Animal &
Plant Health
Agency

Asiantaeth
Iechyd Anifeiliaid
a Phlanhigion

Animal & Plant Health Agency (APHA) report on the delivery of badger trap and test operations on chronic TB breakdown farms in Wales in 2019

Report for project TBOG0235

(Year 3)

Animal and Plant Health Agency (APHA) report on the delivery of badger trap and test operations on chronic TB breakdown farms in Wales in 2019.

Report for project TBOG0235 (Year 3)

1. Overview

In 2017, following public consultation, the Welsh Government (WG) published its Wales bovine tuberculosis (TB) Eradication Programme and its associated Wales TB Eradication Programme Delivery Plan (Welsh Government 2017). WG's aim is to develop processes to break the transmission cycle between wildlife and cattle on farms and the Delivery Plan states that: "As part of the ongoing Action Plan process, where the Welsh Government views that badgers are contributing to the persistence of disease in chronic herd breakdowns, badgers will be trapped and tested on the breakdown farm and test positive badgers will be humanely killed. Persistent herd breakdowns will be focussed on initially". The Delivery Plan also states that "WG will continue to assess the most appropriate deployment of the badger BCG vaccine if and when it becomes available".

In 2017 (Year 1) the Animal and Plant Health Agency (APHA) was tasked by WG with developing a programme of work to implement these proposals through trapping, testing and removing test-positive badgers on persistent breakdown farms. For a report of year 1 see Animal & Plant Health Agency (2018).

In 2018 (Year 2) APHA continued the work on persistent breakdown farms. The programme involved trapping and testing badgers on farms. Test positive badgers were humanely euthanased, while test negative badgers were vaccinated and released. For a report of year 2 see Animal and Plant Health Agency (2019).

In 2019 (Year 3) APHA continued the work, using the same protocol as in 2018. This report summarises the work of year 3.

2. Preparatory phase

The farms were selected for intervention by WG in conjunction with APHA veterinary field staff. Subsequently APHA staff undertook badger sett-surveys at each farm between May and November 2019. An operational plan was then developed for each farm.

3. Licensing

WG has authority under Section 10(2) and (3) of The Protection of Badgers Act 1992 to issue licences to kill or take badgers or to interfere with their setts for the purpose of preventing the spread of disease. WG also has authority on behalf of the Natural Resources Body for Wales, to issue licences under section 16(3)(g) of the Wildlife and Countryside Act 1981 (as amended) to trap badgers. In 2019 APHA were granted licences to undertake trap and test operations on six farms. Licensing inspectors attended all farms during trapping and testing operations. During each intervention, data was collected that could be used to monitor badger abundance, capture efficiency and diagnostic test performance.

Field operations involved a collaborative approach between APHA Science Directorate and Service Delivery Directorate. APHA laboratories carried out a range of diagnostic tests for TB on blood samples, *post mortem* (PM) examinations on the euthanased animals and tissue culture.

Each intervention consisted of the following sequential activities:

- Badger activity survey.
- Deployment and pre-baiting of traps.
- Cage trapping and sampling trapped badgers.
    - Anaesthesia by intra-muscular injection.
    - Microchip insertion subcutaneously for identification purposes, if the animal is not already microchipped
    - Blood sampling:
        - sample for immediate Dual Path Platform (DPP) test on whole blood (referred to as the field DPP test).
        - samples for subsequent laboratory-based DPP test (on serum) and Interferon Gamma Release Assay (IGRA), to inform future operations.
    - Euthanasia by lethal injection of animals positive to the field DPP test.
    - Euthanasia by lethal injection of animals that had tested positive to the laboratory DPP test or IGRA test that were conducted following a previous intervention.
    - Release of animals that were negative to the field DPP and any previous laboratory based tests, following BCG Sofia vaccination. Animals were vaccinated only once. Any animal that had been vaccinated during a previous capture event was not re-vaccinated.
- PM examination of test positive animal carcasses, culture of tissue samples and subsequent spoligotyping and whole genome sequencing of any *Mycobacterium bovis* isolates obtained.
- Collation of data from laboratory tests to inform future interventions.

Standard Operating Procedures (SOPs) for all key activities were agreed with WG and shared with the WG licensing team. These were based on the approved SOPs used by APHA on other projects, but were adapted for WG requirements. For example where APHA SOPs referenced Home Office licenced staff to undertake regulated procedures, WG SOPs were altered to refer to MRCVS registered vets only to undertake all relevant procedures. All SOPs, risk assessments and documents relating to the Control of Substances Hazardous to Health (COSHH) were made accessible to relevant staff prior to operations and staff were required to read the relevant documentation for their roles.

