**Review**

**Bovine tuberculosis: the genetic basis of host susceptibility**

A. R. Allen\(^1,\)\*, G. Minozzi\(^2\), E. J. Glass\(^3\), R. A. Skuce\(^1\),
S. W. J. McDowell\(^1\), J. A. Woilliams\(^3\) and S. C. Bishop\(^3\)

\(^1\)Veterinary Sciences Division, Bacteriology Branch, Agri-Food and Biosciences Institute, Stormont, Stoney Road, Belfast BT4 3SD, UK

\(^2\)Parco Tecnologico Padano, Via Einstein, Polo Universitario, Lodi 26900, Italy

\(^3\)The Roslin Institute and R (D) SVS, University of Edinburgh, Roslin, Midlothian EH25 9PS, UK

The prevalence of bovine tuberculosis (BTB) in the UK remains a significant economic burden and problem for the agri-food industry. Much effort has been directed towards improving diagnostics, finding vaccine candidates and assessing the usefulness of badger culling. The contribution that host genotype makes to disease outcome has, until recently, been overlooked; yet, it is biologically untenable that genetic variation does not play a role. In this review, we highlight the evidence, past and present, for a role of host genetics in determining susceptibility to BTB in livestock. We then address some of the major issues surrounding the design of future studies tasked with finding the exact causative genetic variation underpinning the TB susceptibility phenotype. Finally, we discuss some of the potential future benefits, and problems, that a knowledge of the genetic component to BTB resistance/susceptibility may bring to the agricultural industries and the wider scientific community.

**Keywords:** bovine tuberculosis; resistance; susceptibility; genetic variation

1. **INTRODUCTION**

Bovine tuberculosis (BTB) is an infectious chronic respiratory disease caused by *Mycobacterium bovis*. Eradication of TB from cattle herds has been successful in countries including Austria, Belgium, France, Canada and most of the USA. Today, the UK is one of the few countries in Western Europe that does not hold an officially BTB-free status (de la Rua Domenech 2006). This is despite the fact that in the 1950s the launch of a national BTB eradication scheme, comprising of annual herd tuberculin testing, animal movement restrictions and slaughter of reactor animals, reduced the number of tuberculin reactors from 16 894 in 1961 to 633 in 1979 (de la Rua Domenech 2006). Since then, herd breakdowns in Great Britain have increased by approximately 14 per cent each year since 1990 (Animal Health 2004; DEFRA). Since the mid-1970s, the potential role of infected wildlife reservoirs of the disease, in the form of Eurasian badgers (*Meles meles*) in the UK, has been a concern (Pritchard 1988; Porphyre et al. 2008). Recent research has demonstrated that badgers are maintenance hosts of *M. bovis* infection for cattle, and consequently an impediment to eradication of disease (Krebs et al. 1997; Gallagher & Clifton-Hadley 2000). The recently completed randomized badger culling trial further confirmed the role that badgers play in infecting cattle.

However, the report concluded that the logistics and costs involved in pro-actively culling across large areas greatly outweighed the modest benefits of reduced BTB incidence in cattle. As a result, a recommendation to focus on improving cattle movement controls was suggested (Bovine tuberculosis 2007).

In countries with well-developed control programmes, infected animals rarely show clinical signs, being able to remain in a subclinical state for an extended period. Annual costs worldwide to agriculture owing to BTB are estimated to be around three billion US dollars (Garnier et al. 2003). In the UK, the costs incurred in attempting to eradicate TB in 2005 were £90 million, and it is estimated that it could reach £1 billion between 2008 and 2013 (The Veterinary Record 2008).

Given the difficulties in eradicating BTB, 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 & Mackintosh 2000) where results of experimental challenge with *M. bovis* evidenced a wide spectrum of responses and a high heritability of resistance to TB (0.48 ± 0.096). This result indicates that approximately 48 per cent of the variation seen in response to infection with *M. bovis* is due to host genetic variation.

