

BOVINE TB AND THE USE OF PCR: SUMMARY OF 12 JULY MEETING

Expert Panel: Professor Bob Watson (Defra CSA – Chair); Professor Cecil McMurray (Chair Diagnostics Programme Advisory Group (DPAG) & ex-Chief Scientist DARDNI – vice-Chair); & Professor Mike Barer (University of Leicester).

Attendees: Staff were present from Defra, the Veterinary Laboratories Agency (VLA), The Food and Environment Research Agency (Fera), the University of Warwick, the University of Surrey, and Enigma Diagnostics Limited

SUMMARY

1. The group agreed that:
 - a. for PCR it is necessary to consider both analytical sensitivity and sampling techniques alongside methods of sample preparation;
 - b. there is potential in the faecal and air approaches but these are not yet at a stage where they can be recommended to Ministers for use; and
 - c. further work could be done on faecal sampling and also air sampling (see paragraphs 23 – 24).
2. Overall, the expert group concluded that PCR was not a test that could be usefully used for detecting TB in badgers based on the current state of knowledge, particularly in the field. If the further research at 1c) was pursued, PCR might have some use for testing badgers for TB in the future. However, the group emphasised that there are no guarantees and that the current uncertainties may not be resolved and a practical (or low cost) test regime developed.
3. If the uncertainties surrounding sample extraction and handling could be resolved, and an integrated assay procedure validated, an automated portable test might be feasible in the longer-term (i.e. over several years). The timescale for this would depend on the outcome of an appropriate experimental programme and the resources applied.

BACKGROUND

4. Professor Watson gave an overview of the background and purpose of the meeting. One of key questions for the Coalition Government is how can the incidence of bovine TB in cattle be reduced and part of this is consideration of a science-led badger cull as part of a control package. One of the techniques regularly raised with the Minister of State (Jim Paice MP) by stakeholders is use of a Polymerase Chain Reaction (PCR) test to identify setts containing infected badgers and potentially allow selective culling of infected badgers.
5. The focus of the experts' meeting was whether a PCR test could be used for badgers now (particularly in the field) and its potential use in the future. Within these broad questions there are queries about the stage research has reached for detecting TB in badgers using the PCR test; the sensitivity of the PCR test; alternative tests; sampling strategies; and the conditions under which a PCR test could be used either as a laboratory-based, or field-based system.
6. Defra officials gave an overview of the background to PCR as a test for detection of *M. bovis* in badgers. Defra had worked with Dr Mike Taylor and VLA since 1999 on a PCR test for clinical samples, including faeces. This was brought to the fore when Warwick University published a paper showing surprising results (including from the Republic of Ireland) which showed a lot of positives and unintentionally raised expectations that a test and cull strategy may be feasible. Further studies were done: the specificity and sample extraction methods were refined and the first ring trial was carried out with Warwick, University College London, and VLA, however, the results could not be reproduced during initial validation. In the second ring trial involving the Madrid EU bovine TB Reference Lab, VLA, and Warwick the test was replicable after the test protocol was modified and standardised.
7. Enigma was involved in discussions from 2006 and again more recently to give the practical perspective, as it has developed a portable PCR system for use with available assays (not TB) in a military context. This has suggested that PCR might be more promising as there is potentially equipment available 'off the shelf' that could be used in the field. While this is true, Enigma would require a fully validated assay protocol which could then be taken onto a field based platform.

USE OF PCR-BASED TESTS TO DETECT *M TUBERCULOSIS* IN HUMANS

8. Professor Mike Barer (University of Leicester) highlighted key points about the use of PCR in TB in humans:
 - a) PCR is not routinely used as a primary method for diagnosing TB in humans.
 - b) Most diagnosis for lung TB in humans in the UK is achieved through a combination of clinical history and a chest X-Ray. Direct detection of the TB bacillus is used for confirmation, epidemiology and drug susceptibility.

