Relatively more soil would be ingested when pasture sward is

short and soil may also contaminate silage. There are limited data on *M. bovis* survival in silage.

Cattle faeces, containing *M. bovis,* were not infectious to guinea-pigs once ensiled with grass for ten weeks in a mini-silo. Lack of oxygen and acidity may have reduced the infectivity. Silage pH declines to approximately pH 4.0, although *M. bovis* can survive for 20 days at pH 4-5 in yoghurt. Temperature during ensiling and storage increases to ~30°C, which is close to optimal for *M. bovis*. There is little evidence that *M. bovis* is killed by ensiling, although they may become dormant. Maize silage is no less likely to maintain *M. bovis* and maize cobs are particularly palatable to badgers. Circumstantial evidence suggests that maize can be contaminated by diseased badgers that contaminate the silage clamp directly or indirectly.

Providing cattle with mineral supplements in the field may reduce the attractiveness of soil. Potentially contaminated soil around badger setts should be fenced off. Silage cannot be excluded as a risk and steps should be taken to avoid contaminating silage fields with slurry and badgers should be kept away from silage pits, particularly maize (based on Phillips and others 2007).

Prevailing weather will dictate the viability of *M. bovis* bacilli on pasture when uninfected cattle graze following infected cattle. *M. bovis* is susceptible to the germicidal UV irradiation provided by sunlight within ~12h duration. In warm, wet and overcast weather contaminated pasture can transmit infection for several weeks following grazing by clinically-infected cattle (Phillips and others 2003). One week after resting pasture, following grazing by infected cows, there was ~6% daily chance of a non-infected cow acquiring infection. After two weeks rest, this reduced to 2% daily. Pathology indicated that infection occurred by both the respiratory and ingestion routes. Infection via the respiratory route could occur following eructation or by aerosol inhalation during grazing. These experiments are quite old and mesenteric involvement and generalized bovine TB is allegedly rare nowadays.

Where infected cattle are detected early and removed, other cattle tend not to become infected with *M. bovis* in the field. In a NZ study, a potentially infectious contact made only 0.007 contacts *per* day. In a large herd with a single reactor, the low rate of infection would not have sustained the disease in the absence of a wildlife reservoir or anergic cow(s) (Barlow and others 1997). If undetected, generalized bovine TB can lead to extensive exposure, infection and disease. For example, in an extensive grazing system in Texas, 27% of a closed herd of suckler cows

were reactors, most of which had gross lesions of a single *M. bovis* molecular type (Perumaalla and others 1999). Infection was probably introduced some 15 years previously, possibly when the herd was first established.

2.7.2. Safe Management Of Animal Effluents

The literature in 1959 suggested evidence that TB could survive in faeces for weeks to months. Reuss (1955) had shown that TB could be cultured from 10% of faecal samples from a herd that was mostly TB-positive. Within faeces the bacteria stayed alive for at least 8 weeks. Messemeister (1958) found by accident that 20 min of direct sunlight killed his TB cultures.

Typically ~10% of cattle with advanced bovine TB were capable of excreting the bacteria in faeces (Schneller 1959), but this could be as high as ~80% (Reuss (1955) cited by Schneller (1959) and DEFRA Husbandry Panel (2003)). Schneller (1959) reviews the potential risk of infecting cattle from grazing areas. In one such study 23 of 42 uninfected cattle picked up TB from a contaminated field.

There is a discrepancy here (or something was lost in translation) because O'Reilly and Daborn (1995) cite the Schneller (1959) study as follows: after 7, 14 and 21 days a total of 56 heifers were allowed to graze plots experimentally-irrigated with 10^2-10^{12} *M. bovis per* ml of water. Only 2 of 14 cattle that grazed the plot irrigated 7 days previously became infected. The remainder showed no signs of infection. Schneller (1959) concluded that the risk of rain washing TB from infected field to neighboring fields was not significant if washed out of the faeces and exposed to germicidal UV light. Schneller (1959) discuss leaving fields empty after cattle for 12, 17 or 34 day periods.

Maddock (1936) had already demonstrated that calves could be artificially infected via ingestion of high doses of an *M. bovis* emulsion. However, no AFB was identified in the faeces of these infected calves. He then dosed these calves with infected whey until such time as they excreted *M. bovis* in their faeces. Interestingly, *post mortem* these calves showed no obvious kidney or mesenteric involvement. These excreting calves were grazed for three weeks following which 2 uninfected calves were introduced to graze for 3 weeks on one of three plots at intervals following removal of the original calves. No signs of bovine TB infection were evident in any of these calves *post mortem*. In a further experiment (Maddock 1936) a cow with TB mastitis and excreting *M. bovis* in her faeces was grazed for 9.5 weeks. Naïve calves were introduced to

contaminated plots at monthly intervals. As before, no infection was demonstrated in such calves.

Clearly, although these are old data the proportion of advanced bovine TB cases excreting may still be right, but hopefully there are now fewer cases with advanced bovine TB. This is probably lower for cattle in the early stages of infection, although the precautionary principle dictates that all infected cattle are considered as potential excretors. Viable bacteria, possibly from swallowed sputum can, on rare occasions, be isolated from the faeces of cattle without lesions in the alimentary canal. Longer inter-test intervals may simply allow more cattle to reach advanced TB, increasing the potential risk of transmission, contaminating slurry etc rather than just allowing the accumulation of reactors.

In faeces on pasture *M. bovis* survival depends on the amount of available sunlight and on the protection afforded by the deposit. Typically, faeces remain infective for up to six months when deposited in winter but only 1-2 months in summer (Mitserlich and Marth 1984) before being biodegraded. Cattle avoid grazing close to faeces initially, preferring to graze mature sward fertilized by the deposit. It seems unlikely that there is any acquisition of *M. bovis* infection directly from faeces deposited by grazing cattle, although badgers will regularly forage such cattle deposits in search of food (eg earthworms). This indirect contact is analogous to reports in the human TB literature of *M. tuberculosis* infection being transferred to immune-competent healthcare staff from cadavers and biopsy material from TB-affected individuals. However, it is noteworthy that such transmission still required the generation and inhalation of aerosols with defined properties.

Solid manure (faeces) is not considered to be a major factor in spread, provided that it is properly composted (for many months) before land-spreading and, importantly, does not result in aerosol production (Collins 2000). *M. bovis* may be excreted in bovine faeces and survive for up to 175 days in slurry. Chemical disinfection should be considered and should not compromise production. Manure and slurry should be stored at least 2 months and 6 months, respectively. 'Thick lime milk' if properly applied should inactivate *M. bovis* within 24h. In addition, slurry should be spread on arable land or grassland destined for silage. Creation of aerosols by rain-gun spreading of slurry is a recognized hazard, including for contiguous farms. Shallow injection and band spreading should reduce the risk of such aerosol drift.

Although not considered high risk, cattle-cattle transmission has been demonstrated experimentally at pasture. Since stocking density influences the probability of transmission at pasture, reducing it should proportionately reduce the probability that cattle contact contaminated grass before any bacteria are destroyed by germicidal sunlight. In ROI studies, up to 40% of infected cattle excreted *M. bovis* in faeces (Christiansen and others 1992). The study identified that grazing slurry-treated pasture, where slurry was stored <2 months, was the main risk factor. However, this risk factor was no longer significant in a subsequent multivariate ROI analysis of persistent TB breakdowns (Griffin and others 1993). These conflicting results suggest that slurry disposal merits further consideration. It will be interesting to see whether slurry management is a significant risk factor in the current DARD Bovine TB Biosecurity Study.

The potential for environmental survival in other mycobacterial pathogens can be illustrated. *M. bovis* is an obligate parasite of animals, and so is *M. avium* subsp. *paratuberculosis* (MAP, Johne's disease) for which environmental contamination is thought to be one of the primary sources of infection for dairy cattle. Unlike *M. bovis*, transmission via ingestion from faecal shedding is the accepted main route and the organism can survive for days to months in the environment, depending on the matrix (Whittington and others 2004). In a recent study in the Netherlands, the environment was sampled following the introduction of two groups of cattle known to be shedding MAP. Bacterial DNA was detected in many sites within the housing, both before and after the introduction of these excreting cattle (Eisenberg and others 2010) and was detected outside the barn in a pattern corresponding to the walking route of the farmer. No viable bacteria were detected before the introduction of excreting cattle, but they were detected in the barn 3 weeks later at 7 of 49 sites and then outside the barn at 15 weeks. This illustrates the potential for widespread contamination of the internal and external farm environment, including the detection of viable bacteria in settled dust, which suggests potential transmission via bio-aerosols.

In a subsequent study, such barns were sampled; with animals present, after destocking, after cold high-pressure washing, after having been kept empty for 2 weeks and after the use of disinfectant (Eisenberg and others 2011). MAP was detected by PCR in 78-86% of samples when animals were present. Viable MAP was detected in 6 of 9 samples and in 3 of 7 samples from different barns. Only 2 samples from each barn were positive for viable MAP after cold pressure-washing and no viable MAP

was detectable if the barn was empty for 2 weeks, or if additional disinfectant was used. No viable MAP was detected in any settled dust samples this time.

Rare and sporadic excretion in most cases suggests that faecal spread of *M. bovis* is probably less important than respiratory spread in the epidemiology of bovine TB (Neill and others 1988). The rate of faecal excretion probably depends on the dose, route, severity and duration of infection. Several older manuscripts describe the viability of *M. bovis* in manure and slurry under various conditions, for various times and at varying times of the year. Taken together, they suggest that *M. bovis* can remain viable for several months under suitable conditions. As before, whether these matrices can be rendered into an infectious aerosol remains to be demonstrated conclusively. Composting farm manure at 60-70ºC for >3 weeks should inactivate *M. bovis*, although it seems unlikely that much solid dung heaps would meet such requirements. Slurry mixing and spreading, especially with a rain-gun, may create aerosols which may be carried for several hundred metres (Hahesy 1995). Movement of slurry tankers within- and between-farm may also be risky (Richards 1972). Although conclusive evidence is unavailable, the spreading of inadequately stored and inadequately inactivated slurry may be a risk factor in repeat breakdowns.

Although considered rare, TB in humans (*M. tuberculosis* and *M. bovis*) can affect the kidneys and bladder, which can lead to high bacterial counts in human urine. Recycled human urine may be used in agriculture and may thus constitute a transmission risk. Studies on the survival of *M. bovis* in the environment report highly variable survival times, mostly long survival. In one such study (Duffield and Young 1985) in Australia, *M. bovis* survived for 4 weeks in non-sterile dry and damp soils in 80% shade, in darkness and in the laboratory. *M. bovis* was not re-isolated at 4, 8 or 32 weeks from any matrix exposed to sunlight or from faeces under any conditions.

If *M. bovis* was spread onto arable land there is a theoretical risk should soil dust be inhaled or ingested. As with all these environmental matrices, it is important to consider under what circumstances such contamination could be rendered into infectious bio-aerosols. A recent study (Vinneras and others 2011) reported a log reduction in *M. tuberculosis* and *M. bovis* viability in human urine containing 7g and 3g NH(3)-N L^{-1}, respectively, in just over 10 days at 4ºC and below 3 days at 22ºC, significantly faster than cited for inactivation of mycobacteria in animal slurry. Storage for 5 weeks below 20ºC, or 2 weeks above 20ºC, is

sufficient to prevent transmission when recycling human urine, values within current WHO guidelines.

Phillips and others (2003) recommend that slurry should be spread on, in order of priority; arable crops, hay crops, silage crops and grazing land. In high risk situations slurry should be stored for >6 months and injection should be encouraged. Farm waste water may be contaminated and could potentially spread infection to cattle, or wildlife. Rain-guns should be discouraged for spreading such water, unless adequately treated. Additional research is needed to investigate whether, and to what extent, slurry lagoons inactivate *M. bovis*.

2.7.3. Infection Via Drinking Water

M. bovis survives in running water and when cultured in buffered saline and egg-based media for 300-400 days and >6 years, respectively (reviewed by Phillips and others 2003). When drinking, splashing could facilitate inhalation of infectious droplet nuclei. Running water could also be directly contaminated with cattle or wildlife excreta. Availability of natural water supplies was not a significant risk factor in a ROI study (Griffin and others 1993), although water troughs may be contaminated with *M. bovis* from cattle sputum or wildlife.

A study of African buffaloes, the maintenance host for *M. bovis* in the Kruger National Park, investigated whether shedding of *M. bovis* in nasal and oral secretions might lead to contamination of ground or surface water and subsequent transmission to other species (Michel and others 2007). *M. bovis* was not detected in trough water, suggesting that diseased buffalo do not commonly shed the organism in high quantities in nasal or oral discharges. Surface water was considered unlikely to be significant in the transmission of bovine TB in this free-ranging ecosystem. However, direct sunlight, temperature, humidity and shade are significantly different in Western Europe.

Regular cleaning and disinfection of water troughs and avoidance of stagnation is advisable, especially if reactors are detected. Where there are signs of badger activity, it would be advisable to prevent badgers from accessing cattle water troughs by raising them to >80cm.

2.7.4. Arthropod Vectors

Arthropod ectoparasites are relatively rare on UK and ROI badgers and would appear unlikely to be vectors of the disease between badgers and cattle (Barrow and Gallagher 1981), although an association between tick-borne encephalitis and human TB has been reported

(Meyerova 1991) but this was more likely complicated by immune-suppression. *M. bovis* has been found in ticks from the skin of infected hosts, and studies in Armenia suggest that ticks can carry viable mycobacteria for months (Blagodarnyi and others 1971, cited in Phillips and others 2003). *M. bovis* may be transmitted through inoculation during tick feeding activities. Although there is a theoretical possibility that ticks may transmit *M. bovis* between wildlife and cattle there is no evidence that modifying husbandry would be effective (Phillips and others 2003).

2.8. Where does cattle-cattle transmission occur?

2.8.1. Within-Herd Transmission In Cattle Housing

Studies of cattle housed indoors with TB reactors confirm that cattle-cattle transmission is possible, although not inevitable. Transmission from experimentally-infected calves to housed in-contact cattle occurred in most cattle within 28 days (Cassidy and others 1999). No infection was reported when 22 infected cattle were housed with 32 uninfected cattle. Twenty of these were housed with 10 infected cattle for 6 months, the remainder for 5 months. Twenty of the 22 index cattle showed gross lesions and 12 of 22 had small lesions (O'Reilly and Costello 1998). In a further study Costello and others (1998) reported that 40% of steers that were initially tuberculin negative developed TB when each was housed with 2 reactors for one year. These studies indicate that, although highly variable, cattle-cattle infection tends not to occur readily. Research in GB in the first half of the 20th century, when herd prevalence was at its height, indicated that transmission was much more likely indoors than at pasture. There may be merit in evaluating within-housing transmission by placing caged guinea pigs in cattle housing.

Today, housing type and quality are risk factors for both human TB and other mycobacterial diseases of cattle, such as paratuberculosis (Collins and others 1994). Whilst the evidence for indoor transmission is convincing, many herds only yield one reactor, indicating that it is not that common; 85% of UK herds (1972-1978) reported only one reactor (Wilesmith and Williams 1986). In ROI, transmission from purchased reactors was also low (Flanagan and Kelly 1996). Cattle kept under good husbandry were less likely to excrete significantly for 4-9 months and may have been detected before they became infectious (O'Reilly and Costello 1988). However, unreactive, anergic cattle may be a persistent

source of within-herd infection and deployment of supplementary humoral immune tests may be advantageous (Clegg and others 2011). High humidity, high cattle density and poor ventilation are ideal for transmission compared to conditions at pasture. *M. bovis* can survive for several months if protected from germicidal UV light and longer if protected in faeces.

An ROI risk factor survey did not find that poor quality housing was a risk factor (Griffin and others 1993), although other studies could not reproduce this (Griffin and others 1996). The ROI study suggested that cows housed in cubicles were more likely to have recurrent TB. Direct contact in cubicles could increase the risk of transmission, especially when the cattle stocking density is high (Neill and others 1989).

M. bovis can be isolated from respiratory secretions in ~20% of reactors in some UK studies, and in the lungs of most reactors with respiratory lymph node lesions (McIlroy and others 1986, Hancox 1999). Infected cattle are regarded as potentially infectious by aerosol. *M. bovis* can be transmitted up to 1.5m in aerosols produced by possums when transmitting to deer (Sauter and Morris 1995). Cattle consuming silage and/or concentrate have been observed investigating their food, perhaps because it was contaminated by previous cattle. Animal behaviour suggests that they may prefer uncontaminated, fresh feed and may reflect the desire to select uncontaminated food (Phillips 1993, cited in Scantlebury and others 2004). Keeping cattle in covered yard housing was a biologically-plausible risk in several studies (Johnston and others 2005, Karolemeas and others 2010) where close contact in shared airspace may increase transmission.