Interestingly, in cattle, Ameni et al. (2007) have demonstrated differences in susceptibility to BTB at the level of genus, indicating that *Bos indicus* cattle are more resistant than *Bos taurus*. Recent findings have also demonstrated significant heritability to susceptibility to BTB in Holstein cattle in the UK (Brotherstone et al. 2010) and in the Republic of Ireland (Birmingham...
Furthermore, as stated by Bishop & Woolliams (2010), field studies are likely to underestimate heritability, owing to unequal exposure to the pathogen as well as incomplete sensitivity of the diagnostic tests used, and thus could cause underestimation of the potential gains for breeding for disease resistance.

In this review, we discuss the wealth of data that now indicates the existence of a genetic basis for resistance to BTB in cattle, explore the strategies, new technologies and resources available that could enable the discovery of the exact genetic variation underpinning this phenotype and discuss the feasibility of exploiting such findings in breeding for resistance.

Understanding how *M. bovis* infects its host, and how the host and bacterium respond to each other’s immune and immuno-modulatory activity, has been the subject of many reviews over the years. We will not cover this exhaustively here. However, it is important to be aware of these interactions since underlying mechanisms may be used to identify candidate genes and genetic loci that can inform future genome scans and fine mapping. A short overview of this area is presented in the electronic supplementary material.

### 2. HOST GENETIC VARIATION

Studies on humans and mice have indicated evidence for host genetic variation in tuberculosis resistance, although there have been difficulties in identifying the causative genes. Recently, excellent reviews on mice and human genetic studies in relation to TB have been published (Hill 2006; Fortin et al. 2007). It is beyond the scope of this paper to focus on the same subject. However, an overview of the evidence from both species is presented in the electronic supplementary material. In this review, we focus on the work performed on livestock species, specifically cattle and deer. We then focus on the opportunities available to further elucidate the mechanisms underlying BTB resistance and the use of this information as a complementary and sustainable approach to disease control.

#### (a) Cattle studies

Despite considerable evidence of a genetic component to TB resistance, modest effort has been directed towards identifying bovine genetic susceptibility loci. Indeed, it is only recently that effort has been directed towards quantifying the host genetic influence (Bermingham et al. 2009; Brotherstone et al. 2010).

Early studies demonstrating that zebu cattle (*B. indicus*) were more resistant to TB were conducted in India and in Uganda in the 1930s. In Uganda, the incidence of TB from 1931 to 1936 was 17 per cent in Ankole cattle and only 0.9 per cent for zebu. Furthermore, a small-scale inoculation experiment was conducted, and while all three Ankole cattle inoculated died, only one out of the eight zebu calves died (Hutt 1958).

Research in Simmental cows found evidence of differences in TB prevalence in daughters of two sires, being 4 and 62 per cent, respectively (Ruppert 1935). Similarly, differences in TB prevalence in daughters of different sires were found in black pied lowland cattle (Hutt 1958).

Also, European *B. taurus* cattle appear to be more susceptible to *M. bovis* infection than *B. indicus* cattle. Amen et al. (2007) presented data from a cohort of 2500 zebras, 1900 crossed (zebu–Holstein) and 900 Holstein cattle. Not only was prevalence of BTB higher in the Holstein population compared with the other two, but severity of pathology in skin test-positive animals was also significantly greater. Disease risks were also estimated, with Holstein cattle 2.32 times more likely to be diseased than zebu cattle (Ameni et al. 2007).

Population-level studies have also demonstrated genetic variation in resistance. The extent of the genetic contribution to variable traits such as resistance may be summarized by the heritability (*h*²), which is the extent to which phenotypic differences between animals are due to additive genetic effects (Falconer & 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 (Petukhov et al. 1998). A recent quantitative genetic study, which analysed TB skin test data from British dairy cattle herds, estimated a heritability for TB resistance of 0.18 ± 0.04 on the liability scale (Brotherstone et al. 2010). Interestingly, in this study, TB susceptibility was not found to be associated with milk productivity in the dairy cattle studied. Further work in the Republic of Ireland has also estimated the heritability of the TB resistance phenotype to be 0.18 ± 0.04 (Bermingham et al. 2009), identical to the estimate from the British study. Pertinently, this research also demonstrated a strong genetic correlation between susceptibility to confirmed *M. bovis* infection and *M. bovis* purified protein derivative (PPD) responsiveness. These data address one of the major concerns of the agricultural industry—will breeding for 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 the latter concern by indicating that selection for animals resistant to PPD responsiveness will indirectly select for resistance to TB infection.

