

Control of Bovine Tuberculosis – Testing, Trading & Vaccination – Part 2

INTRODUCTION - TESTING

Tests for diseases like bovine Tuberculosis are biological in nature and unfortunately no biological test is 100% accurate.

Some biological tests are better than others though and the standard test for detecting bovine TB the tuberculin skin test is actually one of the better ones.

Studies have shown that the test is on average 80% sensitive at standard interpretation rising to 93.5% at severe interpretation with specificity in excess of 99.9%. In general terms for all diagnostic tests there is a trade-off between sensitivity and specificity.

i.) Sensitivity: is the ability of a test to correctly identify an infected animal i.e. not to classify an infected animal as uninfected (a “false negative”).

ii.) Specificity: is the ability of a test to correctly identify an animal that is free from infection i.e. not to classify uninfected animals as infected (a “false positive”).

TESTS FOR TUBERCULOSIS

Many disease tests depend on detecting antibodies to the disease organism. These are proteins that circulate in the blood and play a part in destroying the invading organisms.

Tuberculosis is different, however. There are almost no antibodies circulating in the blood of an infected animal until the very last stages of the disease.

The object of disease testing is to detect disease early to decrease its spread, and therefore another method of identifying infected animals is needed.

SKIN TESTING

TB stimulates a reaction by the white blood cells. If protein from TB organisms, called tuberculin, is injected into the skin, it causes an allergic type of white blood cell reaction in the skin and a swelling develops.

This is the basis of the tuberculin test, the normal test for bovine TB which is used all over the world

and which has been responsible for the eradication of TB in many countries.

Countries where there is a wildlife reservoir capable of spreading infection have experienced problems with eradication (e.g. UK, Ireland, USA, and New Zealand).



Fig 1: Vet measuring skin thickness, and Fig 5: Vet injecting bovine tuberculin.

The tuberculin test carried out in the UK involves two injections of tuberculin which are made into the skin of the animal's neck after the skin thickness has been measured with callipers.

One is tuberculin from the bovine TB bacterium (*Mycobacterium bovis*), and, if the animal has been infected with bovine TB, a reaction will develop at the injection site. Unfortunately if the animal has been in contact with bird (also called avian) TB (*Mycobacterium avium*), which is quite common in the UK, or a few other closely related bacteria then a reaction will also develop. This is why a second injection is given. This is also tuberculin, but is made from the avian TB bacterium.



Fig 2: Avian and Bovine Tuberculin.

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The injection sites are examined three days after the injections and the thickness of the skin is measured to see if there have been any reactions. If there are, then the increases in the thickness of the skin at the two sites are compared, and it is this difference that determines the result of the test.

Under normal circumstances, at standard interpretation, if the increase at the avian site is the same or larger than the increase at the bovine site, then the animal has passed the test as the reactions are probably due to infection with avian TB or one of its cousins. If the bovine increase is larger then the animal is either a reactor or an inconclusive reactor depending on the size of the difference in reaction at the 2 sites.

If infection has been confirmed in the herd then, although the test is carried out in exactly the same way, the interpretation that is used is known as severe interpretation.

This approach increases the sensitivity such that virtually any bovine reaction is classed as positive. But while the sensitivity is increased to detect all possible infected animals the specificity is decreased. Being a biological test, occasionally infected animals are missed. The most likely reason for this is that animals have early infection – it can take several weeks before the test will identify these as reactors. In an environment where TB is known to be present, it is likely that some of the inconclusive reactors come into this category, so they are slaughtered.



Fig 3: An example of a TB reactor.

TB lesions are not always found in reactors to the tests when post mortem examinations are carried out. Samples from these animals go for culture, to

see if TB organisms can be identified by growing them, and most (but not all) NVL (No Visible Lesions) reactors are culture negative.

These NVL reactors are a concern to those who have lost their animals as there is always a feeling that the cattle may not have been infected. Skin testing has been carried out for many years now, and it is evident that herds that have only had NVL reactors are found in the TB problem areas in amongst reactor herds where TB has been confirmed. It seems highly likely, therefore, that many of these reactors are, in fact, infected and it has just not been possible to see the lesions, either because they were too small or were in a location that was not examined.

After all, it must be remembered that when the animal is first infected the lesion is literally microscopic and takes some time to grow to a size where it can be seen.

(Note: research into NVL's has found that fine slicing of the lungs, using a bacon slicer, has shown small early TB abscesses in virtually all cases).



Fig 4 : TB in a bovine lung.