Transmission has been associated with slow air exchanges, either in loose boxes, or in bio-containment facilities, which do not adequately simulate natural air circulation or ventilation (Costello and others 1998). These experiments illustrate that transmission is reduced when ventilation improves. When calves, infected by the intra-nasal route are introduced to penned bovine TB-free calves, transmission occurs if they mix soon after initial infection, but is much less likely if mixing is delayed ~12 weeks. In a study in Ethiopia, the severity of bovine TB was also significantly higher in cattle housed indoors at higher stocking density than in cattle kept on pasture. This close direct contact facilitates the generation and transmission of infectious aerosols between animals (Ameni and others 2006).

Cattle are housed for ~6 months annually in the UK, possibly ~3 months in the ROI (Olea-Popelka and others 2006). However, continuous (12 month) housing of dairy cattle is common practice in countries where the climate does not favour grazing or where land is at a premium. Only ~5% UK herds are kept indoors continually. In Europe this ranges from 0% housed in parts of Sweden to >50% in Alpine regions (Wilkinson and others 2011). Plans to establish very large dairy herds (mega-dairies) in GB have met with mixed reactions. Whilst potentially these farms present challenges with respect to waste management, carbon footprint, pollution, disease, welfare and the behavioural needs of cattle they also present opportunities for greater biosecurity and more skilled husbandry. However, concerns have also been raised about 'the Fourth Freedom' in continually-housed cattle; the freedom to express their normal behaviour (FAWC 2011).

Milk yield is highest in continuously housed systems, followed by mixed systems and then pasture-based systems (Wilkinson and others 2011). Whilst grazing may be the more natural behaviour, it can deprive cattle of shade and expose them to periods of heat and hunger stress. Given the choice, cattle actually prefer to be fed indoors, especially when temperature and humidity are at their highest, and they also prefer to graze pasture at night, probably for the same reasons. High quality grazing alone does not provide a sufficiently balanced diet for optimal milk production. Many dairies are relatively old (Grove-White 2004) and cattle size has increased progressively, making these farms unsuitable for continuous housing. Health and welfare have also declined in recent years (increased mastitis, lameness etc).

Adequate ventilation is very important, but only if stocking density is controlled. Opportunities for transmission will be influenced by ventilation, the configuration of the accommodation etc. Phillips and others (2000) make several recommendations. For cows in straw yards a total of 7.5-9m^2 *per* cow is recommended, and just under one third of this should be hard standing, the remainder bedding. Cubicle housing requires at least one cubicle *per* cow. Respiratory disease is more likely to be transmitted indoors and herd-keepers may wish to consider tuberculin testing on housing to reduce the number of reactors *per* breakdown.

Our opinion is that the type of farm housing may actually influence the opportunity for transmission, especially direct transmission, due to contact rates and limited ventilation etc. For example, small groups of tethered cattle housed in traditional 'byres' may

only be in direct contact with their direct neighbours. Theoretically, larger groups of ~20 cattle kept together in 'pens' could have direct contact with all their pen-mates. ~200 cattle grouped together in a 'cubicle' set-up could also contact all their group mates. In these circumstances, cattle social behaviour, hierarchy and grouping may be quite different from the other housing systems. Hence, the structure of the herd and the contact networks supported within (and between) herds will influence the chain(s) of transmission.

Hygiene and disinfection are important measures to help maintain high herd health status. Depending on the amount of organic material, cleaning should provide a 3 log reduction in bacterial counts, disinfection provides a further 3 log reduction (Bohm 1998). Whilst 'cleaning and disinfection' (C&D) would be expected to be beneficial overall, we were unable to source any specific supporting evidence of their value in bovine TB. High pressure cleaning was also noted to generate secondary aerosols which were undesirable when mycobacterial zoonoses were identified (Bohm 1998).

2.8.2. Within-Herd Transmission At Pasture

Studies in the early 20th century, when bovine TB was much more prevalent, suggested that transmission and infection were much less likely at pasture, and beef cattle that were permanently at pasture were rarely infected (Schneller (1959) cited in Phillips and others 2000). Cattle are most severely infectious 6-20 months after infection, ranging from 87 days to 7 years depending on nutritional status, physiological stress and concurrent disease(s) (Barlow and others 1997). Cattle infected by the respiratory route may initially be mildly infectious before mounting an immune response, a period when *M. bovis* has been isolated from nasal mucus (Neill and others 1992). The period to maximum immune response ranges from 8-65 days post-infection (Phillips 2000) and most UK cattle are believed to be mildly or non-infectious under the present testing scheme.

Such early pasture contamination experiments also showed a different pattern of lesion distribution. Many infected animals produced lesions only in the alimentary tract. *M. bovis* infection in UK cattle is now considered to be primarily a pulmonary disease and the main route of transmission is likely to be via inhalation of infectious aerosols. The infective dose by this route is very low, perhaps as little as one organism when delivered by the principle route and to the correct location (Griffin and Dolan 1995), compared with the oral route, where several million

bacilli may be required to establish infection (O'Reilly and Daborn 1995). In some situations infection by both routes may be possible. For the respiratory route, the size of aerosol droplets appears to be more critical than the absolute numbers of bacilli and is likely to vary between animals and incidents. Cattle do not normally appear to excrete large numbers of *M. bovis* in the early stages of infection and most should be detected and slaughtered before then.

Little onward cattle-cattle transmission was reported from purchased cattle in the ROI (Flanagan and others 1999). Cattle-cattle transmission has been demonstrated experimentally in calves (Cassidy and others 1999), steers (Costello and others 1998) and in grazing heifers (pasture contaminated with *M. bovis*), although no infection was recorded in groups of adult cattle exposed to infectious cattle for 4-7 months at pasture in open-sided pens (O'Reilly and Costello 1988).

In a more recent field study in ROI, cattle tended to be at pasture for ~9 months (March-November) and in housing for ~3 months annually (Olea-Popelka and other 2006). It would seem reasonable to assume that relatively limited opportunity exists for cattle-cattle transmission at pasture, given the enhanced ventilation, reduced humidity and exposure to germicidal sunlight. However, external conditions may not always limit transmission potential and cattle behaviour often results in cattle remaining in close, direct contact outside and occupying a small percentage of the available space. For example, there is ample opportunity for close direct infectious contact between cattle in a large dairy herd mustered outside for daily milking.

2.8.3. Between- (And Within-) Herd Spread

Since the 1970s there have been increases in herd size (Grove-White 2004, TheDairySite 2011) and mobility, providing ample opportunities for both local and long range spread by movement of infected cattle. In a recent GB case-control study the failure to provide outside shelter for cattle at pasture was associated with a decreased risk of a confirmed breakdown, possibly by reducing the opportunities for cattle to be in sustained close direct contact (Johnston and others 2011).

Cattle movements in GB, particularly those from areas where bovine TB was reported, consistently outperformed all other variables as the main predictor of bovine TB disease occurrence (Gilbert and others 2005), supporting the ISG conclusion that cattle-based measures alone should control bovine TB in cattle. However, other analyses of cattle

movement data suggest that between 26% and 85% of cattle herd breakdowns were not caused by cattle moving into the index herd, a percentage that could have been attributed to local effects, possibly infectious wildlife or undetected infectious cattle (DEFRA SE3117). The SGM (2008) were concerned that bovine TB might be self-sustaining in badgers and recommended investigation of bovine TB in badgers, specifically where there were no cattle (or at least no bovine TB).

Despite gaining OTF status in 2000, some regions of France have seen recrudescence of bovine TB in recent years. Having identified that 35% of cases were attributable to local risk (neighbourhood contamination), one such region, the Cote d'Or *department* (Burgundy), was investigated by questionnaire and social network analysis (Dommergues and others 2011). Wildlife (boar, badger etc) populations were dense and were known to contact cattle frequently. The authors suggest that cattle-cattle contact at pasture occurs mostly during the first 3-4 days, until cattle become familiar with each other. The frequency of such contact decreases thereafter, but might still occur, for example if a cow is on heat. Grazing time was ~7 months (=30 days of regular within-herd contact). The study focussed on the consequences of cattle contacts rather than on their frequency. Contacts with badgers were considered to be 'indirect'; comprised of cattle grazing badger latrines or badgers using winter cattle feed and was modeled as of longer duration, equivalent to a short period of very close contact. Farmers perceived the risk of borrowing cattle as less than that of cattle purchase, when the risks were essentially the same and the same control measures should apply. Straying of cattle was under-reported and unrecorded local cattle movements might also contribute to the local risk.

The various between-herd cattle contacts for 22 fragmented, grazing 'store' beef (Charolais) herds were investigated and weighted by expert consensus. The experts proposed that the introduction of cattle constituted the greatest risk, although there was a notable lack of consensus on the relative risks of close proximity to badgers and contiguous cattle-cattle contact. However, no between-herd cattle movements were recorded in the study period, although farmers did exchange cattle with herd-keepers outside the study area. Farms had 0-7 (median=1) adjoining contacts and the total number of contacts was 0-15 (median=3), indicating that farms likely differed in their relative risk. Analysis of potential contact between contiguous cattle at pasture led to a reappraisal of boundary fencing; 38% of fences would not have allowed

contact, whereas 45% would have. Since >80% of between-herd 'barriers' were roads or paths the authors conclude that cattle-cattle grazing contact could be reduced quite easily. The median number of cattle involved ranged from 2-55 (median 18). Their analysis implied that network fragmentation was over-estimated (ie. network cohesion was under-estimated). Considering both the number and strength of ties in the network; cattle contact at pasture dominated the model, followed by badger contacts. The study suggested ample opportunity for between herd cattle-cattle contact; a challenge for control. Nearly all contact types were linked, such that any control measure would be likely to have an effect on several contact types (Dommergues and others 2011).

The delay in removing reactors from herds during the FMD epidemic of 2001 was associated with a significant increase in prevalence of *M. bovis* infection in RBCT-culled and non-RBCT RTA-killed badgers. The correlation between an increase in the number of bovine TB infected cattle due to lack of removal of reactors and the number of bovine TB-infected badgers is a strong indication that there is also transmission of *M. bovis* from cattle to badgers (Bourne and others 2007).

2.8.4. Spread Across Farm Boundaries
The risk of having a reactor increases in contiguous herds (Denny and Wilesmith 1999), but this could be due to a common wildlife source, rather than between-herd spread. 25% of 3,975 breakdowns and 23% of 504 breakdowns in ROI were attributed to 'lateral spread' (Griffin and Dolan 1995), although it is not clear how these distinctions were made. A study in Canada showed that cattle farms sharing a common boundary with a TB-affected herd were more likely to have a breakdown than farms within the locality without fence-line contact, suggesting that cross-boundary transmission between cattle was possible (Munroe and others 1999). Collapsed walls and damaged fences were routes by which cattle could mix uncontrollably, potentially spreading *M. bovis* infection.

Contiguous spread was also suspected in 2 of 11 farms involved in the substantial recent multi-herd outbreak in Germany discussed previously (Section 2.1). Animals were known to mix across poorly-maintained fencing (Probst and others 2011). The existence in NI of sufficient contact between contiguous and associated herds is illustrated by the degree of contiguous spread of *Brucella abortus*, although it should be noted that *B. abortus* is significantly more transmissible than *M. bovis* (Abernethy and others 2011).

The risk factors influencing between-herd spread of bovine TB in Canada were modeled using logistic regression. Two main risk factors were identified; herd size (yet again) and a proxy measure that indicated reasons to consider outbreaks as epidemiologically-linked. The odds ratios were highest for herds identified by 'trace forward'; those receiving reactor(s) (OR 57.8), followed by herds with pasture or fence contact (OR 31.8) and finally herds identified by 'trace back'; a source herd for another herd (OR 14.9) (Munroe and others 1999).

Double fencing of boundary fields will prevent physical contact between cattle on neighbouring farms, but cattle may still lean over and exchange aerosols. Current advisory leaflets (DEFRA, DARD, DAFF) suggest use of double fencing to prevent the spread of bovine *M. bovis* infection between cattle. Fencing with shrubs between the two fence lines will keep cattle apart and may reduce the risk of cattle-cattle transmission. Such fencing might also help to keep cattle off the field perimeter used by badgers for defaecation. However, this may only be short term, since growth and thickening of the shrubbery might also alter badger behaviour. Separation of cattle in contiguous farms should be seen as good management practice, to be encouraged wherever possible. Farmers should be aware of potential aerosol drift from slurry spreading between neighbouring farms.

2.8.5. Local Persistence – Bovine TB Clusters

Bovine TB is a low incidence infectious disease with, on average, an apparently low transmission rate between cattle. The importance of cattle-cattle transmission underpins the long established control measures, such as immediate movement restrictions (Bourne and others 2007). Reactor removal has successfully reduced bovine TB incidence in most of the UK.

The local persistence of infection within small populations, such as cattle herds, depends on the maintenance of unbroken chains of transmission or the import of infection from a source(s) external to the herd (DEFRA SE3230). Regardless of its original source, infection can persist in cattle herds despite regular tuberculin testing, and this facilitates amplification within herds and cattle-cattle spread (Karolemeas and others 2011). It is important to consider the extent to which cattle-cattle transmission, whether driven by susceptibility or not, contributes to maintenance (persistence) of infection and what measures could mitigate or minimize the risk of further transmission. A New Zealand study estimated that a new susceptible cow would be infected

every 4-5 months for each infectious cow in the herd (Barlow and others 1997). For example, one reactor in a 4-yearly tested parish would generate 20% reactors in a herd of 200.

Molecular epidemiology (strain typing) studies demonstrate that distinct genotypes of *M. bovis* predominate in different localities, being found in both cattle and badgers, and associated with local herd breakdowns. A number of distinct clusters occur and have increased or decreased in size locally over time (Skuce and others 2010). Marked geographical localisation of *M bovis* genotypes has also been reported in GB (Smith and others 2006b) and confirmed by type-specific probability mapping (Diggle and others 2005). Clustering would tend to imply that there is ongoing transmission and the emergence and spread of what are clearly new variant *M. bovis* genotypes (R Skuce, unpublished) would tend to support this; their transmission is not being interrupted sufficiently by local controls.

Potential explanations for the geographical localisation phenomenon have been proposed (Hewinson and others 2006). The clonal population structure of *M bovis* (the lack of recombination with other bacterial cells) would tend towards clustering of the same or similar genotypes. The natural and imposed movement of cattle and the local and national efficacy of disease control measures would be co-variables, as would the extent to which sub-sections (compartments) of the cattle population were actually structured and managed separately. The possibility that geographical localisation simply reflects the underlying spatial segregation of the disease in wildlife (Woodroffe and others 2009, Kelly and others 2009), and the possibility that the environment is directly contaminated (Courtenay and others 2007) with potentially viable mycobacteria (Ghosh and others 2009, Mba Medie and others 2011), cannot be currently excluded. Under certain conditions, long-term survival structures (endospores) and even non-acid fast forms (Velayati and others 2011) have been detected in anaerobic culture for both *M. tuberculosis* (Ghosh and others 2009, Singh and others 2010) and *M. avium* subspecies *paratuberculosis* (Lamont and others 2011) and *M. tuberculosis* and *M. marinum* can also infect experimental amoeba hosts (Hagedorn and others 2009).

New breakdowns in geographically-separate and previously TB-free regions can be linked by genotyping to existing clusters. Appearance of TB strain types in distant locations probably results from unwitting movement of TB-infected cattle. Badger involvement is much less likely since badger behaviour and genetics indicate that they tend not to make

very long-range movements. Irrespective of the source of infection, there is a risk that such breakdowns will go on to develop into new endemic clusters if infected animals remain undetected for whatever reason. It can be difficult to determine the source of a TB breakdown in a cattle herd. However, in low incidence areas, there is evidence that cattle-cattle transmission could be responsible for around 80% or more of cases. The situation may be quite different in high incidence areas of GB where 85-90% of confirmed breakdowns arise.

The unwitting purchase of infected animals may account for ~7-16% (Green and others 2008). Infectious wildlife is a source of herd breakdowns (Bourne and others 2007) and in some incidents may be a more important source than cattle. It is impossible currently to put precise figures on these possible sources (SGM 2008). The spatial and temporal correlation reported in breakdowns between neighbouring regions in the RBCT may be due to farm-farm transmission and/or farm-badger-farm transmission where bovine TB persists on farm but not in cattle (Mill and others 2011).