4. Delivery of field and laboratory operations

4.1 Timing

Work was completed on six selected farms between May and November 2019.

4.2 Field survey and cage trapping

Each farm was surveyed for badger activity by appropriately skilled APHA field staff. Cage traps were also positioned by APHA staff at locations where there was most badger activity and were pre-baited with peanuts before the trapping phase. Trapping lasted between 2 and 4 days per farm. Captured badgers were anaesthetised and blood samples taken for diagnostic testing (see below).

4.3 Badger sampling

Following capture, an assessment of the condition of every badger was undertaken by the individual checking the trap. This involved visual assessment of the demeanour, respiration, body condition, any injuries present and movement of the animal. Any departures from normality would result in immediate examination by the veterinarian.

Badgers were sampled trap side, unless there were adverse weather conditions in which case they were transported to a central sampling facility in holding cages. All procedures from anaesthesia through to monitoring until release were conducted by a veterinarian. Badgers were anaesthetised by intra-muscular injection with a mixture of ketamine, medetomidine and butorphanol. Balanced anaesthesia is usually induced within 5 to 10 minutes of injection and lasts for about 30 to 50 minutes.

During sampling, the location, sex, body weight and condition, temperature and reproductive status were recorded. Blood samples were taken via vacutainer from the anterior jugular vein and blood tests were performed as described below.

4.4 Blood tests

Two immunological blood tests were used for TB diagnosis in badgers (see Appendix 1 for detailed description):

The DPP test was undertaken on whole blood in the field to provide a rapid result so that animals could be identified for release (negative) or euthanasia (positive). The test was assessed qualitatively and was deemed positive if a line was observed at band 1 only.

The DPP test (on serum) was conducted subsequently in APHA laboratories so as to inform future field operations. The test was assessed qualitatively and was deemed positive if a line was observed at band 1 only.

The IGRA was also conducted subsequently in APHA laboratories so as to inform future field operations. Two IGRA responses were measured: B-A and C. E. Cocktail.

For further details of blood tests see Appendix 1.

4.5 Vaccination and release

Badgers that tested negative to the field DPP were vaccinated by intramuscular injection with 1 ml of reconstituted BCG Sofia vaccine. Animals were vaccinated only once. Any animal that had been vaccinated during a previous capture event was not re-vaccinated. This was facilitated by individually identifying each badger by scanning the microchip through the cage.

Each captured animal that was destined for release, was given a temporary identification mark by cutting a small area of hair on the rump and spraying it with coloured stock marker. All animals were given time to recover in a holding cage, before being released at the point of capture.

On future days of trapping within the same trapping phase, any recaptured animal was recognised by the temporary coloured stock mark and fur clip, and was individually identified by scanning the microchip through the cage. The animal was then released immediately following a welfare assessment without further sampling.

4.6 Euthanasia, *Post mortem* examination and tissue culture

Badgers that tested positive to the field DPP test were euthanased following standard operating procedures. Badgers that had been captured during a previous intervention and had tested positive to the laboratory DPP test, or the IGRA test were also euthanased without any further sampling. The animal was anaesthetised by intra-muscular injection and sodium pentobarbitone was subsequently administered by intravenous injection into the jugular vein, at a dose of 1 ml per 1.4 kg body weight.

All euthanased badgers were submitted for PM examination and histological investigation of tissues using a detailed PM examination protocol (Crawshaw *et al*., 2008). Tissue samples were cultured for *M. bovis* for 12 weeks. Any isolates were characterised by spoligotyping and whole genome sequencing (WGS). Such characterisation may be used to provide insights into transmission dynamics when combined with sequences from cattle on the targeted farms.

5. Results

5.1 Badgers trapped and sampled

A total of 117 individual badgers were sampled in 2019 (Table 1). On all farms two phases of trapping were conducted several weeks apart. Some badgers were therefore sampled more than once, resulting in 155 sampling events in 2019. (A 'sampling event' is the sampling of a badger, and occurred when a badger was caught for the first time within a trapping phase. A 'recapture' is a badger that was caught a second time within the same trapping phase, and was therefore released without sampling).