These encouraging findings indicate a role for genetics in a wider risk management strategy. For example, in dairy cattle, exploitation of genetic variability has already been established and used in selection programmes for mastitis resistance (Rupp & Boichard 2003). Although the heritability of clinical mastitis is low and has an adverse correlation with production traits, selection for mastitis resistance is nevertheless implemented in selection programmes in many countries, notably in Scandinavia (Heringstad et al. 2003). In principle, the same could be done for resistance to TB in cattle breeding programmes. The idea of breeding TB-resistant animals is not new, having been raised before the modern genomics era. In the early years of the twentieth century, 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).
Deer are found, as both wildlife and livestock, in New Zealand, Australia, USA and Canada (Griffin & Mackintosh 2000). They are susceptible to BTB and can act as wildlife reservoirs for cattle. Differential susceptibility to TB has been observed in deer during severe outbreaks and differential disease transmission rates have been attributed to the host genetic background (Mackintosh et al. 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 (Griffin & Mackintosh 2000).

Mackintosh et al. (2000) estimated a heritability of TB resistance in deer to experimental infection with M. bovis of $0.48 \pm 0.09$. This result suggests 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, as discussed above. Further, it was the first rigorous study to confirm that TB resistance is a heritable trait in a livestock species.

3. FINDING TB SUSCEPTIBILITY LOCI IN THE BOVINE GENOME

It seems apparent that resistance to infection and disease caused by the Mycobacterium tuberculosis complex of organisms is under polygenic control in a number of species (electronic supplementary material). To date, candidate gene case-control studies and heritability estimates across these species have laid much of the groundwork upon which the next phase of TB resistance research in cattle will build. At their most basic level, these genetic epidemiology studies will have a common strategy—to compare the genomes of disease-affected case animals with those of unaffected control animals to find allelic variants that segregate and are associated with either phenotype. Below, we discuss some of the methodological considerations that should accompany any attempt to further dissect the TB resistance phenotype in cattle using such case-control methodologies.

(a) TB diagnosis in cattle

It is important to address the area of TB diagnostics in this review as it has major implications for the design of any genetic epidemiology study tasked with determining the heritability of TB susceptibility and the individual loci contributing to this phenotype. The identification of the TB-susceptible (case) or TB-resistant (control) phenotypes will include information derived from the diagnostic test itself, along with other co-variables.

In countries implementing BTB eradication programmes, routine screening of herds to detect the presence of animals exposed to M. bovis is based on intra-dermal tuberculin testing. A positive test is generally followed by animal movement restrictions and compulsory slaughter of test-positive animals in an attempt to remove the disease from the herd. Intradermal skin testing uses the antigenic PPD mixture, known as tuberculin, from heat-killed bacteria (Waddington 2004). PPD was initially isolated and purified by Robert Koch. Bovine PPD (PPD-B), prepared from M. bovis, has become the frontline diagnostic reagent in the eradication of TB. The PPD-B test is an in vivo assay and consists of intra-dermal inoculation of tuberculin; results are then assessed by the degree of delayed-type hypersensitivity (DTH) response observed. DTH is the cellular acquired immune response to specific antigens resulting in an inflammatory response that causes swelling of the skin at the site of inoculation.