Increasingly, the importance of local persistence of bovine TB has been recognized in published ROI studies. There is a strong association with previous herd history, location and future risk (Griffin and others 2005, Olea-Popelka and others 2008), perhaps as a consequence of persistent infection in cattle (residual infection in cattle and local cattle-cattle transmission, Clegg and others 2011). Reasons for local persistence may include residual infection in cattle and/or an infectious local wildlife source. Recent ROI research concludes that bovine TB is still primarily a disease of cattle (Kelly and More 2011). The observation that bovine TB remains clustered spatially in cattle herds in the ROI Four Areas Project sites subjected to proactive badger culling indicates residual infection in cattle, which is increasingly recognized as a significant feature in the ROI epidemic (Kelly and More 2011).

The local persistence and spread of bovine TB in cattle, which has resulted in clusters of infected herds, has typically been ascribed to the existence of local wildlife reservoirs of infection. The part that cattle play in local spread and persistence of TB in cattle herds is not yet quantified. Cattle movement data highlight the extent of local cattle movements that typically take place as a result of normal farm trading practice and suggest that this represents a considerable risk of spreading disease (Mitchell and others 2005). There are high levels of cattle movement between farms in high TB risk areas. The replacement of reactor cattle, and the sale of cattle following de-restriction, creates its own momentum

and due to the poor sensitivity of the tuberculin test, as applied, some of these moved animals are potentially infectious. Moving undetected, infectious cattle serves to shift the TB risk from the seller to the purchaser.

Following the FMD epidemic in GB in 2001, a study on the potential for cattle-cattle transmission in completely restocked herds was undertaken (Carrique-Mas and others 2008). Routine skin testing and cattle movements were suspended during the epidemic. Mathematical modelling identified basically three risk factors associated with increased risk; increasing herd size (OR 1.38-10.75), a bovine TB history in the restocked farm (OR 2.9-5.9) and the purchase of cattle from high risk herds (OR 1.35-9.27). Therefore, purchase of cattle from high risk herds and persistence on farm were associated with TB risk. Whilst it could be argued that routine tuberculin testing should have removed most of these infected cattle, subsequent multi-level analysis on farms in SW England concluded that cattle were more likely to react to the tuberculin test if they were present at a previous test(s) where reactors were disclosed and this was correlated with age and number of tests. Cattle on restocked farms were significantly less likely to react to tuberculin tests than cattle on continuously stocked farms and newly-formed herds presented a reduced risk, highlighting exposure to infected cattle (at or around prior test(s)) as a source for cattle becoming reactors at subsequent tests (Ramirez-Villaescusa and others 2009). Observational VSD data would support this finding. Confirmed home-bred cases, with the same *M. bovis* genotype, are often identified as present, and tuberculin negative, or inconclusive, in previous tests which disclosed reactors (TR Mallon, pers comm).

The Warwick study concluded that cattle-cattle transmission was still important and that further targeted control would be beneficial, in GB at least. The observed increased risk decayed exponentially over time following the last breakdown and although the distribution of reactors at the first test post-FMD was highly skewed, it was consistent with the risk of infection *per* animal depending on the number of infected cattle. The variation observed was best explained by invoking cattle-cattle transmission. There is an associated 'stationary' breakdown risk for persistence on farm, outside of cattle, which decayed with time since last breakdown. Further cohort study analysis of continuously-stocked and re-stocked herds following FMD indicated that an observed lower risk of herd breakdown in the first year after restocking might be due to a temporary reduction in the infectious load on farm. However, this

reduction did not persist following the (re)introduction of cattle, suggesting that cattle themselves were contributing significantly to the observed persistence (Ramirez-Villaescusa and others 2009).

3. SUSCEPTIBILITY

3.1. Nature And Nurture

Patterns of infection at the population scale are determined largely by susceptibility to infection and the impact of infection on infectiousness. Several risk factors (biological, behavioural, environmental and genetic etc.) converge to influence susceptibility (Morens and others 2004). However, risks can broadly be separated into genetic and non-genetic (environmental) risk factors, which act jointly to influence susceptibility. This is the classic nature *versus* nurture scenario (van der Eijk and others 2007). Given that there is observable variation in the outcome of exposure and infection, how much of that variation (difference between individuals) is attributable to 'genetic' effects (ie. heritability) and how much is due to environmental factors? Genetic and non-genetic risk factors are intimately linked, such that environmental factors can maximize the genetic potential and heritability of a population for a given trait, such as TB resistance, and *vice versa.*

In population-based studies it may be possible to estimate the size of this genetic component, if any. This is known as heritability (h_2) and essentially indexes the degree to which progeny resemble the characteristics of their parents. There are a number of different ways that h_2 can be estimated and it is usually recorded on a scale of 0.00-1.00. More correctly, heritability is a measure of the extent to which the observed variation in the measured characteristic is determined by genetic variation in the host. For example, human height has a heritability $h_2=0.8$, which is exceptionally high. However, it also recognizes that 0.2 (20%) of the variation is influenced by non-genetic or environmental factors such as diet and living conditions etc. This is important, because it implies that both genetic and non-genetic components are amenable to manipulation to improve the outcome.

Similarly, milk yield in dairy cows has significant heritability ($h_2=0.30$), which has allowed the selective breeding for increased milk yield seen over recent decades. A further example is resistance to mastitis, which has a relatively low heritability ($h_2=0.06$). Despite this and the observation that breeding for improved mastitis resistance is associated with some detrimental effects (antagonisms) it is still the focus of selective breeding, especially in Scandinavia. Heritability has traditionally been estimated using quantitative genetics approaches with large industry performance and pedigree databases. However, this is only one way to estimate heritability and it may not always produce accurate

estimates. With the advent of high-throughput cattle genotyping it is becoming possible to estimate heritability more directly having determined the actual genetic relatedness within the sampled animals. This may become a more appropriate estimate in future.

It may be possible to reduce the potential susceptibility of cattle to infection by selective breeding and by the maintenance of high herd health status to minimize the impact of concurrent disease.

3.2. Human TB Susceptibility

Human TB and bovine TB have much in common. Hence, it is valuable to be aware of those risk factors which influence susceptibility to human TB.

3.2.1. Human TB – Genetic Susceptibility

With hindsight it is interesting to note that even before Louis Pasteur developed his 'germ theory' in the late 1800s and before the modern understanding of genetics, TB was viewed as essentially an inherited condition (Alcais and others 2005). Despite the recognition of the aetiological agent (*M. tuberculosis*), Pasteur maintained that predisposition to infectious diseases also had a genetic component.

Modern epidemiological studies in regions where human TB is highly endemic reveal a consistent pattern of host population structure (stratification). ~20% of individuals retain negative tuberculin tests throughout their lives, despite repeated exposure to TB bacteria. It would be highly unlikely that all such individuals were immune impaired, so a substantial proportion of these individuals are likely to be naturally resistant to infection (Alcais and others 2005). There are actually two major clinical forms of human TB, corresponding to two age-dependent peaks of incidence; disseminated disease in young children and lung infection in adults (reactivation of latent TB from a silent primary infection), which may be viewed as two distinct 'genetic' diseases (Alcais and others 2005). A recent review concludes that transmission among close schoolchildren contacts (aged 3-11 years) in school outbreaks tended to be higher if the index case was a child rather than an adult (Roberts and others 2011).

Although millions of new cases of human TB occur annually, not everyone exposed to the bacterium becomes infected and nor does everyone infected develop signs or symptoms of disease. Genetic susceptibility to TB infers that 'genes' make someone susceptible to developing TB when exposed to the TB bacterium. Research has not yet

revealed why most human TB-infected individuals never progress to active human TB although it is known that host genes, the environment and the bacterium may all contribute to the final outcome of infection (Moller and Hoal 2010).

The contribution of genetic variation (heritability) for human TB is substantial, with h_2 estimated at <0.71, depending on the phenotype or trait measured. Consequently, genetics researchers now consider that *"predisposition to TB is largely inherited"* (Alcais and others 2005). This derives from classical quantitative studies in twins and other populations. Identical twins have significantly higher concordance rates for human TB than do non-identical twins. Comstock (1978) estimated a 2.5-fold higher concordance for human TB in identical twins compared to non-identical twins and implied inherited susceptibility to be a major risk factor. Despite this and earlier studies being re-evaluated to conclude that environmental risk factors (especially exposure to pathogen) were more significant risk factors for human TB, they still demonstrate a significant role for genetic susceptibility (van der Eijk and others 2007).

Concordance rates among family relatives also increase with the closeness of the blood relation to an extent not easily explained by non-genetic factors. Recent genetic mapping studies identified genetic variation that determined whether or not the individual would react to the tuberculin test. A subsequent study identified further genetic variation that explained some of the range of possible tuberculin test outcomes (Alcais and others 2010). Genome-scale studies are now possible (Newport and Finan 2011) and the race is on to identify the structural human genetic variation associated with TB susceptibility. The first such report identified a susceptibility locus on human chromosome 18 (18q11.2, Thye and others 2010) in a population from West Africa (The Gambia). This association has since been replicated in another West African population sample (Ghana, Thye and others 2011).

It is worth noting at this point that there is currently a significant disparity between the heritability estimated from twin and family studies and that detected in the genome-wide association studies, which only explain ~5% of the variance; the so-called "*missing heritability problem*" (Furrow and others 2011). Encouragingly, advances in understanding other sources of genetic variation, including that present in the vast amounts of non-coding "junk" DNA as well as expressed (epigenetics), as opposed to structural, genetic differences and environmental interactions are closing this gap (Furrow and others 2011). Sequencing advances,

including 'exome' sequencing, have been such that it may soon be cost-effective to 'simply' sequence entire genomes for cases and controls to minimize this missing heritability.

3.2.2. Human TB – Non-Genetic Susceptibility Factors

Selected non-genetic risk factors merit special attention (Sbrana and others 2011). In terms of concurrent infection, the most significant by far is the very dangerous liaison with HIV/AIDS (Mofenson and Laughon 2007). Co-infection with HIV constrains the lifetime risk of TB to an annual risk and whilst death certificates may state that the case was HIV+, it was often TB that killed them. HIV is a significant risk factor, especially in Asia and sub-Saharan Africa. Indirect evidence suggests that HIV promotes immune-suppression, making the case especially susceptible to lethal and otherwise non-lethal infections. Immune-suppression caused by HIV may also drive the reactivation of latent TB and may also modify the infectiousness of HIV-TB co-infected patients. Molecular epidemiology studies clearly demonstrate the explosive potential for TB to progress to disease and spread amongst HIV+ individuals (Burgos and Pym 2002) and that some HIV/TB co-infectees are exceptionally infectious in contact networks.

Several non-genetic susceptibility factors are linked to non-communicable diseases (NCDs), which are now alarmingly on the increase (Creswell and others 2011). Whilst it is beyond the scope of this review to discuss those factors in any detail, it is worth listing them at least: concurrent infection, malnutrition (especially protein deficiency), low body mass index, poor sanitation, poor ventilation, over-crowding, social and environmental factors, alcohol-related conditions, smoking-related conditions, drug-related conditions, vitamin D deficiency, types 1 and 2 diabetes, age, chronic renal failure, chronic obstructive pulmonary disorder (COPD), solid organ transplant, fibrotic lesions, silicosis, cancer (disease and treatment), mental illness etc. (Creswell and others 2011, ECDC 2011, Vento and Lanzafame 2011, Murray and others 2011, Dye and others 2011). In several human populations, Malawi and India for example, young women and older men are at increased risk of human TB (Crampin and others 2004), although TB risk was not associated with pregnancy. This apparent sex difference in risk for younger women may actually better reflect malnutrition, rather than a genuine sex-linked risk.

The location of social interaction associated with some of these conditions or lifestyles may also increase TB risk. For example, in

Minneapolis in 1992 population-based TB genotyping demonstrated that one substance-abuser was responsible for 35% of all new active TB cases, including significant spillover into the general population (Burgos and Pym 2002). The population-attributable fractions of some of these risk factors are significant and population-level interventions to reduce the frequency of NCDs could have a dramatic effect on human TB prevalence.

In many European countries the impact of NCDs is much higher than for HIV (Creswell and others 2011). Some of these risk factors are likely to be relevant for bovine TB susceptibility in cattle. Interaction between vitamin D-deficiency and type 2 diabetes has also been reported in humans and evidence supports a role for both as risk factors for human TB (Goldhaber-Fiebert and others 2011). Vitamin D deficiency is a leading example of a non-genetic, environmental (nutritional) risk factor and human TB is listed as one of the vitamin D-sensitive diseases (Grant 2011, Realegeno and Modlin 2011). Martineau and others (2011) found a significant association between vitamin D deficiency and susceptibility to TB, the impact of which was greater in HIV+ than HIV-individuals. They also described a striking temporal relationship between vitamin D deficiency and TB. Vitamin D, an immuno-regulatory hormone, has an important role in regulating calcium metabolism. Human TB cases had lower serum vitamin D concentrations than matched control contacts, indicating that other factors, such as abnormal processing of vitamin D, were contributing to vitamin D deficiency in TB cases (Sita-Lumsden and others 2007).

Vitamin D deficiency seems to be a risk factor for several other infectious and non-infectious diseases (Fabri and others 2011). Vitamin D would appear to be protective in a *M. bovis* mouse model as well as in human association studies with *M. tuberculosis* (Hart and others 2011). It has even been proposed that increasing serum levels of 25-hydroxyvitamin D *via* oral therapy or increased UVB irradiation (sunlight) would be a cost-effective way to reduce global mortality rates for several diseases, by between 7.6% and 17.3% (Grant 2011). Micro-nutrient deficiencies and indeed excesses may cause secondary immunodeficiency and infection-related morbidity in humans. Iron excess is associated with increased susceptibility and more aggressive disease in human TB (O'Donovan and Milburn 2010).

To complicate matters further, intriguing interactions are reported between helminth parasites (Potian and others 2011), which are highly prevalent in developing countries, and diseases including human TB,

type 1 diabetes, asthma, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease (Liu and others 2010). With modern changes in lifestyle some of these ancient interactions, which helped to maintain homeostatic inflammatory responses have been broken down, leading to a rise in chronic inflammatory and allergic diseases; the "hygiene hypothesis" (Rook 2009).

Chronic exposure to helminths conditioned the immune system to tolerate "the unavoidable" (Ehlers and Kaufmann 2010). Interactions between helminths and microparasites can influence susceptibility to HIV, human TB and malaria. Evidence from animal models suggests that helminths can prevent autoimmune and allergic inflammatory disease, but worsen protective immunity to various infectious pathogens, including *M. tuberculosis* (Elias and others 2005) and may contribute to reduced potency of BCG vaccination.

Whilst the underlying mechanism has not been identified, it is known that a pre-existing helminth infection can impact on immunity to *M. tuberculosis* infection. Potian and others (2011) showed that experimental co-infection of mice with the helminth parasite *Nippostrongylus brasiliensis* enhanced the persistence of *M. tuberculosis* infection and infection with *N. brasiliensis* compromised protection against subsequent *M. tuberculosis* infection (Leavy 2011). There are also interactions between helminths and HIV in humans (Borkow and others 2007).

3.3. Bovine TB – Genetics And Susceptibility

"All animals are equal, but some animals are more equal than others."
George Orwell (Animal Farm)

Although all animals can become infected and diseased, some animals appear to be more susceptible than others. Genetics research aims to find out the extent to which differences in susceptibility (or resistance) are due to the genetic makeup of animals, and then to find the gene variations responsible for those differences. The contribution that both host and pathogen genetic backgrounds make to disease outcome has, until recently, been largely overlooked (Comas and Gagneux 2011). This subject has recently been reviewed (Allen and others 2010, Moller and Hoal 2010, Driscoll and others 2011, Berry and others 2011a).

It is biologically untenable that genetic variation in both host and pathogen does not play a role in the outcome of exposure to TB bacteria. Research in this area should lead to greater understanding of disease mechanisms, inform production of new diagnostic tests and vaccines and lead to identification of sire families and genetic markers associated with disease resistance. Genetic variation may be expressed in resistance to infection, in the response to the diagnostic tests, or both, and it may also determine infectivity once infected. It also implies that not all animals will respond in the same way to exposure, disease and diagnosis. There is anecdotal evidence that certain familial lines of cattle show particular susceptibility to bovine TB. Breeding for resistance to bovine TB in the national cattle herd could produce significant benefits and complement existing control measures relatively quickly. Allen and others (2010) discuss how host and pathogen genetic variation can affect TB disease status and discuss the opportunities now available to exploit modern advances in quantitative genetics, molecular and genetic epidemiology and genomics to ascertain mechanisms underlying the interplay between both organisms.