Table 1 Number of badgers caught at six Welsh farms in 2019

Farm	Trapping phase	No. of badgers sampled	No. of capture events of a non-target animal[3]	No. of recaptures[4]
1	1	35[1]	3	9
1	2	17	1*	2
3	1	17	3	5
3	2	5	0	3
4	1	29	0	5
4	2	16	0	4
5	1	5	1*	0
5	2	2[2]	0	0
6	1	7	0	1
6	2	7	1	2
7	1	11	0	6
7	2	4	0	0

[1]Two of these animals were sampled for the first time on Farm 1 in phase 1, however they had already been sampled on other farms in 2019.
[2]Two of these animals were sampled for the first time on Farm 5 in phase 2, however they had been sampled on other farms in 2019.
[3]Non target animals were released immediately following a welfare assessment. All non-target animals were foxes, except on two trapping events, indicated with a *, where the species was not recorded.
[4]Animals recaptured during the same trapping phase were released without further action following a welfare assessment.

During initial assessment of badgers in traps, none were found in need of veterinary examination. Of 155 sampling events, there were 19 instances when animals had minor injuries that were likely to have arisen while being in the trap. The injuries reported were abrasions or scratches to the: claws (6), knuckles (1), face, mouth or teeth (8), snout (1), forehead or neck (3) or hair loss to the shoulders or armpits (1). The only other injuries observed were bite wounds. Of the 155 sampling events, there were 25 instances when bite wounds on the animals were recorded. Of these, 20 were old or healed, four were open and fresh, while the condition of one was not recorded. All of the individuals were considered to be fit and healthy for sampling and for release where appropriate.

5.2 Summary of badger sampling and diagnostic test results

Results of blood tests are summarised in Table 2; complete results are provided in Appendix 2. During operations there were 155 sampling events, which resulted in the removal of 35 animals; 20 due to a positive field DPP result and 15 due to having returned positive laboratory results at a previous sampling event. One additional animal tested positive to the field DPP test, however this animal was released for welfare reasons because it was a lactating female. Of the 20 field DPP positive animals

that were removed, 16 subsequently tested positive to the laboratory DPP test, two tested positive to the IGRA (B-A) test and none were positive to the IGRA (C.E. Cocktail). Four of the 20 field DPP positive animals tested negative to all laboratory blood tests. The animal that was released after a positive field DPP test subsequently tested positive to the laboratory DPP test and negative to the IGRA B-A and C.E. Cocktail test.

Of the 155 sampling events, on 120 occasions the animal was released. (A total of 118 tested negative to the field DPP; one animal was a lactating female noted above; one animal was released without testing because a sufficient volume of blood could not be collected, although this animal was caught and sampled at a later trapping event). Of the 118 occasions when the animal tested negative to the field DPP, 12 animals subsequently tested positive to the laboratory DPP, while none tested positive to the IGRA (B-A) test or the IGRA (C. E. Cocktail). The remaining 106 sampling events that resulted in a negative field DPP test returned negative results for all laboratory blood tests.

Table 2 Summary of field and laboratory blood tests from badgers trapped at six Welsh farms in 2019 (page 1 of 3).

Farm			Vaccinated previous to this phase	Laboratory DPP		IGRA: B-A		IGRA: C. E. Cocktail	
				POS	NEG	POS	NEG	POS	NEG
Farm 1 Phase 1									
No. badgers	35	→	14						
No. positive to field DPP	4	→	2	4	0	0	4	0	4
No. negative to field DPP	29	→	10[1]	3	26	0	29	0	29
No. positive on previous laboratory test	2	→	2						
No. vaccinated	21								
No. released	29								
Farm 1 Phase 2									
No. badgers	17	→	14						
No. positive to field DPP	4	→	3	3	1	1	3	0	4
No. negative to field DPP	11	→	9	0	11	0	11	0	11
No. positive on previous laboratory test	2	→	2						
No. vaccinated	2								
No. released	11								
Farm 3 Phase 1									
No. badgers	17	→	7						
No. positive to field DPP	1	→	0	1	0	0	1	0	1
No. negative to field DPP	15	→	6	2	13	0	15	0	15
No. positive on previous laboratory test	1	→	1						
No. vaccinated	9								
No. released	15								
Farm 3 Phase 2									
No. badgers	5	→	5						
No. positive to field DPP	1	→	1	1	0	0	1	0	1
No. negative to field DPP	3	→	3	0	3	0	3	0	3
No. positive on previous laboratory test	1	→	1						
No. vaccinated	0								
No. released	3								
Farm 4 Phase 1									
No. badgers	29[2]	→	19						
No. positive to field DPP	2	→	2	1	1	0	2	0	2
No. negative to field DPP	21	→	11[3]	2	19	0	21	0	21
No. positive on previous laboratory test	5	→	5						
No. vaccinated	10[4]								
No. released	22								
Farm 4 Phase 2									
No. badgers	16	→	11						
No. positive to field DPP	4	→	3	4	0	1	3	0	4
No. negative to field DPP	10	→	6	2	8	0	10	0	10
No. positive on previous laboratory test	2	→	2						
No. vaccinated	4								
No. released	10								

Table 2 continued (page 2 of 3).