There will be a few cases unfortunately, where the reactors are true 'false positives'. In these cases, the animal may have been infected with closely related bacteria that caused a reaction but was not TB. However, the specificity of the comparative tuberculin test is very high, meaning that there are very few false positive cases.

BLOOD TESTING

A second test for bovine tuberculosis was introduced in the UK in 2002. This is the gamma

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interferon test. Blood from cattle that have come into contact with bovine tuberculosis contains a number of different substances - one of the substances is a protein called gamma interferon. If the amount of interferon is over a certain level, the animal is judged to be a reactor.

The gamma interferon test is thought to identify infected animals a little earlier than the skin test, but may also identify more “false positives. Scientific research has shown that the average specificity (accurate identification of uninfected animals) of the gamma interferon test is 97% - which is only slightly lower than the 99% plus for the skin test. Performance evaluation carried out in a number of countries shows that at the laboratory cut-offs used in GB the gamma interferon test has sensitivity comparable to or marginally better than the skin test – between 73 and 100%, with a median value of about 87%.

It is used as an ancillary test to the skin test in an attempt to speed up the clearance of infection from herds with particularly severe TB problems and also in new confirmed incidents in 3 or 4 year testing areas where infection has been confirmed.

TESTING INTERVALS

The frequency of routine tuberculin testing depends on the level of bovine tuberculosis in the area. It ranges from annual skin testing in the worst areas to 4 yearly testing in the areas which are considered clear of TB. In the past this has been determined on a parish-by-parish basis but for 2010, a large area incorporating the South West of England, much of the West Midlands and parts of the East Midlands were all put onto annual testing to better reflect the risk of disease in this part of the country, with areas immediately buffering put onto bi-annual testing.

Annual and two-year testing areas are considered high risk, and animals from these have to undergo pre-movement testing within 60 days of the movement and the owner has to pay for this.

TESTS (FOLLOWING REACTORS)

The testing that is needed after a reactor is found on a farm depends on the individual circumstances but in the simplest case movement restrictions are imposed and a further tuberculin test is carried out after 60 days. If the reactor and the most common number to have is 1 (50% of

breakdowns have only one reactor) is an NVL and samples are negative for TB then it may be possible to remove restrictions if this test is clear.

If a slaughterhouse case is found then an immediate check test of all the cattle on the farm is carried out. If infection is confirmed at a post mortem examination or by the laboratory growing bovine TB organisms however, two 60 day tests are needed and these are at severe interpretation. In 3 and 4 yearly testing parishes, a gamma interferon blood test might also be carried out.

60 day testing continues until no further reactors are found, but restrictions cannot be taken off until any inconclusive reactors (IR's) have been re-tested and are clear. This can all take a long time. Twenty years ago many herds were released after one (if NVL) or two tests, but more herds are now taking longer to clear.

This may be due to increased levels of re-infection from wildlife, or continued presence of disease in the cattle herd. Repeated skin testing (i.e. short interval testing) helps prevent this.

Before the farm returns to its normal testing routine, additional tests are needed 6 months and 12 months after the restrictions are released.

There may well be additional testing carried out because infection has been found. Neighbouring herds may need testing as they are “contiguous”, to the infected herd and infection could have spread to them.

If the reactors were purchased from elsewhere, then cattle on the farm of origin will also need to be tested. Similarly, any cattle moved on from the herd will be traced and tested by Animal Health i.e. in case they have taken infection with them.

APPROVED FINISHING UNITS (AFU's)

Extended TB restrictions can cause considerable pressure on housing facilities and resources as well as health and welfare problems. In order to help overcome these, special units can be set up to take cattle that have tested clear on the reactor farm. The cattle have to be licensed to move from the TB farm to the unit by Animal Health. The units are set up privately and have to comply with conditions before they can be approved. All the cattle held in AFUs go for slaughter (see part 5).

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EXEMPT FINISHING UNITS (EFU's)

These can be set up to take cattle from areas where pre-movement testing is needed (i.e. annual and 2-yearly testing areas). Cattle can be moved from the farm to the unit without a pre-movement test. Cattle can also be moved in from areas exempt from pre-movement testing. Like the AFUs these are privately set up and all the cattle have to go for slaughter.

APPROVED QUARANTINE UNITS (AQU's)

Approved Quarantine Units have been introduced to provide a route for calves from restricted farms lacking sufficient facilities to rear their calves. Once approved, they enable calves up to the age of 10 months to be moved into the quarantine unit with the view to them gaining official TB free Status in the future.

When all the calves/cattle on the unit have received two consecutive tests with negative results restrictions can be removed.