Mycobacterium bovis PPD is known to contain antigenic components that are shared by many other mycobacteria (Andersen et al. 2000), including members of the M. avium intracellulare complex (Biet et al. 2005) that are commonly found in the environment, often confounding interpretation of diagnostic tests (Pollock et al. 2001a). This lack of specificity has been addressed in the UK and Ireland by making the tuberculin skin test comparative. By inoculating with both PPD-B and PPD from M. avium (PPD-A), practitioners can determine difference in DTH response and make an informed judgement on whether the animal is infected by M. bovis or sensitized to environmental mycobacteria.

Sensitivity of the test has been shown to be variable with median values of 80 per cent (de la Rua-Domenech et al. 2006). Some animals do not respond to the skin test, despite presenting physical signs of TB infection such as lesions post-mortem (Doherty & Cassidy 2002). Consequently, post-slaughter inspection of carcasses and culturing of mycobacteria from tissue are also important measures for confirming field diagnosis. Conversely, many animals are positive to the skin test, but lesions are not visible at slaughter (Doherty & Cassidy 2002).

The American Thoracic Society (2000), Young et al. (2009) and Barry et al. (2009) broadly agree that, when combined with pathology and bacterial culture data, both positive and negative responses to a TB diagnostic skin test in humans can be indicative of a spectrum of phenotypes. These are:

- pathogen-exposed individuals with negative skin test and no evidence of pathology or bacterial culture. These individuals are the most resistant to TB having eliminated the pathogen via the innate immune response as evidenced by their lack of DTH.
- Individuals with positive skin test, but no evidence of pathology or bacterial culture. These individuals have presumably also resisted the development of the disease, but have had to rely on the acquired immune response to do so.
- Individuals with positive skin test lack overall signs of pathology and exhibit bacterial culture with bacteria persisting in the host in a non-replicating form. These individuals may represent the latent or quiescent phase of TB infection.
- Individuals with a positive skin test lack overall signs of pathology and exhibit bacterial culture with bacterial replication in the host maintained albeit at a sub-clinical level. These individuals may represent the active phase of TB infection prior to clinical disease.
- Individuals with a positive skin test exhibit evidence of pathology and bacterial culture. These individuals represent active infection and clinical disease.

This classification is a departure from the view that tuberculosis 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.

In addition to the BTB skin test, the 1980s saw development of the gamma-interferon (IFN-γ) test for the detection of BTB (Rothel et al. 1990). This test is an enzyme-linked immuno-sorbent assay performed on whole blood and detects cell-mediated release of IFN-γ in response to incubation with PPD-B (Neill & Pollock 2000). Studies undertaken throughout the world have indicated the test’s sensitivity is superior to that of skin testing while specificity is poorer (Neill & Pollock 2000). These findings have influenced the perception that eradication schemes should make use of both tests with the general view being that the tuberculin skin test is superior at detecting infected herds, while the IFN-γ test is better for detecting individual infected animals within herds (Neill & Pollock 2000). Both the skin test and IFN-γ test detect evidence of an adaptive immune response to BTB infection. However, both tests appear to detect infection at different stages, leading to the possibility that both tests may define potentially overlapping, but distinct disease phenotypes. While disease-susceptible and -resistant phenotypes defined using IFN-γ are useful for genetic epidemiology studies, the IFN-γ test is not as widely used in cattle populations in the UK as the skin test. As a result, it is much easier to collect samples from skin-tested animals.

(b) Phenotyping and exposure to pathogen

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 more complex. In a case–control scenario such as this, ensuring that the controls have had equal (or even 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.

Defining TB-resistant and -susceptible phenotypes in cattle for genetic epidemiology studies could be informed by the spectrum of phenotypes proposed by Young et al. (2009). By extrapolating Young et al.’s (2009) spectrum, we propose that the most susceptible animals are defined as those that 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 are those that 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. There is always the possibility that some potentially resistant controls could in reality be infected, despite giving a false-negative skin test result, and are only detected in the abattoir at time of slaughter by detection of lesions. However, with modern cattle movement and epidemiological databases, such false negatives can be detected and retrospectively removed from the control cohort. The latter case and control definitions occupy the opposite ends of the spectrum proposed by Young et al. (2009) and as such may represent the greatest likely genetic differences that are easily detectable.