Given the difficulties in eradicating bovine TB, additional or complementary control measures should be considered. One approach could be to exploit the host genetic variation in response to TB, as seen in studies conducted in red deer (Griffin and Mackintosh 2000) where results of experimental challenge with *M. bovis* evidenced a wide spectrum of responses and a high heritability of resistance to TB ($h_2 = 0.48 \pm 0.096$). This result indicates that approximately 48% of the variation seen in response to infection with *M. bovis* is due to host genetic variation.

Interestingly, in cattle, Ameni and others (2007) demonstrated differences in susceptibility to bovine TB at the level of genus, indicating that the humped-back cattle indigenous to India, Africa and South America (*Bos indicus*) were more resistant than European breeds (*Bos taurus*). Holstein cattle showed higher skin test prevalence and disease severity compared to their zebu herd-mates. Experimental studies have indicated that subtle differences in cytokine expression exist between Holstein and Sahiwal zebu cattle, with TB infection-induced IL6 significantly elevated in the more susceptible Holstein cattle (Vordermeier 2011). Reduced susceptibility in zebu cattle may also be a function of the much longer co-existence of bovine TB with *Bos indicus* than with *Bos taurus*.

Recent findings have also demonstrated significant heritability to susceptibility to bovine TB in Holstein cattle in the ROI (Bermingham and others 2009) and in the UK (Brotherstone and others 2010). Furthermore, as modeled by Bishop and Woolliams (2010), field studies are likely to underestimate true heritability, due to unequal exposure to the pathogen and incomplete sensitivity of the diagnostic tests and could underestimate the potential of breeding for disease resistance. Developments in genetic selection technologies (reviewed by Berry and others 2011b) may be especially useful for traits with relatively low heritability that are only evident after exposure to pathogen or environmental risk factors as adults (Berry and others 2011a). A potential breed effect has been suggested (Vial and Donnelly 2011) and inbreeding has been observed to increase susceptibility to bovine TB in other mammals (Trinkel and others 2011).

3.3.1. Host Genetic Variation

"Mutation is the ultimate source of all genetic variation."

Ernst Mayr

Studies on humans and mice have indicated evidence for host genetic variation in TB resistance, although there have been difficulties in identifying the genes which underpin these outcomes. Recently, excellent reviews on mice and human genetic studies in relation to TB (*M. tuberculosis*) have been published (Hill 2006, Fortin and others 2007). Despite considerable evidence of a genetic component to bovine TB resistance, only modest effort has been directed towards identifying the DNA sequences which underpin bovine genetic susceptibility.

Over millennia cattle have been acted upon by Darwinian natural selection, some of which was probably driven by a response to pathogens. For natural selection to act there needs to be genetic variation in the population and a difference in reproductive fitness between cattle which differ in that trait. Post-domestication, they have also been subjected to significant artificial selection. It has been proposed that for human TB, natural selection against 'TB susceptibility genes' could explain different resistance to TB in different populations. Many centuries of host-pathogen contact in Europe may actually have selected a more resistant human population (Lipsitch and Sousa 2002).

Deer are found as both wildlife and livestock in New Zealand, Australia, USA and Canada (Griffin and Mackintosh 2000). They are susceptible to bovine TB and can act as wildlife reservoirs for cattle.

Differential susceptibility to TB had been observed in deer during severe outbreaks and differential disease transmission rates had been attributed to the host genetic background (Mackintosh and others 2000). Responses of deer to experimental infection with a low dose *M. bovis* inoculum resulted in a continuous normal distribution pattern of response to disease with animals ranging from apparently highly resistant to highly susceptible and estimated a heritability of TB resistance in deer to experimental infection with *M. bovis* of 0.48 ±0.09. This result suggested that it may be feasible to select for TB resistance in deer, and that the high estimated heritability in deer under experimental challenge could indeed be more accurate than heritabilities estimated in cattle under field conditions. Further, it was the first rigorous study to confirm that TB resistance is a heritable trait in a livestock species.

Early studies demonstrating that zebu cattle (*Bos indicus*) were more resistant to TB were conducted in India and in Uganda in the 1930s. In Uganda, the incidence of bovine TB (1931-1936) was 17% in Ankole cattle and only 0.9% for Zebu. Furthermore, a small-scale inoculation experiment was conducted and whilst all 3 Ankole cattle inoculated died, only 1 of the 8 zebu calves died (Hutt 1958). Research in Simmental cows found evidence of differences in bovine TB prevalence in daughters of two different sires, being 4% and 62%, respectively (Ruppert 1935). Differences in prevalence in daughters of different sires were also found in Black Pied Lowland cattle (Hutt 1958).

European *Bos taurus* cattle appear to be more susceptible to *M. bovis* infection than *B. indicus* cattle. Ameni and others (2007) presented data from a cohort of 2,500 zebus, 1,900 crossed (zebu-Holstein) and 900 Holstein cattle. Not only was bovine TB prevalence higher in the Holstein population, but also the severity of pathology in skin test positive animals were significantly greater. Disease risks were also estimated, with Holstein cattle 2.32-fold more likely to be diseased than zebu cattle.

Much is made of the potential impact on the bovine TB epidemic of the introduction of substantial Holstein/Friesian genetics into the UK and ROI in recent decades (Grove-White 2004). However, studies are really only now in a position to address this suggestion. Current genetic studies are focused on the Holstein/Friesian cattle. It will become important to replicate such studies in other breeds.

3.3.2. Quantitative Genetics Studies

Critical to these quantitative and molecular genetics studies are the accurate indexing of disease status (known as 'phenotyping') and the availability of large animal-level test, movement and performance datasets (Bermingham and others 2009). TB in cattle, as in all animal models and human studies, presents as a spectrum of infection outcomes (American Thoracic Society 2000, Young and others 2009, Barry and others 2009). This classification is a departure from the classical view that TB infection has a binary outcome. Extrapolation of this spectrum to cattle would more clearly define animals as having susceptible or resistant phenotypes in future epidemiological studies. It is crucial to define the properties of 'cases' and 'controls' for these genetic studies. Errors in phenotype assignment lead to reduced power to detect associations and reduced ability to replicate findings in other populations. With many complex traits or inherited diseases, phenotype definition is straightforward, provided that the trait can be measured or an objective scoring system can be applied. With host susceptibility to infectious diseases, this becomes significantly more complex. In a case-control scenario such as this, ensuring that the controls have had equal (or greater) chance of exposure to pathogen as the actual cases is difficult and yet crucial to correctly classifying individuals as having susceptible or resistant phenotypes.

We propose that the most **susceptible** animals are those which have an acquired immune response to *M. bovis* antigens, exhibit evidence of pathology in the form of granuloma and lesions and are culture positive for *M. bovis*. The most **resistant** animals have exhibited multiple negative skin tests despite having been raised contemporaneously with skin test positive animals with evidence of pathology and culture. Provided these potential control animals were present in the TB-affected herd for sufficient time prior to disease breakdown, and are of a similar age to the TB-affected cases, these criteria serve as an effective control definition since both sets of animals occupy the same epidemiological group and are likely to have been equally exposed to the pathogen (Allen and others 2010).

Population-level studies have also demonstrated genetic variation in resistance. The extent of the genetic contribution to variable traits such as resistance is summarized as heritability (h^2), which is the extent to which phenotypic differences between animals are due to additive genetic effects (Falconer and Mackay 1996). Heritability is an important parameter since it is one of the factors determining the potential success

of breeding schemes in livestock production. A heritability of 0.06-0.08 for resistance to TB in black and white cattle has been estimated in South Siberian populations, where TB incidence was recorded for many years. As discussed previously, there may be other ways to estimate heritability directly from genotyping data.

The first large-scale quantitative genetic study, carried out in the ROI, estimated heritability of TB resistance to be 0.18 ±0.04 (Bermingham and others 2009). A parallel study in GB dairy herds estimated heritability for TB resistance to be 0.18 ±0.04 on the liability scale (Brotherstone and others 2010). The use of field data is predicted to underestimate the true role of genetics. Due to the anticipated unequal exposure in an infectious disease and the sub-optimal performance (especially sensitivity) of diagnosis (Bishop and Woolliams 2010) these heritability estimates have been been adjusted upwards to h_2=0.24. This brings heritability of tuberculin response and disease closer to that for milk yield, which has been a major driver in genetic selection in dairy cattle in recent decades. This also suggests that reduced susceptibility should be achievable through genetic selection.

Interestingly, in the GB study, TB susceptibility was not associated with milk productivity in the dairy cattle studied. This implies that the recent GB bovine TB epidemic was unlikely to have been driven by the breeding goal of increased milk yield over recent decades. Conversely, it implies that breeding towards bovine TB resistance should not adversely affect milk yield. In hindsight, it is noteworthy that the ISG, in response to suggestions from the Independent Husbandry Panel (IHP, Phillips and others 2000), were sceptical of the potential role of cattle genetics (Bourne and others 2007) and felt that such a breeding programme would take a very long time, and may not successfully maintain the characteristics currently selected for in agriculture.

The ROI and GB quantitative genetic studies demonstrated a strong genetic correlation between susceptibility to confirmed *M. bovis* infection and *M. bovis* tuberculin responsiveness. They address one of the major concerns of the agricultural industry; will breeding for bovine TB resistance, based on diagnostic testing results, merely produce cattle that still become infected but fail to be detected because of a lack of response to the diagnostic test? These findings allay those concerns by indicating that selection for animals resistant to tuberculin responsiveness will indirectly select for resistance to bovine TB infection. The correlation between bovine TB tuberculin result and confirmed disease status was very high and these results infer that the national

tuberculin programme is effectively removing the most susceptible animals from the population (Bermingham and others 2009).

Intriguingly, a subsequent ROI study determined that reactors, rather than being the most productive cattle in the dairy herds studied, tended to be relatively poor milk producers, at odds with frequent herd-keeper claims that bovine TB always affects their best producers. The study also suggested that their under-performance preceded their infection with *M. bovis* (Boland and others 2010). The biological basis for this observation might be associated with increased susceptibility, for whatever reason, among lower producing dairy cows in this study. However, further work found no significant association between somatic cell count (SCC), a proxy for udder health, and tuberculin test reactivity. In this relatively small study, udder health was not associated with bovine TB infection status (Boland and others 2011). Perez and others (2011) reported a reduction in daily milk yield in Argentine dairy herds, but only during the lactation period when cows first tested positive. Such cows were also more likely to abort (OR=45) for a second time, than cows that were continually tuberculin test negative. On average, reactors were not pregnant for 17 days more than test negative cows. The authors don't speculate whether this is association or causation.

Furthermore, a recent ROI study investigated the interaction between bovine TB resistance and other economically-important production and quantitative traits. Most of the associations, with the slight exception of increased 'survival', appeared to be 'neutral'; selective breeding towards bovine TB resistance, based on tuberculin test results, should not have a significantly negative impact on other desirable production traits. The authors caution a balanced approach to managing the various breeding goals of productivity, animal health and welfare (Bermingham and others 2010).

One of the significant read-outs of these large-scale quantitative genetics studies is that it should ultimately be possible to rank the bovine TB risk of individual sires (sire relative risk), based on the bovine TB status that follows in their progeny. This value is likely to be built into the breeding index or estimated breeding value (EBV) for individual cattle, which is calculated by quantitative geneticists. Sire relative risk rankings for Holstein-Friesians in the ROI and UK are currently being developed and are likely to overlap significantly, since the same proven sires tend to be used in both regions. Implementation of selective breeding should be possible in the near future, even without

understanding the structural or expressed genetic variation that leads to variation in the resistance/susceptibility phenotype.

These encouraging findings indicate a role for genetics in a wider risk management strategy. They provide highly significant evidence of genetic variation in the ultimate fate of a cow during a bovine TB breakdown. For example, in dairy cattle, exploitation of genetic variability has already been established and used in selection programmes for mastitis resistance (Rupp and Boichard 2003). Although the heritability of resistance to clinical mastitis is relatively low (h_2 = 0.05 in an ROI study, Berry and others 2010) and has been adversely correlated (antagonistic) to some production traits, selection for mastitis resistance is nevertheless implemented in selection programmes in several countries, most notably in Scandinavia. The same could be considered for resistance to bovine TB. The idea of breeding cattle with increased resistance to bovine TB is not new, having been raised well before the modern genomics era. In the early 1900s efforts were already underway to increase resistance to TB by breeding (Waddington 2004). Indeed, Dutch cattle breeders in the 1940s, convinced that artificial selection for production traits alone was ill-advised, developed the modern Friesian breed as a more robust dairy cow since anecdotal evidence suggested older lineages were more susceptible to disease (Theunissen 2008).

3.3.3. Molecular Genetics

Resistance to infection and disease caused by members of the *M. tuberculosis* complex is likely to be under the control of many genes (polygenic). To date, human and mouse candidate gene case-control studies and heritability estimates have laid much of the groundwork for the next phase of research into the genetics of TB resistance. These genetic epidemiology studies have a common strategy; to compare the genomes of affected 'case' animals to those of matched unaffected 'control' animals to find stretches of variable DNA (allelic variants) that segregate and are significantly associated with one or other phenotype.

The identification of significant heritability to tuberculin test and infection outcome is an important milestone. Sufficient variability and sire relative risk exists that selective breeding could soon be considered. Indeed, genetic gain is predicted to be relatively quick (Brotherstone and others 2010) and could take the form of promoting breeding from lower risk sires or avoiding the use of high-risk sires. For the industry, this would depend on the performance of breeding animals for competing production traits. Susceptibility or resistance could be built into the EBV

for pedigree cattle. This should be achievable relatively soon. Hence, genetic progress could, in theory, be made without understanding the genetics which underpins the resistance or susceptible phenotypes.

However, there are significant design issues, including population stratification (structure), study power etc. (Stein 2011) and studies, if run at sufficient scale (power) can be expensive, resource- and data-intensive. To control for stratification, it is reasonable initially to concentrate on powered studies in one breed. There are probably two types of genetic variation, those that act within a breed and possibly others that act across different breeds.

However, stunning recent advances in genetics, genomics and DNA sequencing and genotyping technologies (Berry and others 2011b) have made these ambitious studies achievable. In fact, current and future capability was unthinkable just 5 years ago. The studies are now capable of indexing at very high density the genetic variation which separates cases from controls. One approach has been to look for genetic variation in biologically-plausible, potential candidate genes likely to be involved in innate or acquired immune responses, and some provisional results are being reported from relatively small studies. Whether these are robust and repeatable remains to be demonstrated (Adams and Templeton 1998, Barthel and others 2000, Driscoll and others 2011).

However, due to the stunning achievements of the bovine genome sequencing project (The Bovine Genome Sequencing and Analysis Consortium 2009, Larkin 2011) and associated genetic marker discovery (Bovine HapMap Consortium 2009), a more shotgun approach can be adopted which does not require any *a priori* knowledge of the role of genetic variation. Genome-wide association studies (GWAS) now index the genetic variation at >700,000 genetic markers (SNPs) in cattle. Consequently, this whole genome strategy should more accurately identify the network of genes involved in variation in resistance and serve to highlight the importance of previously undiscovered mechanisms and pathways crucial to the host-pathogen interaction in bovine TB resistance.

The first of these studies has just reported preliminary findings (BBSRC 2011) which indicate that some degree of resistance to bovine TB is inherited. Whilst the project team did not expect to find "*the bovine TB gene*", the GWAS did identify a number of genetic markers associated with the risk of acquiring bovine TB if exposed; some were associated with increased risk and some with reduced risk. Subject to further research and validation, these provisional results suggest that it might

be possible to selectively breed cows which are more resistant to bovine TB and members of the research team are now working with an industrial partner, DairyCo, to explore the possibility of implementing selection for increased resistance in commercial dairy cattle. It may also be possible to develop DNA tests which have predictive value for susceptibility. Further structural genetic variation, such as DNA repeat copy number variation (CNVs) or insertion/deletion events (InDels) could be investigated in future. Analysis of expressed genetic variation (epigenetics) may also indicate associations with susceptibility or resistance phenotypes. This genomics approach offers a clear route to full exploitation of the genetic variance in selection (JA Woolliams pers comm).

If bovine TB susceptibility is a complex genetic trait, which we currently think it is, governed by the combined input of small effects spread over many genes or non-coding control regions, then a large number of cases and controls will be needed to achieve the statistical power to detect variants eliciting small effects on relative risk of disease. Having a properly designed study of a large enough size and power is critical. Too small a study size can lead to spurious associations between genotype and phenotype, exacerbating the so-called *"Beavis effect"* or *"winner's curse"* (Xiao and Boehnke 2009) in which significant associations are biased upwards. As a result, these studies generally require several hundred or thousand case and control samples.