EXEMPT MARKETS

These are approved by Animal Health for taking cattle from farms in annual and 2-yearly testing areas without pre-movement testing. All the cattle have to go for slaughter, to an AFU or EFU or back home (except for cattle from 2 and 3 yearly testing areas which cannot go home). Details and lists of these units and markets can be found on the Animal Health website - [here](http://www.defra.gov.uk/animalhealth/managing-disease/bTb/movement/index.htm).

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TB, BADGERS, EVIDENCE

When it comes to bovine tuberculosis, evidence suggests that badgers in certain parts of the country form a reservoir of disease.

THORNBURY TRIAL

Some evidence supporting this is the Thornbury trial which took place from 1975 – 1981. In the Thornbury trial, all badgers were proactively eliminated from the area, and TB reactors in cattle disappeared in the area for 10 years (see figure 9 below). It was only when badgers

recolonised the area that TB reactors reappeared in cattle.

STEEPLE LEAZE

At another site at Steeple Leaze, in Dorset, TB cases ceased for 7 years after the elimination of badgers. The area then changed to arable farming.

These two studies showed that in these areas TB badgers were the sole source of TB outbreaks in the cattle. But the badgers were culled by gassing the setts so no infected badgers were dispersed. This method contrasts with the later use of trapping after badgers emerge from the setts which will always result in an incomplete cull and some dispersal.

Outbreaks of TB in an area are most commonly associated with a single spoligotype of TB, and an identical spoligotype found in badgers in the same area, strongly suggesting a joint reservoir of infection.

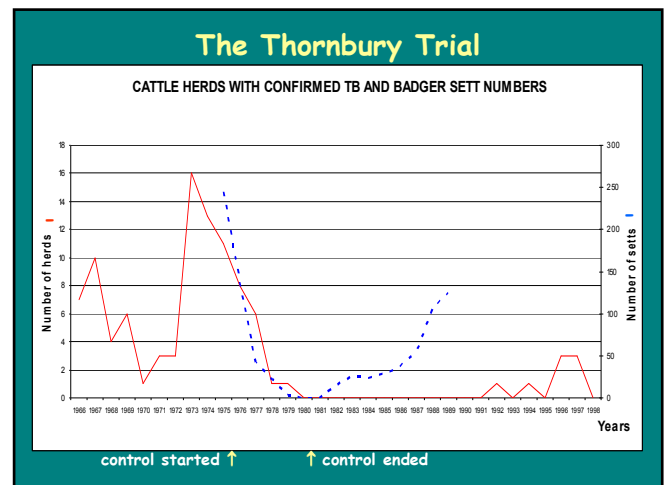


Fig 5: Graph depicting the correlation result of the Thornbury Trial.

THE RANDOMISED BADGER CULLING TRIAL (RBCT)

The ISG's RBCT trial started back in 1998 but the reactive cull was discontinued in 2003 prior to its projected end date (05/06). The reason for the suspension of the reactive culling was a proposed 'perturbation effect', where it was shown that although TB was reduced by 23% in the proactive cull areas, TB increased in the surrounding areas by 24%.

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Some of the ISGs conclusions were considered to be ‘temporally implausible’, because the ISG were comparing badger culling in one year with the incidence of TB in cattle in the same year, whereas any change in cattle incidence would not have been expected until a few years later.

This effect was confirmed by Imperial College London 2008, who reported that in the second year after the cessation of the RBCT new bovine TB breakdowns in the proactive RBCT cull areas fell by 60.8%. The paper points out that over the final three years of the RBCT, once culling methods had been improved, and in the two years following the cessation of culling, new bTB breakdowns in the proactive areas began to reduce significantly year on year. In addition there was no longer any evidence of a ‘perturbation effect’ (i.e. the incidence of TB reactors did not rise in adjacent areas, and in fact it fell).

The latest scientific research published in 2010 by Imperial College London as part of the ongoing review of the RBCT data and following a negative cost benefit analysis of culling suggests that a properly managed badger cull has a positive effect and reduces the incidence of confirmed breakdowns in proactive culling areas by 37% and within 2km of the culled area by 3.6%.

Importantly the beneficial effects observed within the trial areas in the first year post trial have reappeared in the last 6 month period analysed (37 – 42 months post trial).



Fig 6: A caged trapped badger.

BADGERS AND TB

Following the Badgers Act 1973, The Mammal Society National Survey showed that badger

numbers increased by 70% in the 10 years from 1980 to 1990 and it was forecast that this rate of increase in numbers would continue.

