



## **The need for enhanced molecular epidemiological data to unravel bovine tuberculosis transmission among and between badgers and cattle**

### **Summary**

- **Fine-scale genetic analyses reveal gaps in the current understanding of bTB epidemiology**
- **Whole genome sequencing can differentiate bTB strains involved in outbreaks on neighbouring farms**
- **Badgers in outbreak areas can have identical as well as very different genetic bTB strains to those among infected cattle**
- **Strain persistence among cattle sampled years apart raise the possibility of latent infections, environmental persistence, inter-herd transmission of bTB**
- **Coarse grained approaches to bTB reduction such as badger culling should await further fine-scaled analyses to elucidate the full complexity of bTB epidemiology**

Epidemiology of diseases is greatly assisted by molecular genetic analyses of pathogen strains. Such fine-scale analyses allow individual strains to be identified and tracked through outbreaks. Recently developed Whole Genome Sequencing for *Mycobacterium bovis* (bovine tuberculosis) has been proven a powerful tool as it can differentiate among strains causing outbreaks at the level of individual herds of cattle in neighbouring farms. Initial studies conducted in Ireland cast new light on the epidemiology of bTB including evidence indicating that sequential outbreaks on single farms involve both different and identical strains over periods of years, and that badgers sampled near herds can either have identical strains to infected cattle, or significantly different strains.



Such fine scale analyses suggest that our understanding of the epidemiology of bTB is not yet well known. More detailed work will be required (analysing strains circulating among badgers and cattle sampled synchronously during outbreaks) but available data already casts doubt on assumptions that badgers have comprehensive involvement in bTB outbreaks among cattle. Badger culling programs should not occur until the epidemiology of bTB is fully understood and risk factors such as latency, environmental persistence of the pathogen, and inter-herd transmission are fully analysed.

### **Molecular genetic studies**

Continued technological improvements and refinements in the field of molecular genetics have led to advances in techniques capable of pinpointing small changes in the genomes of pathogens. This allows for unequivocal analyses of epidemiologic processes, as single genetic strains can be traced among hosts involved in outbreaks.

Long in use for epidemiologic studies of HIV-AIDS, influenza, and human tuberculosis for example, such techniques have only recently been applied to studies of the epidemiology of bovine tuberculosis (bTB).

Indeed, as recently as 2011, a report by the Bacteriology Branch, Veterinary Sciences Division, Agri-food and Biosciences Institute (<http://www.dardni.gov.uk/afbi-literature-review-tb-review-badger-to-cattle-transmission.pdf>) concluded that “the extent to which infectious badgers contribute to TB in cattle remains un-quantified and indeed may be un-quantifiable.” With state-of-the-art techniques it is now indeed possible to quantify such contributions.

Molecular genetic techniques used to elucidate bovine tuberculosis epidemiology have until recently relied on two main techniques – spoligotyping and VNTR (variable number of tandem repeats). A combination of both techniques has proved to be superior to either test alone to investigate the transmission of disease between badgers and livestock (<http://www.hindawi.com/journals/vmi/2012/742478/>). However, these techniques lack the fine-scale capability of differentiating among the closely related strains of *Mycobacterium bovis* present in the UK and the Republic of Ireland that genetically differentiate though clonal expansion. Spoligotyping and VNTR analyses have a “coarse” resolution capability on the order of hundreds of square miles.

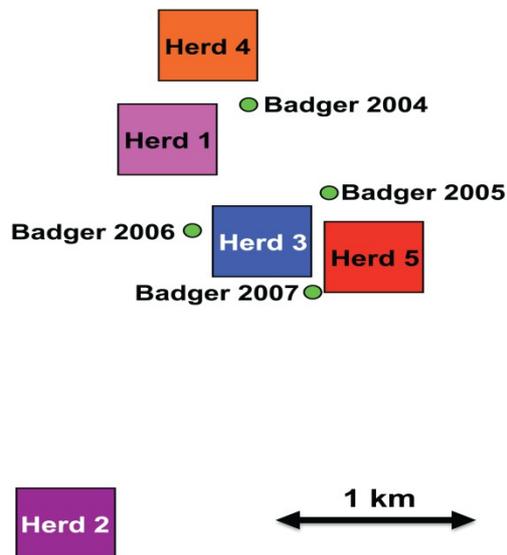
To adequately assess fine-scale transmission patterns that occur at the individual herd level and associated badger populations Whole Genome Sequencing (WGS) has recently shown considerable promise. With this technique it is now possible to provide much more detailed evidence of possible transmission chains linking the two hosts (cattle and badgers) at the