The genome is now viewed as a single entity whose component genes interact, contributing to specific phenotypes (Moller and Hoal 2010). Recent advances are taking the field of genetic epidemiology beyond the concept of associating individual genetic polymorphisms with phenotypes. Daetwyler and others (2008) reported on the possibility of using novel methodologies to associate whole genome variation with disease phenotypes. This technique attempts to estimate the maximum value of the genetic component of a phenotype by including all loci genotyped. In this way, the effect of multiple variants whose low relative risk may have meant they went undiscovered, are included in the whole genome prediction. Such predictions may then be made on non-phenotyped animals, enabling selection of resistant animals with a greatly reduced need for large-scale phenotyping. This concept is an extension of genome-wide or genomic selection using SNP arrays (Meuwissen and others 2001), which are now commonplace in most major advanced dairy cattle breeding programmes.

It is possible that *M. bovis* and cattle may have reached an evolutionary stalemate, as has been proposed for humans (Muse Davis and Ramakrishnan 2009). Whilst this proposed tolerance of host for pathogen and *vice versa* may well be an example of co-evolution to maximise the survival of both, some commentators suggest that the balance of power still resides with the pathogen (Paige and Bishai 2010, Cardona and Ivanyi 2011), thereby hampering eradication. By improving the genetic resistance of the national herd, the balance in the evolutionary arms race could be tipped back in favour of the host. This genetic approach could well be more sustainable than the current test and slaughter protocol. Furthermore, identification of the novel biochemical pathways and networks involved in host response to pathogen could inform future efforts to produce better diagnostic tools and vaccines. It represents a powerful, valuable and responsible use of industry and competent authority data.

There may be other benefits to this strategy. Several other obligate intracellular bovine pathogens, notably *Brucella abortus*, *Salmonella enterica*, and *Mycobacterium avium paratuberculosis*, may interact with their host using similar mechanisms to those likely to be discovered for *M. bovis*. It is conceivable that by selecting animals to be more resistant to bovine TB, one could also select serendipitously for increased resistance to other pathogens.

However, some concerns have been raised about breeding for improved resistance (Berry and others 2011a). How sustainable would selection for resistance be when the pathogen is so adaptable? The *M. tuberculosis* complex bacteria are some of the most successful pathogens on the planet and have proven highly-adaptable in new hosts and ecological niches throughout their evolution. Could altering host resistance to disease in national cattle populations result in future populations of *M. bovis* eventually evolving to better infect 'resistant' cattle? We speculate that the outcome of this may depend on the relative speed of host genetic gain *versus* the ongoing evolution of the pathogen. Evidence in other systems would suggest that the pathogen has not yet won that race. If selection for disease resistance was based on multiple genetic variants this would reduce the probability of the pathogen rapidly evolving to circumvent all resistance mechanisms.

Might breeding for resistance to bovine TB potentially make animals more susceptible to other pathogens? TB resistance in cattle may involve bolstering the adaptive immune Th-1 response, potentially at the expense of the Th-2 response, which could potentially leave cattle

more vulnerable to infection by parasites such as *Fasciola hepatica* (Flynn and others 2009). Basing resistance on the innate immune phenotype may address these concerns. Tuberculin test results are used in genetic studies as a proxy for the true predisposition to disease. Whether this holds true in future remains to be demonstrated. Simply improving 'tolerance' to bovine TB would not be desirable (Berry and others 2011a).

Breeding for resistance to bovine TB in the national cattle herd is likely to produce significant benefits relatively quickly, particularly through the use of AI semen from low-risk sires. Such schemes would also complement existing eradication schemes, providing a more sustainable strategy for reducing incidence and would have no direct impact on wildlife populations. We consider that genetic improvement could form part of the control package, but not the sole part, since we are assuming (for now) that the resistance phenotype is not absolute; the aim is to breed cattle that are more resistant (or more resistant for longer) to the average exposure commonly encountered in the field.

3.3.4. Pathogen Genetic Variation

The pathogen itself represents an important source of genetic variation, which might also influence the outcome of infection (Verhagen and others 2011) and consequently the efficacy of control. Recent studies in human populations (Kaufmann 2008, Caws and others 2008, de Jong and others 2008) demonstrate that the genetic make-up of bacteria and their hosts are now seen as important sources of variation, whose interplay should always be taken into account. For example, the *M. tuberculosis* Beijing genotype has been shown to have increased transmissibility and pathogenesis (Hanekom and others 2011).

The relationship between host genotype, mycobacterial strain and the development of human TB continues to be tested (Caws and others 2008). Six major genetic lineages of *M. tuberculosis* have been identified and are highly geographically localized (Coscolla and Gagneux 2010). A recent study suggested that the early interactions between *M. tuberculosis* and the human host were determined by the lineage of the infecting strain (Krishnan and others 2011). Thye and others (2011) suggested that pathogen variation may explain some of the difficulties inherent in finding the genetic variation that underpins susceptibility to human TB.

Results of a human TB study in Vietnam indicated an association between variation in the TLR2 (Toll-like receptor-2) gene and individuals

with meningeal TB infected with the Beijing lineage of *M. tuberculosis*. Similarly, variation in the human IRGM gene, which is involved in autophagy (cell self digestion), was shown to contribute to protection from *M. tuberculosis* disease, but not by *M. africanum* (Intemann and others 2009). Also, de Jong and others (2008) determined that the distinct *M. africanum* lineage could transmit equally well between humans but was less likely to progress to disease than *M. tuberculosis*. Recent evidence suggests that the interplay between host and pathogen is highly tuned, adapted and evolved. For example, different host response mechanisms were detected in mouse strains to two minor variants of *M. bovis* BCG (Di Pietrantonio and others 2011).

The extreme clonality of members of the *Mycobacterium tuberculosis* complex means that they are prone to population genetics effects, such as population bottlenecks, selective sweeps and genetic drift (Smith and others 2006b). Sampling from one population seeds a new ecological niche or geographical region and can lead to the emergence of strains with dramatic, geographically clustered variation in the pathogen population (Smith and others 2006b). The striking phylo-geography disclosed recently for the major lineages of human-adapted *M. tuberculosis* has important implications for lineage-lineage phenotypic differences (Gagneux and Small 2007) and will likely impact current and future regional TB control, epidemiology, diagnosis and vaccinology (Hershberg and others 2008). However, to date, these observed inter-strain differences in behaviour are more convincing in experimental studies than they are in population-scale studies (Coscolla and Gagneux 2010).

In truth, these bacteria are remarkably similar and the null hypothesis would be that no significant differences in pathogenicity (virulence) should be expected, especially at a local level. Similar research to define the population structure and genetic family tree (phylogeny) of *M. bovis* in the UK, ROI and Europe is underway (NH Smith and others, unpublished). The UK and ROI is dominated by a specific lineage or clonal complex now known as European 1 (EU1, Smith and others 2011). It is marked by a specific genome deletion. There is evidence that it differs from that found in much of western Europe, which is dominated by another clonal complex of *M. bovis*, known as EU2 (Rodriguez-Campos and others 2011).

Furthermore, we now have genetic evidence that EU1 may have been exported from the British Isles to regions of the world comprising the former British Empire, probably from the 1800s onwards and

possibly in British beef breeds, such as the Hereford (Smith and others 2011). If this is the case, bovine TB has obviously not remained exclusively within that breed, providing further evidence for cattle-cattle transmission. However, the export routes are not entirely straightforward. Similarly, a recent *M. bovis* genotyping study in China suggested transmission from local cattle to other cattle imported from OTF countries (Sun and others 2011). Given the evidence that *M. tuberculosis* lineage affects the outcome of infection and disease, *M. bovis* lineage should be included as a co-variable in epidemiological studies tasked with elucidating the mechanisms underpinning the variability observed in bovine TB infection of cattle.

Preliminary GB analysis revealed some intriguing differences across different molecular types of *M. bovis* (DEFRA SE3020). It showed differences in numbers of inconclusive reactors across spoligotypes, and revealed that particular spoligotypes were more frequently detected on repeat testing. This suggested that clonal groups of *M. bovis* may have distinct phenotypes that could be relevant to control strategies (Goodchild and others 2003). This is supported by experimental data which indicated that metabolic fingerprints and genotypes were highly congruent (Winder and others 2006). *M. bovis* genotypes derived from different hosts and from different countries have recently been shown to produce different pathological lesions and had different virulence in an experimental mouse model. This provides further evidence that *M. bovis* can also show different virulence (Aguilar Leon and others 2009). It will be important to index pathogen variation in future epidemiological studies.

Genotyping of *M. bovis* in the UK has revealed that the population consists of a small number of readily-identifiable clones which appear to be clustered (geographically localized) (Smith and others 2003, Skuce and others 2010). Within geographical areas a single clone dominates or has reached fixation (endemic clones). Further GB analysis indicated that the population structure was not compatible with a process of random mutation and drift, and could best be described by a series of clonal expansions where different genotypes rose to high frequency, either as a result of sampling (founder effects) or selection. The geographical localization of different strains can be exploited to determine the source of *M. bovis* in outbreaks as a result of purchasing infected animals (Skuce and others 2010). Badgers and cattle in the same area tend to share identical genotypes. The geographical clustering of strains has not changed significantly in recent decades. Bovine

tuberculin is made from *M. bovis* AN5, a 1948 GB isolate. Relatively few close relations of AN5 have been identified in current UK and ROI sampling, although AN5 belongs in the EU1 clonal complex, unlike the vaccine *M.bovis* BCG strain. However, AN5 may still not be optimal for the detection of modern, locally-prevalent *M. bovis*. This could be investigated systematically.

3.4. Bovine TB – Non-Genetic Risk Factors For Susceptibility

Animal-level risks have been very well reviewed by Phillips and others (2002) and more recently by Humblet and others (2009 and 2010). Further research is required to better understand the relative importance of different transmission routes before more precise husbandry recommendations can be made (Phillips 2000).

3.4.1. Age

Numerous studies in various countries identify age as a significant animal-level risk factor (Humblet and others 2009). Age is a biologically-plausible risk factor because the duration of exposure increases cumulatively with age. Several studies show that older animals are more likely to have been exposed than younger ones. Older cattle may be more at risk and maybe should be given particular attention. Age might affect the probability that an animal tests positive. However, the age distribution of older reactors in GB is quite similar to the age distribution predicted for British cattle, indicating that individual cattle appear to be at equal risk of being a reactor regardless of herd size or age.

A cross-sectional study in ROI identified that calves were significantly less likely to be tuberculin reactors at a population level (Griffin and others 1996). However, young animals may well be infected depending on prevailing circumstances and practices. Whether or not a <42 day-old infected calf is detected by the tuberculin test depends on how soon after birth it was infected and any individual variations in the ability to mount a response to tuberculin (TBAG 2009). Therefore, it is generally considered ineffective to TB test young calves, as reflected in the GB rules for pre-movement and pre-export TB testing. An increase in prevalence with age was recorded in Latvia (mean age of onset 6 years, Petukhov 1981) and in the UK (relative risk to cows >8 years old was 12-fold that of 1-2 year olds, Benham 1985). Most bovine TB-infected Mexican cattle are adult females and in fair-good body condition (Milian-Suazo and others 2000). Humblet and others (2009) cite older work (Francis 1946) – *"the evidence suggests that even when young cattle are*

pastured with heavily infected old stock, the incidence in the former remains low until they enter the cow shed."

It is also interesting to note that a 'cull for age' policy for all breeding cows, based on experience which showed that older cattle were more likely to be infected and less likely to respond to the tuberculin test, made a significant contribution to the latter stages of bovine TB eradication in Australia (Radunz 2006).

3.4.2. Gender

Gender was not a significant risk in ROI studies of reactor herds (Clegg and others 2008) but mostly appears as a risk factor in published African studies (Humblet and others 2009) and opinion is divided on how such findings are replicated between studies. Relative differences in susceptibility may be masked by differences in longevity of beef and dairy cattle and the different between- and within-herd movements and contacts experienced by both genders. Males have potentially more contact with other herds during breeding, which may increase their risk (Humblet and others 2009). Whether or not the apparent differences in detectability between genders relates to physiological differences remains to be settled.

3.4.3. Breed

Breed has been identified as a risk factor, again mostly in African studies, where European breeds, imported to help develop the dairy industry, may be less resistant than the indigenous breeds, such as zebu. However, apparent differences between breeds may be more influenced and better explained by different management. Imported dairy breeds are retained under extensive systems (Elias and others 2008). Perhaps variability in tuberculin test outcome between breeds might also be a factor. This is testable with modern animal test and movement databases (Humblet and others 2009). A potential contributory factor to the observed difference in risk for dairy and beef enterprises may be breed-related (Vial and others 2011), although no evidence of a breed effect was reported in susceptibility to *M. bovis* infection in GB (Benham 1985) or NI (Denny, oral evidence to Phillips and others 2000). A recent GB bovine TB genetic susceptibility candidate gene investigation did not find a breed effect in markers associated with bovine TB reactors (Driscoll and others 2011) and breed was not a significant risk factor in a recent ROI study of reactor herds (Clegg and others 2008).

3.4.4. Body Condition Score (BCS)

BCS is familiar to cattle breeders: it records body conformation, including musculature, fat coverage, bone structure etc. Such measurements are recorded and used by the major cattle breed societies to inform matings for desirable traits in progeny in selective breeding programmes. The data are now integral to calculating the estimated breeding value (EBV) of performance-recorded cattle and increasingly is being exploited to drive genetic gain via the new discipline of genomic selection (Olson and others 2011). Low BCS was associated with increased risk of tuberculin reactivity in a cross-sectional study in Zambia (Cook and others 1996).

Whilst most economically important traits investigated had a negligible association with *M. bovis* infection, tuberculin responsiveness was correlated with fat production (0.39) and BCS (0.36), and negatively correlated with somatic cell score (−0.34) and survival (−0.62). Therefore, selection for increased survival may reduce susceptibility, whereas selection for reduced somatic cell count, increased fat production and BCS may increase susceptibility to *M. bovis* infection (Bermingham and others 2010).

3.4.5. Physiological State

This potential risk relates to pregnancy, lactation, parturition etc. Pregnancy has been associated with tuberculin test anergy, with skin reactivity depressed for ~15 days around parturition (5 days pre-calving to 10 days post-calving) (Kerr 1949). Buddle and others (1994) reported a similar reduction in tuberculin reactivity and a temporary reduction in IFN response post-calving. No direct association between pregnancy and susceptibility to infection/disease was reported (Buddle and others 1994), although well-documented peri-parturient immune-suppression in dairy cows, which may be linked to other deficiencies (Kehrli 1998, Burton and others 2003), might be the mechanism.

The potential link between periparturient immune-suppression and mastitis susceptibility in dairy cows was investigated in the USA (Burton and others 2003) where experimental and field evidence suggested that systemic and local (mammary) immune responses were deficient around parturition, supporting the logical hypothesis that immune deficiency was behind the heightened susceptibility observed in periparturient cows. Dietary (anti-oxidant) supplementation and vaccination has been shown to boost immunity sufficiently to reduce mastitis severity and to restore normal milk yield.

3.4.6. Stress

The role of 'stress' (neuroendocrine) hormones in the susceptibility of animals to bacterial infections is also becoming clearer (reviewed by Verbrugghe and others 2011). Previously the main focus of stress research had been on the direct effect of stress hormones on immunity and intestinal barrier function. The duration of stress seems to play a significant role in the complex interplay between stress, host immunity and the pathogen. Now it is understood that chronic stress can shift immunity from Th1 (cell-mediated) towards Th2 (humoral-mediated) which could alter susceptibility and disease course. Stress hormones can influence the macrophage-pathogen interaction and probably affect the outcome of mycobacterial infections. However, there are relatively limited data on the role of husbandry, housing, socializing, weaning, handling, movement etc on stress in cattle. A recent ROI study showed that weaning induced an acute stress response in calves and actually enhanced the immune response (O'Loughlin and others 2011). Male calves and calves penned away from their dam were more sensitive to weaning stress.

3.4.7. Cattle Enterprise Type

The age profile and contact networks established within beef, dairy and mixed enterprises are likely to be quite different. For example, there is limited contact between dam and progeny in dairy herds, compared to beef herds where there is significant contact between adults and calves.