(c) Candidate gene versus whole-genome approaches

Having assembled a panel of well-phenotyped case and control animals, options are to genotype markers in candidate genes only or to genotype markers from across the entire bovine genome. Previously, candidate gene studies based on biological plausibility of involvement with the phenotype, but no prior genomic regional association, have been applied to study populations. These studies have had limited success in finding disease susceptibility loci. Such candidate gene approaches in BTB resistance research have concentrated mainly on the bovine natural resistance-associated macrophage protein (NRAMP1) gene owing to the ubiquity with which it has been identified as a candidate in mouse and human studies (electronic supplementary material). A microsatellite in the 3’ untranslated region (UTR) of the bovine NRAMP gene has been found to be associated with natural resistance to brucellosis infection in cattle (Adams & Templeton 1998) and in macrophages the ‘resistant’ allele appeared to be associated with the survival of M. bovis BCG (Qureshi et al. 1996). In one small study, comprising 33 cattle with positive results to the tuberculin test, of which nine were assigned a resistant phenotype and 24 a susceptible phenotype, no association was observed between the bovine 3’ UTR microsatellite and resistance to M. bovis infection (Barthel et al. 2000).

Other biologically plausible potential candidate genes, primarily from the immune system, the interface of host–pathogen interaction, have been suggested. The bovine MHC, or BoLA region, has shown association with several diseases in cattle (Lewin et al. 1999) and, in particular, BoLA DRB alleles have been associated with variation in T-cell responses to M. bovis antigens studied in vivo in a population of 47 Holstein calves (Casati et al. 1995). Similarly, the bovine orthologue of human DC-specific ICAM-3 grabbing non-integrin C-type lectin (DC-SIGN) gene has recently been identified and functionally characterized (Yamakawa et al. 2008). Results demonstrate that the DC-SIGN receptor interacts and binds to M. bovis BCG (Yamakawa et al. 2008). It is worth speculating that in cattle, as in humans, polymorphic variation in the DC-SIGN gene may be associated with TB resistance.

As can be seen above, when compared with the human and mouse fields of tuberculosis susceptibility loci research (electronic supplementary material), comparatively few actual genetic epidemiological studies have been done in cattle populations. Studies tend to be small, focused on plausible candidate genes only and very probably underpowered to detect genuine associations. Future efforts are more likely to use a whole-genome approach, exploiting high-density single
nucleotide polymorphism (SNP) arrays to discover disease-associated variants more common in affected than unaffected animals, for which there was no a priori link to the phenotype. 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 TB resistance.

(d) Detecting association in whole genomes
Mammalian genomes are large and complex. Association studies have been developed that make use of the variability of the genome as a mapping tool. Whole-genome case–control association studies make use of large numbers of phenotyped case and control individuals, and genotype them for specific polymorphic markers spread across the entire genome (Lander & Schork 1994). The concept of linkage disequilibrium (LD), i.e. non-random allelic association between linked loci, is central to this endeavour. By genotyping many polymorphic markers across whole genomes, researchers aim to find the segment of chromosome that segregates with the phenotype they are interested in. A particular allelic variant of a genotyped polymorphic marker occurring more commonly in the diseased case population than in the control population is indicative that they have been successful. After identifying the particular segment of the chromosome that is of interest, researchers then have the challenge of finding the actual causative DNA variation underlying the phenotype. However, by knowing the identity and function of genes in the implicated segment, one can determine candidate genes that could plausibly contribute to the phenotype.

The ubiquity with which case–control genetic association studies have been applied in the field of human epidemiology has permitted refinement of methodology and study design. This is likely to be of great benefit in designing a study to find TB susceptibility loci in cattle. Below are listed some of the major design issues that affect the ability of such a study to detect genuine genetic effects.