- c) PCR and related DNA detection methods are extremely effective for virus detection but have so far received limited routine application in the diagnosis of bacterial infections. Chlamydia is a notable exception, and some other bacteria are regularly assayed by PCR in several institutions (e.g. MRSA, *Clostridium difficile* and *Neisseria meningitidis*), but the biology of these organisms is very different from the TB bacillus.
- d) Conventional TB lab confirmation is difficult given the time it takes the TB bacillus to grow. For this reason PCR tests have been seen as highly desirable and considerable effort has been invested in their development. Despite this, the situation remains as stated in a).
9. PCR gives at least 98% diagnostic sensitivity on sputum samples **where the bacillus can be seen by microscopy**. These samples are from heavily infected patients. PCR is not used to diagnose people who are infected but have not yet progressed to a severe infection. These represent the majority of infected people and probably badgers. It could be possible to replace microscopy with PCR. However, in comparison: microscopy can take as little as 15 minutes; the minimum PCR takes is 45 minutes with the most advanced systems. Moreover, the negative predictive value of PCR is not good, PCR costs more than microscopy, and it is not the current industry standard to detect human TB. However, it was noted that PCR could provide further useful information when run in parallel with microscopy, and PCR systems could be designed which required a lower operator skill level than microscopy.
10. PCR is useful to confirm TB rather than detect it, and to detect key drug resistances, so it can be useful particularly in instances where there is high drug resistance. It is not used as a primary test for TB in humans. Immunological tests for TB infection are more sensitive than PCR for the detection of infected individuals.
11. In recent studies in Peru the PCR testing of human faecal samples gave 86% sensitivity overall in adults with active pulmonary TB (clinical disease) whereas in children, who excrete lower amounts, the sensitivity was 38%. Other sample frameworks (spinal, tissue etc) do not have sensitivities above 80% for clinically diagnosed TB. A recent report suggested above 80% sensitivity is possible but this has not yet been replicated.
12. The group acknowledged that veterinary medicine used a herd based disease control approach based on infection whereas human medicine was focused at an individual and their infectious level.

POTENTIAL USES OF PCR-BASED TESTS TO DETECT *M. BOVIS* IN BADGERS

Sensitivity

13. Most PCR tests validated under laboratory conditions would have an analytical sensitivity of 10^4 genome copies/ml of aqueous sample for the whole process (sample extraction, purification and amplification), because of the inherent sample error and statistical validation at low target copy numbers. Under field conditions, sensitivity may be 2 or 3 orders of magnitude lower because of the sample properties. The group agreed that based on what is currently known (because of variable extraction efficiencies, inhibitors etc), the analytical sensitivity of a PCR test for *M. bovis* from badger faeces was unlikely to be improved beyond 10^3 organisms per gram of faeces.
14. The group discussed how PCR is presented relative to other tests such as STAT PAK. It agreed that care is needed in making relative comparisons between the sett-level sensitivity of PCR from one sampling event and the sensitivity of other tests where animals have been tested several times in one year. One problem of sampling for TB is that there is intermittent excretion of very few organisms. This could possibly be overcome by changing the sampling strategy. The group agreed that for PCR it is necessary to consider both analytical sensitivity and sampling techniques alongside methods of sample preparation. The group considered that more effort was required to devise a rigorous and optimised protocol suitable for field use.
15. It was recognised that the primary purpose of the Warwick University study was not to look at sampling strategies or using the PCR test for detecting infected or infectious badgers but rather to determine the analytical sensitivity and reproducibility of the use of PCR for detecting *M. bovis* in faecal samples
16. **Cost:** There was a brief discussion of the potential cost of PCR which suggested £500 per sett (not including sampling time and using 10 replicates per sett, reagent costs at £10/assay) but the current test is not yet at a stage where it could be considered for wider field use.

Sampling

17. The group took the view that an improved sampling strategy and sample preparation could possibly improve overall sensitivity.

Faeces

18. There are several factors that affect faecal sampling, e.g. the use of latrines by badgers is variable depending on time of year and how active they are. Furthermore, *M. bovis* is not distributed homogeneously within the faecal droppings. There are a range of issues that are not yet resolved when sampling faeces.

Air

19. The Health Protection Agency (HPA) has been using PCR on air samples for a few diseases (especially viral). HPA tried using PCR for TB in air and did not get very far. The TB Programme may wish to contact HPA to explore further.
20. Air sampling could be difficult based on experience to date. VLA have tried unsuccessfully to isolate and culture *M. bovis* from infected cattle breath samples. Badger setts can be large and complex and for such large volume sampling there is a question of how localised infected aerosols from badgers would need to be, although there could be some infected dust particles within the sett. From routine air quality sampling in towns etc the evidence shows that some bacteria eg *Helicobacter pylori*, can be detected in the air but this does not necessarily map to infection.
21. Enigma gave an overview of military applications of PCR where it provides a holistic measurement of the environment. Air sampling was pioneered by the HPA at Porton Down (then PHLS) many years ago and is useful for detection of various antigens at ports of entry, etc. There are no data to provide evidence for use of air-sample based PCR tests for badger setts but this should not be dismissed without study.
22. From a practical perspective air samplers come in all shapes and sizes eg 2ft by 2ft powered off a car battery; and there are also air quality detectors across the country. The group raised a concern about a potential (perceived) health and safety risk from drawing out air from a badger sett anticipating it will contain aerosolised *M. bovis* (a Class 3 hazardous microorganism). It was clarified that samplers draw air into a liquid, and it therefore does not present a significantly different risk compared to other sampling as the resulting air is purified.
23. Professor Watson asked what work would have to be done to see if testing air using PCR was viable. A proof of principle experiment was suggested. VLA pointed out that there would be an opportunity in the autumn using clinically infected badgers (large doses of *M. bovis*). The experiment would take around six months with results available in the second quarter of 2011.