DEFRA project SE3003 showed that beef cattle herds were the least likely to have a bovine TB breakdown in the GB data. Mixed dairy and beef enterprises were 3-fold more likely to have a TB breakdown than beef herds and dairy herds were 5-fold more likely. Dairy herds have a higher 'transmission coefficient' than beef herds due to their longevity and their more intensive management system, which often results in closer confinement.

Farming systems (Thornton 2010) have recently increased production intensity, especially increased milk yield and growth rates. However, ROI studies indicate no significant differences in the risk of herd breakdown by enterprise type (dairy, suckler and drystock units, Fallon 1994, Mairtin 1994). There appears to be no evidence to suggest that changing cattle enterprise type following a breakdown would be beneficial.

3.4.8. Immune Status

Immune-suppression is a significant predisposing risk factor in many diseases of man and animals (expanded below).

3.4.9. Concurrent Infection

Most hosts are infected by multiple parasites and pathogens at any given time. It is important to remember that infection is not simply the interaction of one host and one parasite or pathogen (Carslake and others 2011). Microbes compete and interfere with each other and can modify each others' life histories and disease course and transmissibility (Raoult 2011). Most control programmes and disease models do not adequately control for these interactions. This could impact disease epidemiology in several ways. It seems reasonable to assume that cattle, exposed to multiple micro-organisms (some of which are pathogenic and potentially immune-modulatory, if not immune-suppressive) would be more susceptible to infection and disease. The complex interplay between host and multiple pathogens may, theoretically, also lead to enhanced infectiousness of bovine TB at the animal-level.

3.4.9.1. Immune-Suppressive Viruses

Susceptibility to bovine TB may be enhanced by concurrent infection with immune-suppressive viruses (de la Rua Domemech and others 2006). Immune-suppressive diseases, such as Bovine Viral Diarrhoea (BVD) and Enzootic Bovine Leukosis (EBL) probably increase susceptibility to infection. However, there are limited data on the interactions between concurrent immune-suppressive diseases and *M. bovis* infection in cattle.

BVD virus is distributed worldwide and is endemic in much of the world. It is more common in young cattle (6-24 months old). Herd-level sero-prevalence in Ireland is 98.7% (Barrett and others 2011). BVD can cause transient infection or persistent BVD-infection (PI). The proportion of PI animals is believed not to exceed 2%. A small co-infection (BVD and *M. bovis* BCG) study showed that BVD could transiently reduce IFN responses to *M. bovis* in the two weeks post-BVD virus inoculation and resulted in a failure to identify bovine TB-infected cattle (Charleston and others 2001). It is unclear whether this is significant under prevailing field conditions or whether any BVD association is causal. Also, it may be the persistently BVD-infected (PI) animals that are more significant in the epidemiology of such co-infections. A recent case report in Cornwall describes a severe outbreak of *M. bovis* infection in housed calves in

which concurrent infection with the immune-suppressive BVD virus was confirmed (Monies and Head 1999).

BVD virus infection probably was widespread in Australia, yet they have successfully eradicated bovine TB. No significant differences were detected between calves experimentally co-infected with BVD and *M. bovis*, compared to *M. bovis*-infected alone (Kao and others 2007). However, this study concluded that if maximum exposure to *M. bovis* was actually critical on occasion for cattle-cattle transmission of bovine TB, even cattle that shed *M. bovis* intermittently could still be important in persistence and spread. The relevant question is not disease costs, but to what extent it is beneficial to control BVD (McInerney 1996). An association between diabetes in cattle and BVD was also reported in Japanese (Taniyama and others), GB (Murondoti and others 1999) and Canadian (Clark 2003) case studies. Concerted efforts are now being taken to better manage BVD infection in Ireland.

Bovine leukosis, caused by Bovine Leukaemia Virus (BLV) is endemic in many USA cattle herds. BLV infection is associated with immune suppression and reduced productivity. A BLV-bovine TB co-infected Holstein cow was reported from a BLV-endemic herd with marked enlargement of visceral and peripheral lymph nodes, which yielded *M. bovis* (Fitzgerald and others (2009). The animal showed no clinical signs, but was in poor condition; one of 21 submitted as suspicious for bovine TB and was the only one with gross lesions. The authors speculate that BLV infection might have predisposed this cow to infection and disease due to *M. bovis*.

Although data are sparse and its immune-suppressive potential is unknown, Bovine Immunodeficiency-like Virus (BIV) may affect susceptibility. BIV has been raised as a potential confounder in our own genetic epidemiology study on bovine TB (BBSRC-funded) where our comparisons are essentially comparing family groups within herds and accumulating the outcomes across herds. If BIV exposure (within a herd) is random, and it predisposes to TB susceptibility, then all this would do is add a bit of 'noise' to the data; it wouldn't undermine the results. We have designed, essentially, a matched case-control study to minimize such effects. The project is based on Holsteins to control for population structure (stratification) and controls are drawn from management-matched cases (same age, sex, epidemiological group etc), so that they have equal opportunity of exposure to bovine TB.

To be a confounder a variable must be related to both the exposure and the outcome, in this case; exposure = genetic make-up of the animal

and outcome = bovine TB infection. If BIV is purely acting as a random effect on immunity, then there may be a relation between BIV and bovine TB occurrence; but we would not expect a significant association between genetic make-up and TB infection. For a confounding effect there would need to be a genetic susceptibility to BIV, in which case the variable could be confounding the association between TB and genetic make-up. That in itself is always a possibility; any unknown immune-suppressive virus to which there is a genetic predisposition could cause exactly that effect. This is not necessarily a fatal flaw; the start (genetic make-up) and the end (TB infection) are still the same, even if the effect is via an intermediate variable (an immune-suppressive virus). This becomes more of an issue when comparing one herd with another and looking at non-genetic factors (Bishop SC and McDowell SWJ, pers comm).

Similarly, haemolytic diseases (Babesiosis or Tick Borne Fever) may also have a role, although this has not been investigated. Diseases which may indirectly affect cell-mediated immune responses (enteropathies/nephropathies, fasciolosis, haemonchosis and ostertagiasis etc) and are not known to be directly immune-suppressive may modulate *M. bovis* infection.

The influence of UK respiratory pathogens, such as *Dictyocaulus viviparous* (Husk), *Pasteurella* spp., *Mycoplasma* spp., *Haemophilus* spp., IBR, BVD, PI3 and RSV on susceptibility to infection with *M. bovis* remains untested. The resulting pneumonia, bronchitis, tracheitis and altered bronchial mucus and secretions may exacerbate *M. bovis* infection by rendering respiratory membranes more susceptible to infection with *M. bovis* and those agents which induce coughing may facilitate increased aerosol spread of *M. bovis*.

3.4.9.2. Parasites – Helminths And Nematodes

Infection by helminth parasites, such as liver fluke (*Fasciola hepatica*), polarizes host immunity and promotes Th2 immune responses (Allen and Maizels 2011). This has been shown to inhibit immune tests based on Th1-type immunity, such as the tuberculin test and IFN (Flynn and others 2007). In a co-infection model (*F. hepatica* and *M. bovis* BCG) the predictive power of tuberculin and IFN tests was compromised. *F. hepatica* infection altered macrophage function. These results question whether *F. hepatica* infection modulates the predictive capacity of bovine TB diagnosis and question how pathogens and parasites interact with the host to modulate susceptibility to infection, disease or infectivity.

The veterinary literature is somewhat ambivalent on bovine TB-liver fluke interactions. DEFRA SE3013 reported that tuberculin reactors and contacts, particularly dairy cattle with antibodies to liver fluke were less likely to show evidence of *M. bovis* infection. It is not yet clear whether this association is causal. Liver fluke infestation might modulate the inflammatory response and reduce the predictive value of the tuberculin test in co-infected animals. Liver fluke antigens are potent stimulators of Th2-type immune responses and concurrent (or previous) exposure to liver fluke antigens might modulate the immune response to tuberculin, a hypothesis that merits further investigation. There is no reported reason why flukicides should interfere with the tuberculin test, although herd-keepers are advised not to administer any drugs (not just flukicides and other wormers) around the time of tuberculin on IFN testing.

Epidemiological and genetic studies have shown that gastrointestinal parasitic nematodes (worms) can modulate immunity to intracellular pathogens (Ezenwa and others 2010). Subsequently paramaterised mathematical modelling predicated that nematode-induced immune-suppression could allow the invasion of bovine TB in African buffalo. In nematode absence, bovine TB failed to invade the system, illustrating the interactions between hosts and pathogens that probably play out in the wild and influence disease dynamics and patterns.

A recent study in inbred mice illustrated that mice, previously infected with the intestinal helminth *Strongyloides venezuelensis*, showed increased bacterial burden and compromised immune responses when co-infected with *M. bovis* (Dias and others 2011). The authors concluded that intestinal helminth infection could have a detrimental effect on TB control and could increase TB susceptibility in co-infected individuals.

3.4.9.3. Other Mycobacteria

Prior exposure to environmental mycobacteria may modulate the diagnostic and disease response to subsequent exposure to pathogenic mycobacteria, by immunological cross-reactivity. Saprophytic mycobacterial species are ubiquitous in many environments (Cooney and others 1997, Rastogi and others 2001). Environmental mycobacteria are also ubiquitous in natural water supply biofilms. For example, the *Mycobacterium avium-intracellulare scrofulaceum* (MAIS) complex mycobacteria predominate in water, dust, and human sputum samples.

Mycobacterium fortuitum complex organisms are common in soil (Kamala and others 1994).

Many environmental mycobacteria potentially induce non-specific reactions to bovine and avian tuberculin (Cooney and others 1997), which may modulate the susceptibility of cattle to *M. bovis* infection. Whilst direct evidence in cattle is lacking, immunological priming of humans and other animals by exposure to environmental mycobacteria is well-established (Donoghue and others 1997). Morris and others (1994) concluded that *"there is no data to suggest alterations in susceptibility due to prior or intercurrent exposure to mycobacteria of different species or to other less closely related organisms."* The possibility that consumption of environmental mycobacteria primes the immune response to *M. bovis* cannot currently be excluded. However, it is not clear why this mechanism did not appear to significantly influence *M. bovis* infection, unless the weight of infection was overwhelming due to lack of control and poor management.

Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis*, is a chronic, insidious disease of cattle and other ruminants which is endemic in much of the UK and beyond. Exposure of cattle to this pathogen can cause cross-reactivity to components of bovine tuberculin, potentially reducing the specificity of the SICTT. Barry and others (2011) investigated the effect of *M. avium* Complex (*M. avium* subsp. a*vium and M. avium paratuberculosis*) infections on routine *M. bovis* diagnostic tests; the SICTT and IFN test using PPDa, PPDb and PPDj tuberculins to diagnose *M. avium* subsp. *avium, M. bovis* and *M. avium* subsp. *paratuberculosis*, respectively. They report that SICTT results were consistent with the infecting organism and that all *M. avium* Complex-infected calves would have been classified as SICTT-. The IFN response peaked later and was more sustained for *M. avium* subsp. *paratuberculosis*-exposed calves compared to *M. avium* subsp. *avium*-exposed calves. There was close correlation in responses to specific PPDs, with PPDa and PPDj being the most similar. (This study is discussed in more detail in Review 3).

UK experiments on calves, pre-sensitised with the close relative *M. avium* subsp. *avium* demonstrated elevated responses to avian tuberculin in the SICTT and IFN tests that might mask *M. bovis* detection, even when specific antigens (ESAT-6 and CFP-10) were used (Hope and others 2005). A study in Spain followed bovine TB and Johne's disease co-infected herds for 3.5 years. The SICTT and IFN tests detected 65.2% and 69.6% of bovine TB culture-positives, respectively (Aranaz and others

2006). These proxy sensitivity estimates were lower than previously-accepted estimates (de la Rua Domenech and others 2006).

3.4.10. Nutrition And Mineral Supplements

Lactating cows are under considerable nutritional stress, especially in early lactation (Grove-White 2004) and care needs to be taken so as not to compromise biosecurity during feeding. A study in the ROI reported that susceptibility to bovine TB was increased when cattle were kept on reduced or unbalanced rations (Griffin and others 1993), although a subsequent in-contact ROI study showed no evidence that a restricted diet affected bovine TB transmission ie. low food intake did not increase the risk of transmitting *M. bovis* infection between steers in a small ROI study (Costello and others 1998). It is currently not clear whether low body condition score (BCS) is a risk factor or a consequence of bovine TB (Humblet and others 2009).

In a Mexican study, *M. bovis*-infected cattle were mostly in fair/good body condition (Milian-Suazo and others 2000). Malnutrition and deficiencies are recognized risk factors in human TB, so it is reasonable to assume that there are nutritional effects in cattle that have not been elucidated. Czech studies suggested that vitamin A, vitamin C, calcium and protein deficiencies, and carbohydrate excess, tended to increase the risk of *M. bovis* infection (Kabrt 1962).

An epidemiological association between mineral licks and *M. bovis* infection was reported in ROI (Griffin and others 1993), with OR=2.7. Risk was higher on farms with rough grazing, possibly due to mineral deficiency in low quality pasture, leading to the hypothesis that mineral deficiencies predisposed cattle to bovine TB. Providing cattle with mineral supplements in the field may reduce the attractiveness of soil. However, further ROI studies found no association between copper, selenium and iodine and prevalence (Fallon 1993) in a relatively large study of housed and pastured herds, even though some cattle showed signs of deficiency. It is plausible that some other micro-nutrient(s) were explaining much of the variation seen and recommended intake for some minerals, such as sodium, magnesium, iron or cobalt may be sub-optimal for dairy cows (Phillips and others 2000). Magnesium status is important for immune-competence (McCoy and others 1993) and is frequently deficient in grazing cattle. Intriguingly, the magnesium status of human leprosy cases is also reduced (Jain and others 1995).

There is evidence that mineral deficiencies play an important role in predisposing animals to mycobacteria. Rodents have low iron status,

which increases their susceptibility to experimental paratuberculosis. Copper deficiency in cattle suggests that competition from iron could predispose cattle to paratuberculosis (Lepper and others 1989). There is some evidence that badgers are susceptible (reduced reproductive rate and health) to increased cadmium in pasture (Van den Brink and Ma 1998). Provision of mineral licks at pasture (which contain zinc to offset high cadmium intake) might benefit badger health more than cattle health and could explain the association between mineral licks and bovine TB breakdowns (Griffin and others 1993). The use of mineral licks for cattle inside farm buildings has also been associated with a decreased risk of prolonged breakdown (Karolemeas and others 2010). The mechanism is maybe due to minimizing shared use by wildlife or it may affect cattle susceptibility directly. Mycobacteria tend to be tolerant of acid soil and are inhibited by the reduced iron availability in alkaline soils (Mitserlich and Marth 1984). Mycobacteria tend not to be good at scavenging and chelating iron (Johnson and others 1997) and *M. bovis* needs to scavenge iron for its survival within the host. O'Donovan and Milburn (2010) reported an association between bovine TB and high levels of iron in the soil in GB. The rock types present in the west of England and Wales were rich in iron and aluminium in contrast to those areas largely free of bovine TB, which tended to be rich in calcium salts and silicon oxides, which is reflected in soil chemistry. However, it was not clear what other factors had been controlled for in this study. Johne's disease is also more prevalent in areas with acidic soils, which increase mineral availability.

Studies examining whether poor nutrition in cattle is linked to susceptibility to bovine TB are probably difficult to do well, because herd-keepers would be unlikely to agree to feed their herds an inadequate diet and the study might not get ethical approval. DEFRA supported two projects investigating the link between trace elements and bovine TB. One looked at whether there was any association between levels of trace elements in soil and the geographical pattern of TB outbreaks in England and Wales and found no obvious association. The other project compared the level of trace elements in blood samples from cattle that are confirmed to be infected with *M. bovis* with the level in samples from cattle that are not infected. In exposed, non-reactor controls lower levels of the seleno enzyme glutathione peroxidase (GSHPx) and higher levels of copper were associated with a higher risk of confirmed bovine TB. No association was detected with vitamin B12, although a stronger association was detected between bovine TB and GSHPx in in-contacts.

Lower liver copper, but not selenium, was associated with elevated risk in reactors. The study indicates that trace nutrients might influence susceptibility and that they merit further studies (Downs and others 2008).

Much of the UK's soil is mineral-deficient. Deficiencies of copper, selenium, cobalt and iodine are reported in farmed animals and mineral supplements for cattle can help alleviate. Some evidence suggests that trace element deficiencies can result in impaired immune responses. The association between *M. bovis* infection and trace elements such as selenium, copper and vitamin B12 status of cattle was investigated as part of DEFRA SE3013. Lower selenium status might increase susceptibility to *M. bovis* infection and there might be an association with copper. However, it was not possible to conclude that the associations observed were factors in the incidence of bovine TB in cattle.