(e) Study size and effect of linkage disequilibrium
If tuberculosis susceptibility is a complex genetic trait governed by the combined input of small effects spread over many genes, 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 study size can lead to spurious associations between genotype and phenotype, exacerbating the so-called winner’s curse (Xiao & Boehnke 2009) in which significant associations are upwardly biased. The power of genetic epidemiology studies to detect allelic association at a specific locus is intimately related to the size of the study population used, allele frequency of the associated variant, the size of the effect of the variant on the phenotype and the amount of LD observed between the causative allele and the marker allele genotyped. As a result, with so many initially unknown variables, power calculations are determined retrospectively. However, as a general rule, increasing size of the study population increases the power to detect low-frequency variants with small effects on phenotype. As a result, these studies generally require several hundred or thousand case and control samples.

There is the possibility that several major gene effects, which are easier to detect, account for most of the phenotype variation, as has been shown for some forms of human cancer. For instance, the BRCA locus has been observed to confer large relative risks of developing breast cancer in carriers. However, it is believed that this locus may only account for 20 per cent of the genetic variation observed in breast cancer development and that the remaining 80 per cent of variation is under the control of smaller gene effects that have lower relative risks, and are therefore, harder to find (Thompson & Easton 2004).

Mapping disease resistance loci relies on using the LD between variants contributing to the phenotype and marker loci. Therefore, the degree of LD between both has an impact on the power of the study, hence the number of markers and the number of cases and controls required to detect these associations. Since LD decays quickly as distance between loci increases, a higher marker density and a larger number of cases and controls are needed to ensure adequate power. LD in the cattle genome appears to stretch over much greater distances than that observed in the human genome, however, and in some cases up to 1 Mb (Kim & Kirkpatrick 2009). This elevated LD has probably arisen as a result of non-random mating (Bovine HapMap Consortium 2009; Kim & Kirkpatrick 2009) and likely reduces the marker density of SNPs needed to cover the bovine genome (Bovine HapMap Consortium 2009). Therefore, the 800 000 SNPs offered in current bovine SNP arrays may provide sufficient genome coverage for effective mapping. The increased bovine LD, compared with humans, will also make it easier to detect true-positive associations without having to drastically increase numbers of case and control animals. A negative aspect of this elevated LD will be that once a putative chromosomal area has been mapped, it will be much harder to fine map the actual causative mutation.

(f) Population stratification
Underlying substructure of a population can cause spurious associations between genetic marker and phenotype, by virtue of the fact that subgroups within populations can have very different allele frequencies (Hamer 2000; Cardon & Palmer 2003). As a result, with human genetic association studies, populations are standardized to use only individuals from the same ethnic group. This maximizes the probability that any observed difference in allele frequency will be due to the disease phenotype under study and not due to demographic differences.

Recently, the Bovine HapMap Consortium has illustrated the great diversity that exists between cattle breeds and the conversely smaller diversity within breeds (Bovine HapMap Consortium 2009). These data suggest that any case–control study undertaken in cattle should endeavour to use case and control animals of the same breed that have the same genetic heritage. In doing this, one maximizes the probability that any difference observed in allele frequency will be due to the
difference in susceptibility to disease alone and not due to population substructure.

(g) Statistical analysis
In genome-wide studies where large numbers of individual polymorphisms are typed across many genes, the spectre of multiple comparisons arises (Romero et al. 2002). To counteract the effect of multiple comparisons, it is usually necessary to apply some form of corrective modification to the p-values obtained (e.g. Bonferroni correction) to account for the number of statistical tests made.

Furthermore, we now view the genome as a single entity whose component genes interact, contributing to specific phenotypes (Moller & Hoal 2010). Recent advances are taking the field of genetic epidemiology beyond the concept of associating individual genetic polymorphisms with phenotypes. Daetwyler et al. (2008) have recently 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 is 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 selection using SNP arrays (Mewissen et al. 2001), which is now commonplace in most major dairy cattle breeding programmes.