24. The experts agreed that testing air samples is conceptually intriguing and there is not much work to date with, therefore, little data. The group agreed this approach was worth evaluating particularly with the opportunity of carrying out trials at VLA under controlled conditions. The experts also emphasised that there were no guarantees and it was important to describe what the critical path of research needed to develop an air based PCR test would be; for example: proof of principle using spiked air samples and air samples from experimentally infected animals in the lab, followed by proof of principle on samples taken from putative positive setts on the field, followed by steps required to achieve OIE validation which includes a ring trial to demonstrate reproducibility and a specificity trial. If any phase failed then Defra would need to re-consider whether it was worth repeating or abandon development completely. As part of the process, there would be an advantage in the TB Programme consulting an expert group on air sampling.

Other diagnostic tests: Culture, Interferon gamma, STAT-PAK

25. The sensitivity of TB tests varies depending on the stage of infection.

26. **Culture:** To collect clinical samples of a good enough quality (sputum, urine etc) badgers need to be anaesthetised and culture takes several weeks. Sensitivity of detection of *M. bovis* in urine is lower than in faeces (data from Woodchester Park show 1.25% of all clinical samples tested are positive on culture of urine for *M. bovis* (97, out of 7,700 catches although most of these animals are not infected with TB). Tracheal aspirates are the best sample for culture to identify infectious badgers as TB is primarily a respiratory disease, and culture based tests only detect infectious animals. The group concluded that using saliva and urine samples held no potential advantage over faeces as these samples are difficult to obtain and are unlikely to contain *M. bovis* in a high proportion of infected animals.

27. **STAT-PAK** has limited sensitivity (50%) and its effectiveness depends on the stage of infection. The STAT-PAK test could be applied as a trap-side test now but it is an antibody-based blood test which means it detects animals mainly in the later stages of disease. The estimated cost is £1,000 including trapping and using the STAT-PAK per social group (i.e. approx 5 badgers).

28. **Gamma interferon** (blood test) is the most sensitive live animal test (at approx. 85%) and identifies animals in an earlier state of infection compared to other tests. It can also detect “latent” infection, i.e. animals that are infected rather than only infectious ones. It would be more difficult to apply on a very large scale as it is a lab based test using live cells (and therefore blood sample viability is time limited) and is more expensive than STAT-PAK (a commercial bovine IFNg test is approx £18 each), plus trapping costs as with STAT-PAK.

What are the advantages of field based PCR test compared to laboratory tests?

29. From Enigma's experience a field-based platform would include automated extraction, standardisation, and speed (to run six PCR tests in parallel takes 40 minutes, no transport time). A key benefit is that the manufactured test is completely automated. The variability created by the human element is removed and a lower skills base is required compared to laboratory-based tests. The results are, therefore, replicable. If commercialised, the test would have to be (re-)validated.
30. The group agreed that if the approach was successfully validated for TB (i.e. using faecal analysis) there could be an advantage in having a cage-side test.
31. 0%-100% of badgers in a sett could be infected, and if a sett-based approach is taken there was little to be gained through identifying individuals since the whole sett or social group would have to be culled because of the disease risk if one animal was found to be infected.

CONCLUSIONS

32. The group agreed that more research is needed to improve infected sett identification using PCR. However, it cannot be guaranteed that such research will result in a practical test or one that is low cost.
33. The group concluded that there is potential in the faecal and air approaches but these are not yet at a stage where they can be recommended to Ministers for use. This will depend on an adequate programme of further work which will also have to be costed. There are two approaches worth looking at:
 - a. **faeces**: Much work has already been done and the group agreed that work on the reproducibility and sensitivity of the technique had been taken as far as it could at this time but should be kept under review. Now work needed to be started on the sampling techniques and on sample handling.
 - b. **air**: the opportunity for preliminary work on experimentally infected badgers at VLA should be followed through.
34. Testing of tracheal aspirates, sputum, saliva, or urine samples is not worth pursuing and could not be justified as these samples are difficult to obtain and are unlikely to contain *M. bovis* in a high proportion of infected animals.