In summary, there is evidence that other pathogenic mycobacteria diseases alter the mineral status of animals, but it is unlikely that the trace elements most commonly believed to be deficient in cattle are related to *M. bovis* infection. Some other macro-nutrients commonly believed to be deficient might explain the association between presence of mineral licks and protection from *M. bovis* infection.

3.4.11. Cattle Behaviour

Other genetically-controlled factors influencing susceptibility to bovine TB may be behavioural. For example, grazing habits with respect to avoidance of excretory products may be under innate genetic influence. The amount of social behaviour that might facilitate cattle-cattle transmission, or investigatory behaviour towards badgers or their excreta, may also be under genetic influence. Specific mechanisms of immunity will almost certainly be genetically influenced (Phillips and others 2002).

Cattle contact patterns are highly variable (White and others 2008) and can be influenced by their relative position in the herd social hierarchy. Cattle form dominance hierarchies and various groupings within herds (Sauter and Morris 1995) which become even more important in low prevalence disease. Some cattle are highly connected within the herd contact network and have the potential to act as hubs in the spread of disease within these complex contact networks. Targeting prevention or control measures to high-contact individuals (or groups) should further enhance disease management. Cattle with higher intra-herd contacts also featured prominently in cattle-badger contacts (Bohm

and others 2009). This concurs with data from New Zealand, where bovine TB reactors tended to be from the top half of the herd hierarchy (Sauter and Morris 1995).

Cattle that are higher in the herd social heirarchy also show greater inquisitiveness and have a higher risk of acquiring infection from cattle introduced to the herd, as well as potentially from direct contact with infectious wildlife (Bohm and others 2009). Lower social status may increase the risk of indirect transmission from pasture contaminated with badger excretions.

Estimates of genealogical relationships within a bovine TB-infected white-tailed deer population indicated that infected deer were significantly more closely related than non-infected deer, suggesting that contact (and genetics) within family groups was a significant mechanism of disease transmission (Blanchong and others 2007). This indicates the intimate interaction between genetics and behavioural risks.

3.4.12. Therapeutics

Corticosteroids may be used to induce parturition or for the treatment of ketosis and are well known for causing immune-suppression. Their production by the calf at parturition may contribute to the dam being unresponsive to cell-mediated immunological tests (tuberculin and IFN) for a period post-calving. Corticosteroids have suppressive effects on the tuberculin test (Kerr and others 1949) and their use may increase susceptibility to infection and enhance infectiousness. Licensed non-steroidal anti-inflammatory drugs (NSAIDs) are now available for cattle, so corticosteroids are now used less commonly. Corticosteroid abuse could potentially conceal infected animals, but this might be ultimately be ineffective and counterproductive.

3.4.13. Climate And Weather

The effect of prevailing weather conditions on the occurrence and transmission of *M. bovis* has also been raised. In one study area the annual prevalence increased in relation to rainfall the previous year (King and others 1999). Climate may contribute to the geographical localisation of bovine TB in south-west England and west Wales. If infection was more likely to occur at pasture than indoors, cattle would be infected in early summer and could transmit to the following winter, leading to high numbers of infected animals being detected early the following year (King and others 1999). Seasonal patterns are probably

obscured by the timing of intense tuberculin testing. Even annual testing is probably insufficiently frequent to determine within-year patterns of infection. Weather and climate has been linked to geographical and temporal variation in bovine TB (Wint and others 2002). Climate may also affect cattle and badger behaviour (Phillips 2000) and hence the likelihood of transmission. It is noteworthy that the introduction of a climatic measure (the North Atlantic Oscillation, NAO) improved the fit of a bovine TB mathematical model (Woodroffe and others 2006), although much of the variation remained unexplained.

4. RISK FACTORS

Not all of the studies summarized below are classical 'risk factor' studies; instead they are observational studies. Some studies include analysis of wildlife-related risk factors, which have not been discussed in any detail here. Risk factors and potential mitigation measures at badger-cattle interfaces have been discussed in more depth in Review 2. It is important to remember that risk factors identified in herd- and animal-level studies are *associated* with the measured outcome and are not necessarily causal.

The following are amongst the risk factors shown to influence the potential of direct and indirect (via faeces and urine) exposure of cattle to the wildlife reservoir when at pasture; stocking regime (set-stocking), rotational *versus* strip grazing, stocking densities, farm habitat types and livestock production intensity (Johnston and others 2011). Sharing feed or water between cattle and wildlife when housed or at pasture, housing type and storing manure indoors are associated with the differential risk of transmission between cattle and wildlife. The most frequently identified risks for herd-to-herd transmission included herd movements and trading, where general trading or purchase from markets or herds in hot-spot areas or from infected herds have all been linked with increased risk for the receiving herd (Johnston and others 2011).

Pathogenesis studies suggest strongly that the route of transmission of bovine TB is largely via the respiratory system, requiring transmission *via* infectious aerosols. Epidemiology also suggests that, on average, multiple, direct close contacts with an infectious case are required for transmission. Hence, it is important to consider those risk factors, such as cattle contacts and movements, which theoretically facilitate such transmission. The identification of risk factors and risk settings for infection and transmission are intimately linked with those factors which affect susceptibility. As an example, classical epidemiological studies have been used to investigate the risk factors and settings which conspire to facilitate human TB transmission in various populations.

Unlike with bovine TB, in human TB epidemiology a distinction is often made between transmission arising from active TB and that arising from reactivated latent TB. This cluster analysis is based on pathogen genotyping data, where clusters of the same (or similar) genotypes are modeled as indicating ongoing transmission, whereas unrelated genotypes are taken as evidence of reactivated latent cases which were acquired elsewhere. Genetic tracking of *M. tuberculosis* is now a

cornerstone of human TB control programmes (Weisenberg and others 2011) and is considered to be essential for understanding the dynamics of transmission (Djelouadji and others 2011) and this is supported by sophisticated web tools (Shabbeer and others 2011). For example, in a recent study in Arkansas USA, a higher proportion of human TB cases in large clusters were non-Hispanic black, homeless, <65 years old, male, sputum positive, alcohol abusers and HIV sero-positive (Talarico and others 2011). The study also indicated that social behavioural patterns might have more impact than the infectiousness of the source.

Without having comprehensive pathogen genotyping data, secondary cases within a household were generally assumed to have resulted from within-household transmission. A recent study in Peru demonstrated a <10% risk that additional cases within a household were actually acquired in the community (Cohen and others 2011). Indeed, up to ~70% of household contacts of infectious pulmonary TB remained tuberculin test negative for longer than others despite equal exposure (Sridhar and others 2011).

In bovine TB several risk factors (e.g. cattle husbandry and environmental practices) have been suggested as predisposing farms to TB breakdowns (Humblet and others 2009). However, they are not amenable to experimental investigation due to the large number of variables, the impracticality and cost of conducting controlled experiments on commercial livestock farms, and the need for data from a large number of representative bovine TB breakdowns. In such circumstances, a 'case-control' study provides the appropriate approach (Bourne 2007).

The odds ratio (OR) associated with the absence or presence of the explanatory variable was calculated for each variable recorded, with its 95% confidence interval. An estimated OR of >1.0 indicates that the factor is associated with an increased risk of a breakdown, and the greater the numerical value of the OR, the greater the risk. By contrast an OR <1.0 suggests that the factor reduces risk and is 'protective' in relation to bovine TB breakdowns. Summaries of the studies assessed as part of this review have been included below in two groups. The first are classical case-control studies, the second are predominantly cohort-based studies or case-control studies focused on specific risk factors. [Intervention studies, focusing on the assessment of badger culling (e.g. the GB RBCT, the ROI 'Four Area Trial) have not been included]. Most of the studies listed under 'other epidemiological studies' used pre-existing

data (e.g. cattle movement and bovine TB test data) and have addressed specific epidemiological questions, such as the role of cattle movement.

4.1. Case-Control Studies

A case-control study of risk factors for bovine TB in NIwas undertaken based on tuberculin test reactors identified between 1990 to 1992 (Denny and Wilesmith 1999). The study involved 427 dairy farms (excluding farms with fewer than 30 cattle and herds with reactors in purchased cattle). Data were extracted from an on-farm questionnaire and the (then DANI) Animal Health Computer System. Variables investigated included the number and nature of farm boundaries, the number of neighbours and their bovine TB history, the number of hedgerows, the presence of badger setts, whether badger carcases had been found on the land, and the possible presence of deer. Two factors were significantly associated with bovine TB breakdowns; the presence of badger setts or carcasses on the farm (OR 2.06, 95% CI 1.27-3.33) and contiguous neighbours with confirmed bovine TB (OR 2.44, 95% CI 1.55-3.86).

A matched case-control study was undertaken in the ROI to provide information on the role of farm management practices, environmental factors and farmer characteristics in the epidemiology of bovine TB. Eighty dairy herds with chronic bovine TB were compared with the same number of herds which had been free of the disease for many years. A standardized questionnaire was used. The study was conducted from August to October 1990, in Counties Cork and Kilkenny. Factors which were identified as possibly contributing to recurrent outbreaks of TB included nutritional factors, cattle purchases (especially bulls), the presence of badgers, and the spreading of slurry. Overall, the findings suggested that intensively managed dairy herds were at greater risk of bovine TB outbreaks than were other herds (Griffin and others 1993).

Subsequently, a case-control study of 200 herds from East Offaly, with cases defined as outbreaks of bovine TB detected at herd test, was undertaken (Griffin and others 1996). The data were based on tuberculin test results and other herd- and animal-level data available from the local DVO, along with badger sett locations. Herd-level risk factors significantly associated with an increased risk of infection were herd size and the presence of TB in a contiguous herd. Differences between animal types (increased risk in cows, heifers and bullocks compared to calves) and a reduced risk (protective) in animals purchased since the

preceding herd test were found at the animal-level. No significant differences were found between cases and controls in the distance to the nearest sett or the nearest main sett.

Herd-level risk factors for bovine TB breakdowns based on cattle farms enrolled within the GB RBCT (prior to FMD 2001) were investigated (Johnston and others 2005). The study (the TB99 study) comprised 268 farms from SW England, with questionnaires on farm management practices completed by staff from the local Animal Health Office. The strongest factors associated with an increased TB risk were movement of cattle onto the farm from markets or farm sales, operating a farm over multiple premises and the use of either covered yard or 'other' housing types. Spreading artificial fertilizers or farmyard manure on grazing land was associated with a decreased risk. The presence of an active badger sett mapped to either the farm land or to within 1km of the farm boundaries, was not statistically significant.

A study carried out in Michigan, USA (Kaneene and others 2002) may have limited relevance to Northern Ireland. The study size was small, involving 17 case and 51 control farms. Major factors associated with increased farm risk of bovine TB were higher bovine TB prevalence among wild deer and cattle farms in the area, herd size, and ponds or creeks in cattle housing areas. Factors associated with reduced farm risk of TB were greater amounts of natural open lands in the surrounding area and reducing deer access to cattle housing by housing cattle in barns, barnyards, or feedlots and the use of electrified wire or barbed wire for livestock fencing.

Marangon and others (1998) undertook a case-control study of bovine TB in the Veneto Region of Italy. The study was relatively small, involving 27 cases and 74 control farms and examined a limited number of potential risk factors. Factors potentially associated with increased risk included running mixed (dairy and beef) enterprises and cattle purchase. Other factors such as herd size, housing system, summer mountain pasture, possible contact with wild animals, and indirect contacts with other herds) did not appear to significantly contribute to bovine TB risk in this study.

Mathews and others (2006) examined the association between farm habitat features and other factors and the risk of bovine TB in two areas in West and SW England and involved 120 dairy herds in total (excluding herds with bovine TB breakdowns due to imported cattle). Variables were derived from pre-existing data sources with land cover information derived from remotely sensed satellite data and hedgerow characteristics

obtained from aerial photographs. Badger road-kill records within 1km and 5km proximity of the farm were used as a proxy measure of badger density. The predictors found to be significant included farmland habitat, topography and indices of badger density and herd size.

A comparative case-control study in England in which risk factors in herds with transient and persistent TB breakdowns were compared to a common set of control herds (229 herds in total) was reported (Reilly and Courtenay 2007). Interviews with herd-keepers were conducted (March 2000-February 2003). Data on farm management practices were obtained from on-farm questionnaires whereas the presence of badger setts and the type of habitat cover were determined by field survey of the relevant farms. The purchase of cows was a risk factor for both transient and persistent breakdown. The purchase of >50 head of cattle and the storage of manure for ≥6 months were risk factors for transient breakdowns, whereas the use of silage clamps increased the risk of persistent breakdown. Rather counter-intuitively, decreased odds of both transient and persistent breakdown were associated with higher stocking densities (>3 cattle/ha). Running mixed herd enterprises compared to beef-only or dairy-only was an additional protective factor against persistent breakdown. Herd size and tuberculin testing interval were also significant risk factors for both transient and persistent breakdowns, whereas active badger sett density and regional location only affected the risk of persistent breakdowns.

Johnston and others (2011) report the results of a matched case-control study (218 of 401 herds available for analysis) in 4 regions of England and Wales in 2005/2006, where case herds had confirmed infection. The significance of association with risk factors varied clearly by location. Overall, they report that; contacts with contiguous herds (OR=2.24), sourcing cattle from herds with a recent bovine TB history (OR=1.90), operating a fragmented farm (OR=2.41), feeding cattle inside housing (OR=4.89) and presence of dead badgers on farm (OR=3.10) were all associated with increased risk of a confirmed breakdown. Case herds were more likely to source cattle from herds with a breakdown within the last 2 years and more likely to have more direct contacts with contiguous herds with more confirmed breakdowns in the previous 2 years among contacted herds. They were also more likely to report finding dead badgers on farm. Providing feed outside of cattle housing was protective (OR=0.41), as was the practice of not providing shelter at pasture for cattle, which may reduce the opportunities for cattle-cattle contacts. Grazing the whole pasture was associated with increased risk, possibly

due to the increased potential for badger-cattle contact at pasture. They conclude that there is an increased local risk related to the occurrence of breakdowns amongst neighbours and/or contacted herds and possibly shared exposure to an external source, such as wildlife. Risk factors tended to vary by region, so control recommendations should reflect local risk.

4.2. Other Epidemiological Studies

Abernethy and others (2010) used APHIS data to investigate the effect of selected risk factors on recrudescence of bovine TB in breakdown herds post de-restriction. Factors associated with an increased risk included the number of reactors at the disclosing test, the number of reactors at follow-up tests, the number of follow up tests, the level of bovine TB in the district council area, herd size, the number of cattle purchased during the post-outbreak interval and a history of bovine TB breakdown(s) within the previous two years.

Using the GB Cattle Tracing System and VetNet data Brooks-Pollock and Keeling (2009) examined the relationship between herd size and persistence of bovine TB on farms. Using a measure similar to the Critical Community Size, the VetNet data revealed that herd size was positively correlated with disease persistence. Carrique-Mas and others (2008) analysed cattle movement data and herd TB history in approximately 4,200 herds, which were restocked post-FMD. Three risk factors were identified in the study; sourcing cattle from herds that were routinely tested for bovine TB more than biennially, a history of TB breakdowns in the restocked farm (1997-2000) and increasing herd size.

Although by design a case-control study, a GB study was undertaken at the animal level and specifically examined the relationship between the selenium, copper and vitamin B12 status of cattle and bovine TB infection (Downs and others 2008). The animals involved were 200 reactors and 200 in-contact animals, selected from herds in England and Wales. The study found that lower levels of GSHPx (Selenium) and higher levels of copper were associated with an increased risk of confirmed bovine TB but there was no association with vitamin B12.

Gilbert and others (2005) assessed the role of cattle movements in the spread of bovine TB in Great Britain using movement records from the Cattle Tracing System data archive. Their study showed that cattle movements, particularly those from areas where bovine TB was reported, consistently outperformed environmental, topographic and other anthropogenic variables as the main predictor of disease occurrence.

Gopal and others (2006) reported on the introduction of bovine TB to NE England by bought-in cattle. Their study investigated 31 herds that experienced confirmed breakdowns between January 2002 and June 2004; nine of which had restocked post-FMD 2001. In all but one of the breakdowns the most likely source of infection was identified as one or more purchased animals. In 17 of the breakdowns, reactor animals were traced to herds from which the same *M. bovis* genotype (spoligotype-VNTR profile) was isolated, and in five breakdowns a different genotype was isolated. Reactors in five of the breakdowns included homebred and purchased animals, providing evidence for the likely spread of the disease by cattle-cattle transmission within the herds on arrival. The lack of geographical clustering of molecular types pointed to the overwhelming source of infection being purchased cattle.