(h) Pathogen genetic variation
Recent studies in human populations (Caws et al. 2008; de Jong et al. 2008; Kaufmann 2008) have demonstrated that the genetic make-up of bacteria and their hosts are now seen as important sources of variation, with interplay between the two needing to be taken into account. The relationship between host genotype, mycobacterial strain and the development of tuberculosis in a Vietnamese population has been tested (Caws et al. 2008), with results indicating an association between variation in the TLR2 (Toll-like receptor-2) gene and individuals with meningeval tuberculosis infected with the Beijing lineage of M. tuberculosis. Similarly, variation in the human IRGM gene, involved in autophagy, has been shown to contribute to protection from disease caused by M. tuberculosis, but not by Mycobacterium africanum (Intemann et al. 2009). Also, de Jong et al. (2008) determined that the phylogenetically distinct M. africanaum lineage of the M. tuberculosis complex could transmit equally well between humans but was less likely to progress to tuberculous disease than M. tuberculosis.

The extreme clonality of members of the M. tuberculosis complex means that they are prone to the effects of population bottlenecks, selective sweeps and genetic drift (Smith et al. 2006). Sampling from one population occurs, which seeds a new ecological niche or geographical region, and can lead to the emergence of strains that exhibit dramatic, geographically clustered variation in the pathogen population (Smith et al. 2006). The striking phylogeography disclosed recently for major lineages of human-adapted M. tuberculosis has important implications for lineage–lineage phenotypic differences (Gagneux & Small 2007) and will likely impact current and future regional TB control, epidemiology, diagnosis and vaccinology (Hershberg et al. 2008). Similar research to define the population structure and phylogeny of M. bovis in the British Isles is underway (Smith et al. 2006). Given the evidence that M. tuberculosis lineage affects the outcome of infection and disease, it would be pertinent to include the M. bovis lineage as a covariable in any future epidemiological study tasked with elucidating the mechanisms underpinning the variability observed in BTB infection of cattle.

(i) Resources available
Today we are in a unique position to identify the loci, markers and genes critical to influencing the degree of host resistance to TB. This is a consequence of two main achievements. First, recent major advances in genomics have opened opportunities to identify genes underlying host genetic variation. The bovine genome has been sequenced to 7.1-fold coverage (The Bovine Genome Sequencing and Analysis Consortium 2009), and 2.4 million putative SNP markers are now available to be exploited (Bovine HapMap Consortium 2009; Matukumalli et al. 2009; Villa-Angulo et al. 2009). Second, the genome information has assisted in the development of SNP arrays that can be used to dissect genetic variation for many complex traits and diseases. In cattle, it is now possible to type animals with a commercially available SNP array that contains a panel of 800 000 SNPs. These tools will allow powerful whole-genome association studies to be conducted, enabling identification of genes associated with TB resistance as well as genomic selection.

However, the application of genomic techniques requires phenotypic data. Further efforts are required at an epidemiological level to collect precise phenotypic information that will enhance the establishment of sound evidence of a host genetic influence on TB. Particularly powerful is the use and collection of field data relevant to the incidence of TB, coupled with post-mortem examination, bacterial isolation and laboratory confirmation. Large existing databases are already established owing to the systematic testing of herds in countries with eradication schemes. These provide the opportunity to refine disease breakdown information, to be used for robust analysis and modelling studies. In principle, health information can then be linked to pedigree and other animal databases and lead to the estimation of direct genetic effects on resistance and also correlated effects on production traits.

Moreover, extensive data have now been collected on the spatial distribution of the various bacterial strains that could allow detailed interrogation of the impact of the pathogen lineage on the genetic control of the response (Smith et al. 2003). Together, these resources, the phenotypes, the genomic sequences and the high-density genomic markers, will help address fundamental questions about host and pathogen genetic factors that influence TB prevalence in cattle.

