Bovine tuberculosis: molecular evolution of *Mycobacterium bovis* in the British Isles

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Introduction Bovine tuberculosis (TB) is a chronic disease of animals caused by infection with the slow-growing, obligate intracellular bacterium *Mycobacterium bovis*. It is the most complex and difficult multi-species endemic disease currently facing government, the veterinary profession and the farming industry in the UK (Reynolds 2006). Despite sustained (and costly) implementation of eradication programmes since the 1950s, bovine TB has not been eradicated from either the UK or Ireland. Indeed, there has been a sustained and largely unexplained increase over the last 20 years in parts of the UK (Gilbert *et al.,* 2005).

In order to optimise control measures it is important to investigate the neutral and selective forces which have shaped the current epidemic and to investigate the influence of genetic variation in the pathogen (Smith *et al.,* 2006). Different classes of bacterial genetic marker, which inform on different evolutionary scales, have been identified recently in sequenced *M. tuberculosis* complex genomes (Gagneux and Small 2007). Deletions and SNPs are well suited to reconstructing the evolutionary history (phylogeny) of these clonal pathogens. This study was undertaken to investigate the molecular evolution of *Mycobacterium bovis* in the UK and Ireland. Knowledge of the evolutionary history and population structure of *M..bovis* in the British Isles could lead to the rational development of population-specific diagnostic tests, genotyping tools and vaccines.

Materials and methods Structured sampling of the *M. bovis* populations in Great Britain (GB), Northern Ireland (NI) and the Republic of Ireland (ROI) was undertaken (476 isolates in total: GB 126, NI 240, ROI 110). SNPs were genotyped by DNA sequencing and SNaPshot assays. Spoligotype- and VNTR-defined genotypes (Skuce *et al.,* 2005) were fitted to lineages along a linear phylogeny separating sequenced *M. bovis* BCG (France) and extant *M. bovis* (GB). We compared the phylogenies from GB, NI and ROI.

Results The backbone linear phylogenies of *M. bovis* isolates from GB, NI and ROI were very similar. The majority of extant GB, NI and ROI *M. bovis* isolates fitted close to the sequenced contemporary GB isolate, although some more ancient genotypes were evident, particularly in the ROI sample. GB, NI and ROI populations are now dominated by *M. bovis* of a particular spoligotype-defined lineage (the SB0140 clonal complex). However, each region appears to have developed its own unique genotypes within the SB0140 clonal complex.

Conclusion The populations of *M. bovis* in GB, NI and ROI are of the same major lineage and descend from a common ancestor. The *M. bovis* populations in each area could have been identical in the past (homogenised) and subsequently barriers to movement were established that allowed each population to evolve independently. These *M. bovis* populations continue to evolve over time and may not be the same as when eradication was nearly achieved in past decades. Traditional control methods, which were highly effective, may no longer be sufficient. In conjunction with cattle movement recording, the identification of region-specific *M. bovis* genotypes also informs outbreak investigations between and within these regions.

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