Green and others (2008) used cattle movement data to construct an individual (premises)-based model of bovine TB spread within GB, accounting for spread due to recorded cattle movements and other causes. Outbreak data for 2004 were best explained by a model attributing 16% of herd infections directly to cattle movements, with a further 9% unexplained, potentially including spread from unrecorded cattle movements. The best-fit model assumed low levels of cattle-cattle transmission. The remaining 75% of infection was attributed to local (wildlife and cattle) effects within specific high-risk areas. Green and Cornell (2005) investigated herd breakdowns in four counties of England and Wales using data from the national database of bovine TB testing history (VetNet). Factors that influenced herd breakdown included calendar time, herd size, number of cattle tested, the test type, the inter-test interval and spatial grouping of farms.

The proximity of farms to badger setts was compared between ROI farms that had experienced a TB breakdown and those that had not, over the 6 year period from 1988 to 1993 (Martin and others 1997). The data were derived from badger removal in East Offaly, which began in 1989 and continued through 1993. By the end of 1990 approximately 80% of all badgers caught in the 6 year period had been removed. The risk of a multiple reactor TB breakdown decreased for herds at least 1km away from an infected badger sett and increased as the number of infected badgers *per* infected sett increased. Despite the significantly reduced risk of a breakdown with increasing distance from infected badger setts, the relationship was not strong (sensitivity and specificity of the model

were in the low 70s%) and explained only 9-19% of bovine TB breakdowns.

A retrospective cohort study with bovine TB test data extracted at CVERA (UCD) (Olea-Popelka and others 2004) investigated breakdown severity as a predictor of future herd breakdowns in the ROI. The hazard (risk) of a future bovine TB breakdown increased directly with number of cattle in the herd, a positive history of previous bovine TB in the herd, and the local herd prevalence of bovine TB. The presence of confirmed bovine TB lesions in reactor cattle was not predictive of the future breakdown hazard when the effects of other factors were controlled.

The ROI study above conflicts with a recent GB study, which showed that ~30% of herd breakdowns extent for >8 months (Karolemeas and others 2010) and consume disproportional resources as well as acting as ongoing sources of infection. Breakdown duration was a function of infection status and test performance. Potential explanations for persistent infection included: sub-optimal performance of the bovine TB tuberculin test, delay in its application or re-introduction of infection. Skin test sensitivity has been estimated at 75.0-95.5% (de la Rua Domenech and others 2006). If the sensitivity was substantially lower, failure to detect and remove infected animals would create potential for within-herd persistence and onward spread.

Factors associated with breakdown recurrence in the ROI, where detailed animal-level data were available (Wolfe and others 2010 and ROI references summarized therein), included slurry spreading, purchase of bulls and cattle, presence of inconclusive reactors in the breakdown, presence of badgers and nutritional status. Where only population-level surveillance data were available, factors associated with recurrence included herd size, reactor number and recent herd bovine TB history (Wolfe and others 2010, Abernethy and others 2010, Karolemeas and others 2011).

In DEFRA SE3230 (The Problem TB Herd – Characterisation, Prediction and Resolution) breakdown confirmation status was by far the strongest risk factor for **persistence** (OR=12.6). They used an improved case definition and concluded that this strong association may best be explained by the tendency to deploy severe interpretation of the tuberculin test in herds with confirmed status and the possibility that true prevalence was underestimated (DEFRA SE3230, Karolemeas and others 2010). Their model could predict earlier those herds most likely to sustain persistent infection. Resources and earlier intervention could be directed at those herds. The model predicted that stopping animal

movements onto the farm during the breakdown and moving salt licks indoors was associated with a small decreased risk. It is also plausible that a number of the unconfirmed herds were not actually infected.

Further analysis from the same GB research group using the RBCT CCS2005 data identified that despite increased testing during and after breakdowns ~21% of breakdowns recurred within 12 months. 60% of these recurrences were disclosed at the 6-month follow-up, suggestive of within-herd persistence. 38% recurred within 24 months (Karolemeas and others 2011). Factors associated with **recurrence** were – reactor number and recent history of bovine TB in the herd, consistent with previous ROI and NI studies (Wolfe and others 2010, Abernethy and others 2010). However, they found a lack of association with the confirmation status of the initial breakdown and recurrence. They conclude that their data support a higher prevalence of infection than observed or residual infection. The main risk factors associated with recurrence in this study ranked as follows: use of 'other housing types' (OR=4.6), number of contiguous farms (OR=3.2) and borrowing animals (OR=2.1) (Karolemeas and others 2011). Protective factors associated with decreased risk of recurrence included the presence of rough grass/moorland (OR=0.3). These recurrent breakdowns may have been re-infected from a local source, such as wildlife, or from cattle movements into the herd. As well as consuming disproportionate resources, the existence of recurrent breakdowns suggests that such herds cannot reliably be cleared of infection and undermines stakeholder confidence in the TB programme.

There is either residual infection within such herds or they are repeatedly becoming re-infected. They concluded that certain farm practices or characteristics may predispose to re-infection and that a combination of factors was associated with recurrence, rather than just one strong factor. Despite variation between farming practices within the British Isles, reactor number and recent history of bovine TB were consistent risks for recurrence in ROI, NI and GB studies (Karolemeas and others 2011). Whether the breakdown was confirmed or not was the major factor in the duration of breakdowns (persistence) in GB (Karolemeas and others 2010), but was not a factor in risk of recurrence, and neither was herd size nor cattle movements (Karolemeas and others 2011). This illustrates that the risk factors for different types of breakdown (sporadic, persistent, recurrent etc) may well be different. Either way, this could increase transmission potential to local wildlife or to local or more distant cattle herds through cattle contacts and

movements, during periods when movement restrictions are not applied. The relative contribution of persistence versus re-introduction to recurrence is unknown (Karolemeas and others 2011) and although their wildlife data were relatively weak, no association was detected between badger presence and recurrence at the 6 month follow-up herd test. To increase the detection of exposed/infected cattle within-herd, there have been suggestions to increase the between-test interval and the duration of herd restriction.

Olea-Popelka and others (2005) attempted to estimate the levels of badger exposure for cattle and to test the hypothesis that increased badger exposure does not increase the risk of bovine TB in selected ROI herds. They used data from the Four Areas Trial badger cull in Kilkenny (1996–1999). The specific location of cattle within each farm, and the length of time that cattle spent in each farm field during the grazing season, and in the barnyard during winter, was used to build an exposure coefficient to quantify the amount of badger exposure that cattle encountered either on pasture or in the barn. The study design was a matched case-control study in which the control herds were selected using incidence density sampling. During the 4-year study period, 543 badgers were removed and of those 96 badgers were bovine TB positive and 96 herd breakdowns occurred. There was a significant association between case herds and having a higher badger sett exposure coefficient during 1996–1998, but no significant association between case herds and having a higher exposure coefficient based on the number of badgers, or the number of bovine TB-positive badgers, during September 1997–December 1999 was found. It would be valuable to take the same approach to quantifying within-herd cattle contacts in housing and at pasture.

Porphyre and others (2008) investigated risk factors for bovine TB on New Zealand cattle farms and their relationship with possum control strategies. Study design was a retrospective cohort based on data obtained from the TB testing surveillance programme. The model showed that, despite intensification of possum control strategies over time, proximity to forest parks (a principal possum habitat in this area) remained a significant predictor of the number of confirmed cases of TB detected *per* farm *per* year. Their analyses showed a significant, three-fold increase in bovine TB risk in dairy cattle relative to beef, conditional on the size of the local possum habitat. Other factors identified included the cattle population size and the presence of previous infection.

Ramirez-Villaescusa and others (2009) examined herd- and animal-level risks associated with bovine TB tuberculin test positivity in cattle in 148 herds in RBCT areas of SW England. Data on cattle on these farms were sourced from the bovine TB VetNet database from1996 to 2004 and from the British Cattle Movement Scheme database. Results showed that cattle were more likely to react to the bovine TB tuberculin test when they had been present at a previous bovine TB herd test(s) where other cattle had reacted. This positively correlated with age and number of tests. Cattle on restocked farms were less likely to react to the tuberculin test compared with cattle on continuously stocked farms. These results highlight the likely importance of exposure to infected cattle present at a previous test as a source of infection to cattle that subsequently became reactors. This suggests that there was a lower risk of exposure to bovine TB to cattle in newly formed herds.

Further analysis (Ramirez-Villaescusa and others 2010) examined herd and individual animal risks associated with tuberculin test positivity in cattle in 148 herds in south west England, 24% were restocked post-FMD and 76% were continuously stocked. Farms restocked for <12 months post-FMD had a significantly reduced risk compared to continuously-stocked farms. The feeding of mineral licks and vitamin supplements was associated with reduced risk. Storing manure and slurry indoors or in a closed container, spreading manure all year, possession of dairy cattle, increased herd size, purchase of cattle from markets and farm location were all associated with increased risk. The authors concluded that whole herd removal might have reduced the infectious load on these premises, but this did not continue once cattle were reintroduced. The method of slurry storage or spread might allow *M. bovis* to persist in the environment in some cases. The increased risk associated with cattle purchase and continuous stocking versus restocking supports a role for undetected infection in cattle as a risk to other cattle.

Roberts (2004), in an MSc dissertation, examined the influence of selected herd factors on the occurrence of bovine TB in one DVO region in Northern Ireland, using data extracted from APHIS. Factors identified as significantly associated with herd breakdown, included herd size, presence of bovine TB in contiguous herds and the median number of cattle movements into the herd.

White and Benhin (2004) used two GB cross-sectional time-series data sets for their analysis. The first was a 'land-use' data set obtained from the Agricultural and Horticultural Census; Small Area Statistics

(excluding minor holdings, June 1988 to June 1996). The second dataset consisted of the number of herd breakdowns and corresponding numbers of badger-removal operations for the period 1986–1996. Variables found to significantly affect the number of herd breakdowns between parish groups were the number of herd breakdowns in the previous 2 years, the number of farmers and the ratio of cattle to regular farm workers.

4.3. Farm-Scale Studies

Most of these case-control studies operate over quite large areas, larger than the individual farm scale, where model predictions would be even more useful. However, a recent study, using RBCT (see Review 2) data from one year into treatment to one year after treatment, presents an analysis of spatial farm-level, herd-based risk factors associated with the probability of a confirmed bovine TB breakdown in herds subjected to proactive badger culling , localized reactive badger culling and no culling (survey-only) treatments (Vial and others 2011). Local farm-level risk factors were controlled for. Within reactive and survey-only areas, the risk of a confirmed bovine TB breakdown was associated with two factors - increasing numbers of active badger setts and having cattle herds within 1.5km. For proactive areas, the strongest predictor of bovine TB risk was the number of *M. bovis*-positive badgers culled initially within 1.5km, suggesting that a risk remained for those herds, which was not removed by badger culling. They provide further evidence that the local infection in cattle and badgers is linked. Within the RBCT data they found that dairy herds were more at risk than beef herds. They also noted that dairy herds tended to rely on one particular breed of cattle, whereas beef farms tended to use a mixture of breeds and crossbreeds. Whilst acknowledging the complex interaction of risk factors, they indicated that a breed effect might operate (Allen and others 2010).

The relative importance of the behaviour of wildlife reservoirs is determined by local ecology, including farm practices (Mill and others 2011) and will likely vary in time and space. Further, recent, mixed modelling and event history analysis were used to investigate individual risk factors in RBCT data analysis, again at the individual farm level. Farm characteristics, in particular herd and farm size, number of land parcels and being contiguous to other breakdowns were significant and consistent risks. They also identified increased risks for those herds subjected to reactive culling and those with increased herd size and increased and fragmented farms (Mill and others 2011). In areas with

previously undisturbed badger populations, risks were reduced for herds within the proactive zones, but the authors point out that they did not evaluate the effect at the cull edges or within 2km of the cull. Risk was actually greater in reactive and survey-only areas by 23% and 18%, respectively, indicating that localized reactive culling was associated with a higher risk than not culling, and this was felt at the local farm level. Whether this risk was sustained over time since last cull remains to be reported. Farm and herd size, number of land parcels and contiguous neighbours were the most consistent risk factors and no consistent risk due to badger- or habitat-related variables was identified at the farm level.

Several herd-level risk factors have been identified in recent GB studies, both inside and outside RBCT treatment areas. These comprise – herd size, historic incidence and farm area (Vial and Donnelly 2011). In summary, the risk factors that have been most consistently identified in relation to bovine TB in the UK and ROI include: cattle movement, occurrence of TB on contiguous premises and/or the level of bovine TB in surrounding areas and herd size. Other factors identified in some studies include: indicators of badgers density/activity, use of multiple premises, housing type, herd type, farmland habitat, fertiliser usage, mineral deficiencies and use of silage clamps. Herd-keeper behaviour is likely to change during an outbreak due to increased risk perception, leading to improved biosecurity measures and risk aversion (Garcia Alvarez and others 2011).

In general, the most consistently identified risk factors are biological plausible and consistent with known transmission routes involving cattle-cattle and badger-to-cattle spread. It is important to note, that epidemiological studies vary in the variables analysed, the exact measures used (e.g. in relation to association with badgers) and study size and power. Not all risk factors would be expected to be identified equally across studies. Risk factors will vary across regions due to factors such as differing farm structures, farm management practices, local TB control and the relative importance of specific risk factors within individual areas. After extensive and iterative risk factor studies on RBCT data, the ISG concluded that important risk factors differed between regions and other case-control studies of bovine TB in cattle had yielded widely differing recommendations (Bourne 2007).

Taken together, these studies illustrate the variation in study design and outcome. It may not be possible to reliably identify particular risk factors which could be widely adopted and predicted to lead to

reduced transmission of disease to and from cattle. More insight can be achieved when risk factors are classified into management, wildlife and environment factors (Bourne 2007). It should be accepted that environmental features are rarely controllable by the herd-keeper. The primary risk factor is cattle density, which increases the probability of transmission via aerosol between infectious and susceptible animals (Humblet and others 2009). Regarding management factors, the results suggest that cattle movements, herd contacts, use of fertilizer, housing and feeding practices may impact on risk. However, study findings identify associations and not necessarily causes. Nevertheless there is sufficient evidence that by applying the broad principles of biosecurity it should be possible to reduce the risk of cattle becoming infected by other animals, including badgers. Account should be taken of cattle movement on and off the premises, minimising contact with other cattle and between cattle and badgers and taking greater care with animal housing and feeding practices. In particular, studies such as the TB99 and CCS2005 analyses of the GB RBCT data indicate that there is no universal solution for farm management to reduce the risk of a herd breakdown. Occasionally a clear cause and effect relationship can be demonstrated by these epidemiological studies, but in most cases the situation is more complex and the research tells us what factors are important concerning a specific question or a theoretical level of risk associated with a particular event, behaviour or contact.

Risk factors have been investigated in case-control studies in Europe and the USA. Historical incidence was a robust predictor of the rate of future breakdowns in UK and ROI herds, suggesting that the disease source was not adequately removed, or that some other factor(s) made them particularly susceptible. Herd size is repeatedly identified as a major risk in many studies. Large herds tend to graze larger areas and may purchase and move more cattle, increasing the probability of having contiguous herds that facilitates cattle-cattle spread. Herd size was a significant predictor of transient and persistent bovine TB in a case-control study in England (Reilly and Courtenay 2007) and, surprisingly, higher stocking density was associated with reduced risk. The number of farm parcels, but not the area farmed, was associated with increased risk. In turn, herd size was linked to management-related risk factors including farm type, feeding regime and herd turnover rate (Vial and others 2011). The higher production stress of intensive management has been associated with increased risk (Griffin and others 1993). Herd breakdowns tend to reoccur, especially in larger herds, possibly as a

result of failing to clear the source and contact with contiguous herds and infectious wildlife.

Larger herds are more likely to have at least one cow with disease. As herd size increases, the probability of at least one case increases and herds of different sizes are therefore at different risks. The observed size distribution of bovine TB-affected herds suggests that animals pose identical risks. Cattle living in different parts of the UK and ROI must experience different risks. There was no consistent indication in the GB TB99 and CCS2005 data to indicate that the presence of any wildlife species, or indeed domesticated species, was associated with the risk of multi-reactor breakdowns (Bourne 2007). There is evidence that increasing herd size for financial gains may actually contribute to increased bovine TB incidence (Brooks-Pollock and Keeling 2009).

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