Bovine experimental models of M. bovis infection have been used before to elucidate the dynamics of infection
and pathophysiological outcomes (Pollock & Neill 2002). Availability of such expertise would also be of great benefit to this work. Once individual marker or whole-genome associations have been made with disease resistance, it would be beneficial to challenge these animals, or their progeny, with experimental M. bovis infections. Such work would serve to test the hypothesis that these animals are indeed resistant under field conditions and perhaps shed new light on in vivo mechanisms that contribute to the phenotype.

4. CONCLUSIONS
The overall aim of this review was to assemble the evidence that will help unravel the genetic components underlying differences in response to TB. Research in livestock host variability in response to BTB could deliver genetic markers such as SNPs associated with resistance. Implementation could be by the use of genetic markers associated with TB resistance/susceptibility in existing breeding programmes or even the identification of genetically susceptible sires to avoid their use in high BTB prevalence regions. The latter approach could help to achieve the goal set by Cox et al. (2005) who argued that even modest reduction in the reproduction number of M. bovis in cattle could bring the current epidemic under control.

It is possible that M. bovis and cattle may have reached an evolutionary stalemate, as has been proposed for humans (Muse Davis & Ramakrishnan 2009). While this proposed tolerance of host for pathogen and pathogen for host may well be an example of coevolution to maximize the survival of both species, some commentators suggest the balance of power may be tilted in favour of the pathogen (Paige & Bishai 2010), thereby hampering eradication. By improving the genetic resistance of the national herd, the balance in the evolutionary arms race between host and pathogen could be tipped back in the host’s favour.

Such an approach could well be more sustainable than the current test and slaughter protocol in the UK and Ireland. Furthermore, identification of novel biochemical pathways involved in host response to pathogen could inform future efforts to produce better diagnostic tools and vaccines.

There may well be other benefits to this strategy of breeding for TB resistance. Several other obligate intracellular bovine pathogens may interact with their host using similar mechanisms to those likely to be discovered for M. bovis. These organisms include Brucella abortus, Salmonella enterica and Mycobacterium avium paratuberculosis. It is conceivable that by selecting animals to be more resistant to BTB, one could also serendipitously select for increased resistance to these other pathogens. In addition, there is evidence (Brotherstone et al. 2010) that there is no genetic association between milk yield and the genetic susceptibility to BTB, indicating that there is little evidence that selection for increased milk yield has increased susceptibility to BTB or that selecting for resistance will reduce productivity.

However, there may also be unexpected difficulties in pursuing this strategy. The tuberculosis-causing family of organisms are some of the most successful pathogens on the planet and have proved adept at adapting to new hosts and ecological niches. Altering the host resistance to disease in national cattle populations could also result in future populations of M. bovis eventually evolving to become better at infecting resistant cattle. If, however, selection for disease resistance was based on several genetic variants, or indeed the entire variation of the genome, this will reduce the probability of the pathogen rapidly evolving to circumvent all resistance mechanisms affected by this variation. An additional problem may be that breeding for resistance to BTB may possibly make animals more susceptible to other pathogens. TB resistance in cattle may involve bolstering the adaptive immune Th-1 leucocyte response, potentially at the expense of the Th-2 leucocyte response. Reduction of Th-2 response could leave cattle more open to infection by parasites such as Fasciola hepatica (Flynn et al. 2009). Basing resistance on the innately immune phenotype at one extreme of Young et al.’s spectrum may however address these concerns by ensuring that the mechanism by which M. bovis infection is resisted occurs prior to the onset of adaptive immunity.

We now stand at a defining moment in the history of agriculture wherein we can use modern genomic tools to subtly influence the future evolution of the animals we have farmed for thousands of years. The contribution that host and pathogen genotypes make to disease outcome has, until recently, been overlooked by policy makers; yet, it is biologically untenable that genetic variation of both organisms does not play a role. Breeding for resistance to BTB in the national cattle herd could relatively quickly produce significant benefits, particularly through the use of AI semen from sires with genomic variants associated with resistance. Such schemes would also complement existing eradication schemes and provide a more sustainable strategy for reducing incidence of this endemic disease.

REFERENCES