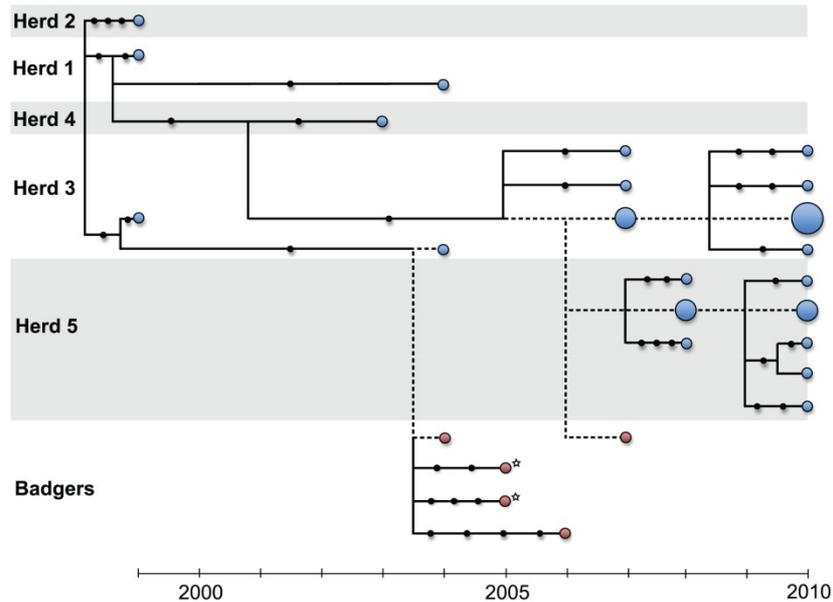
level of local farms and individual outbreaks (<http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003008>).

### Initial data from a small-scale study

Results of that study in Ireland (*Whole genome sequencing reveals local transmission patterns of Mycobacterium bovis in sympatric cattle and badger populations*. Biek, R. et al, 2012, PLOS Pathogens 8(11)) using 26 infected cattle (5 herds) and four infected badgers revealed some highly interesting patterns:



Geographic relationship among cattle herds and badger samples



**Maximum likelihood relationships among *M. bovis* samples from badgers and cattle – black dots represent single nucleotide substitutions, dashed lines indicate branches without mutations, size of the circle indicates the number of samples sharing the same genotype. Badger 2005 (starred) was co-infected with two different strains. Rate of mutation = 0.147 substitutions per genome per year.**

**Results show that –**

1. *M. bovis* isolates from cattle (e.g. Herd 3 and Herd 5 in 2010) revealed that outbreaks involved genetically distinct isolates demonstrating the ability of WGS to track *M. bovis* at the herd level;
2. Repeated outbreaks within the same herd mostly involved closely related isolates falling within the same genetic lineage. The authors suggest this could result from reintroduction of the same strains from neighbouring herds, environmental persistence, latent infections, or alternative hosts;
3. Of the four badger isolates two were genetically indistinguishable from those occurring in herds the same year while the other two exhibited unique strains not circulating among cattle and not involved in cattle outbreaks in subsequent years. WGS cannot determine the source of outbreaks when cattle and badgers are infected with identical strains;
4. WGS data shows that local strains are the primary culprit of outbreaks among specific herds;
5. WGS based on more extensive sampling during outbreaks can be used to directly determine the role of badgers in the maintenance of bTB in cattle;

6. WGS results raise the possibility of independent bTB cycles operating within cattle and badger populations – such sylvatic cycles are well documented for other pathogens (e.g. rabies).

#### **Downsides of WGS -**

1. Cost to be balanced against detail of the information.
2. Limited knowledge of rate of back mutations especially when transitional (purine->purine, pyrimidine->pyrimidine) versus transversional (purine <-> pyrimidine) changes are involved. The authors do not indicate whether mutations are transitions and/or transversions.
3. Limited number of laboratories capable of performing the analyses.
4. Absence of a forensic database to determine involvement of previous strains in local bTB outbreaks, but reconstruction is possible if original samples have been banked.

#### **Conclusions –**

Using the precautionary principle, considerable further fine-scale analyses of the involvement of badgers in local outbreaks need to be undertaken before controversial programmes of badger culling are undertaken. Initial WGS data from Ireland show that inter-species transmission is far from being proven a major cause of persistent outbreaks, suggesting that inter-herd transmission is a persistent factor despite existing bTB controls and tracking of individual cattle.