

Follow up comments on PCR testing from a question from Owen Paterson MP.

Can PCR be used to identify bTB infection in badgers from the sampling of faeces and latrines?

What is PCR?

PCR test (Polymerase Chain Reaction) is a highly sensitive test that can detect small amounts of DNA or RNA (genetic material) in a sample of blood, tissue or body fluids/excretions. PCR is a common molecular technique which uses enzymes to separate and copy the existing DNA/RNA in a sample, multiplying the genetic material up so that any bacteria can be easily detected by comparison with samples of known bacteria.

As with any diagnostic test, the key factors are sensitivity and specificity:

Sensitivity: How often can PCR detect DNA specific to the group of tuberculosis bacteria in a sample which is known to be positive?

PCR is a very sensitive technique and will probably almost always pick up tuberculosis bacteria or even parts of bacteria in a sample if they are present

Specificity: Has the test picked up the presence of DNA from *Mycobacterium bovis* (*M. Bovis*) specifically i.e. the tuberculosis bacteria that causes bovine tuberculosis? At present the answer is we don't know. Current PCRs detect the presence of a number of possible tuberculosis bacteria (see below), not just *M. bovis*.

Detection of *M. bovis* by PCR is a developing science and has great promise. However at present there are issues which limit its usefulness. Therefore further research is required before it can be considered as a viable option to detect the presence of bovine Tuberculosis in Badgers via the sampling of faeces and latrines.

These issues include:-

False positive results - The current PCR tests pick up the presence of other bacteria in the mycobacterium tuberculosis complex (which includes *M. bovis*, *M. tuberculosis*, *M. africanum*, and *M. microti*, *M. canetti* and *M. bovis* BCG), not just *M. Bovis*. This is due to the fact that bacteria within this group have some common genes (mpb64 and mbp70), and these are currently used in the test to differentiate between mycobacterium tuberculosis complex bacteria and other bacteria. It is also suspected that other bacteria found in the soil may have the same or similar genes, for example it is known that isolates of *Mycobacterium kansasii* have a homologue of the gene mpb70 (ref 1).

False negative results

Studies (ref2) have shown that excretion of *M. bovis* in faeces from infected badgers is intermittent and often occurs at low levels, which means that the bacteria are not uniformly distributed within the stool. This could mean that, due to the small size of the

sample taken from faeces or a latrine, you could miss the infection which may be present.

The danger here is that you would not be detecting all of the infected badgers present, unless sampling was very extensive.

You cannot differentiate *M bovis* or specific strains of *M bovis* using PCR

It is not currently possible to reliably differentiate *M bovis* from other mycobacterium tuberculosis complex bacteria using PCR.

In addition to this, even if you could figure out how to detect *M bovis* specifically, we would not be able to differentiate between strains of *M bovis*. Techniques based on culture of *M bovis* such as spoligotyping as opposed to gene technology as with PCR can identify and differentiate between strains in the laboratory. PCR would therefore not be able to trace the origin spread or interaction of a strain within the cattle or wildlife population.

The need for more research

Testing methods to resolve these issues need to be developed before PCR can be considered in the field and there are a number of research projects running on the use of PCR for the detection of bTB. There are also cost and practical issues which may limit the usefulness of this test in the field. Recent studies completed at Warwick University (ref 3) have shown an improvement in the possible use of PCR in the field, and a possible reduction of the costs involved, however, the studies do not address the fundamental issues above, therefore further studies are required before PCR can be considered as a viable option for the effective diagnosis of bTB in badgers.

References

1. FEMS Microbiol Letts (1997) 148:43-48
2. PCR as a Diagnostic Tool, February 2005, TB Forum Paper TBF 118
3. Is Mycobacterium bovis in the environment important for the persistence of bovine tuberculosis?
Biology Letters O. Courtenay et al, March 2006