Badgers have a different immune system to cattle, and as a result there are more opportunities for excretion, not only from contaminated sputum and faeces but also from urine and discharging bite wound abscesses (Gallagher 2008). In the badger, counts equivalent to 5×10^6 bacteria have been recorded in the urine of an advanced TB case (Gallagher and Clifton-Hadley, 2000). This together with contaminated sputum where badgers have been foraging for worms represents a potentially significant contamination of pasture and these discharges present a serious source of infection for other animals using that pasture. It is important to note however that *M. bovis* is readily killed by UV light (i.e. sunlight) so will not last long on pasture when subjected to direct sunlight. However, the bacteria is thought to be viable for anywhere between 3 – 70 days depending on the time of year and weather conditions in more shaded areas. The actual route of spread from badgers is still being investigated.

CATTLE AND TB

In the 1920's and 30's clinical (or severe) cases of TB were common with cattle showing signs of emaciation due to lung disease. Cattle were housed in close confinement in poorly ventilated cow sheds and calves developed TB by drinking their dam's milk. Due to the current intensity of TB testing 'open' or severe cases of TB in cattle are quite rare. TB cases at post-mortem at an abattoir are identified by lesions in the chest cavity or by large caseous lesions in the lung tissue itself. These lesions may well have excreted bacteria.



Fig 7: TB lung lesion.

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CATTLE TO CATTLE TRANSMISSION

There is evidence that bTB is spread from cattle to cattle, although evidence suggests this spread is limited. Using naturally infected reactor cattle Costello et al, (1998) showed that when 10 sentinel animals were housed with two reactors for 12 months, four of the ten became infected proving that prolonged close confinement can therefore result in transmission. However, there is considerably more evidence that shorter term exposure is less likely to lead to transmission of bTB. O'Reilly and Costello (1998) found no transmission between 22 naturally infected reactor cattle and 32 non infected contacts kept together for 4 – 9 months in paddocks or open housing, despite many of the reactors having gross lymph node lesions.

In a separate project in the ISG trials, 200 reactor cattle were tested for infectiousness by culturing samples from nasal mucous, all samples were negative for *M. Bovis* (Report, 2007, appendix1, p234). Overall the evidence is that cattle to cattle transmission is limited, which may explain why in the South West of England, one of the worst bTB endemic areas in the UK, over 80% of cattle herd breakdowns have less than 3, usually 1, reactor. This has remained constant since the 1960's.

BADGER VACCINATION

In March 2009 DEFRA announced plans for a Badger Vaccine Deployment Project (BVDP), as an integral part of the overall bTB control strategy. The project was to assess the practicality of using a licensed injectable badger vaccine in six trial areas in TB 'hot spots' in England however as of June 2010 the project was scaled down to just one area near Stroud, Gloucestershire. Jim Paice, MP said at the time *"We're committed to carefully-managed and science-led badger control as part of a package of measures, and we're looking carefully at badger vaccination and culling as part of that. It makes sense to review the Badger Vaccine Deployment Project to keep our options open and to ensure best possible use of taxpayers' money. By going ahead with the training in Stroud, we'll maintain capacity to train lay vaccinators while we consider how best to deploy vaccines as part of a badger control policy."*

It is still hoped that the remaining area will help to assess all practical aspects in regards to vaccinating badgers and will provide knowledge

that will help in the move towards a long-term goal of an oral badger vaccine. An oral vaccine will not be available until at least 2014.

The Food and Environment Research Agency (FERA) are managing the project area in which those farmers, who are taking part, have had their land surveyed to identify badger setts. Trained and accredited contractors have been licensed to trap and vaccinate badgers in the area with BCG vaccine before releasing them. The project started in June/July 2010. The operation takes on average around 12 days per farm, 10 days of pre-baiting followed by 2 days trapping and vaccinating. This process is to be repeated annually on each farm for the next 5 years.

Vaccination will reduce the risk of badgers catching bTB and so reduce the level of disease in the badger population and overtime in the cattle population. It is recognised that to vaccinate already infected badgers will not cure them and will not stop the excretion of disease from these individuals, but as infected badgers die off vaccination will steadily build up group immunity. DEFRA will monitor the incidence of TB in the cattle population but the BVDP is not a scientific study and it may be a number of years before any discernable effect in cattle can be detected.

CATTLE VACCINATION

Vaccination of cattle against tuberculosis is currently illegal under EU Directive. The reason for this is that vaccinated cattle cannot be distinguished from infected cattle by the present skin test. In 2008 the UK Government announced additional funding for vaccination projects and this included cattle vaccination. Research work to develop a vaccine is at an advanced stage, together with a tuberculosis test which will differentiate between vaccinated and infected cattle. This is a complex and difficult subject and we cannot expect a vaccine to be available until 2015 at the earliest.

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