Farm			Vaccinated in a previous phase of trapping	Laboratory DPP		IGRA: B-A		IGRA: C. E. Cocktail	
				POS	NEG	POS	NEG	POS	NEG
Farm 5 Phase 1									
No. badgers	5	→	1						
No. positive to field DPP	1[5]	→	0	1	0	0	1	0	1
No. negative to field DPP	4	→	1	0	4	0	4	0	4
No. positive on previous laboratory test	0	→	0						
No. vaccinated	3								
No. released	5								
Farm 5 Phase 2									
No. badgers	2	→	2						
No. positive to field DPP	1	→	1	1	0	0	1	0	1
No. negative to field DPP	0	→	0	0	0	0	0	0	0
No. positive on previous laboratory test	1	→	1						
No. vaccinated	0								
No. released	0								
Farm 6 Phase 1									
No. badgers	7	→	3						
No. positive to field DPP	1	→	0	1	0	0	1	0	1
No. negative to field DPP	6	→	3	2	4	0	6	0	6
No. positive on previous laboratory test	0	→	0						
No. vaccinated	3								
No. released	6								
Farm 6 Phase 2									
No. badgers	7	→	3						
No. positive to field DPP	0	→	0	0	0	0	0	0	0
No. negative to field DPP	6	→	2	0	6	0	6	0	6
No. positive on previous laboratory test	1	→	1						
No. vaccinated	4								
No. released	6								

Table 2 continued (page 3 of 3)

Farm			Vaccinated in a previous phase of trapping	Laboratory DPP		IGRA: B-A		IGRA: C. E. Cocktail	
				POS	NEG	POS	NEG	POS	NEG
Farm 7 Phase 1									
No. badgers	11	→	0						
No. positive to field DPP	0	→	0	0	0	0	0	0	0
No. negative to field DPP	11	→	0	0	11	0	11	0	11
No. positive on previous laboratory test	0	→	0						
No. vaccinated	11								
No. released	11								
Farm 7 Phase 2									
No. badgers	4	→	4						
No. positive to field DPP	2	→	2	0	2	0	2	0	2
No. negative to field DPP	2	→	2	1	1	0	2	0	2
No. positive on previous laboratory test	0	→	0						
No. vaccinated	0								
No. released	2								

Arrows indicate the link between animals in column 1 and their vaccination status and subsequent blood test results. For example, in phase 1 on farm 1, column 1 shows that four animals tested positive to the field DPP. Of these, two were previously vaccinated (Column 2). Subsequently all four tested positive to the laboratory DPP (column 3). None tested positive and four were negative to the IGRA (B-A) (column 4). None tested positive and four tested negative to the IGRA (C. E. Cocktail), (column 5).

[1]Of these 10 previously vaccinated animals, two received a vaccine dose during this trapping phase. The animals were believed to be unvaccinated at this time. Their microchips and identity were only discovered after the second vaccine dose had been administered.
[2]Blood test results were unavailable for one animal because a sufficient volume of blood could not be collected. This animal is known to have been vaccinated previously and was captured and sampled subsequent to its capture here.
[3]Of these 11 previously vaccinated animals, one received a vaccine dose during this trapping phase. The animal was believed to be a new capture because a microchip was not found when scanned. The animal's microchip and identity were only discovered after the second vaccine dose had been administered.
[4]One animal was released before vaccination could be administered and another was mistakenly vaccinated despite having been vaccinated previously, (see [3] above).
[5]One animal was positive to the DPP test, but was released for welfare reasons because it was a lactating female.

5.3 *Post mortem* examination results

A total of 35 badgers were euthanased during the operations and all were submitted for PM examination (Table 3). *M. bovis* was isolated from tissue samples from five animals, but was not isolated from the remaining 30. Isolates were characterised by spoligotyping and whole genome sequencing (WGS). Three of the *M. bovis* cultures were identified as spoligotype 9, one was spoligotype 17, and one was spoligotype 1 (BCG). Note that culture is insensitive and that although a positive result confirms TB infection, a negative culture result does not necessarily confirm absence of infection.

Table 3 Summary of *Post mortem* results from badgers trapped at six Welsh farms in 2019.

Farm	Trapping phase	No. of badgers sampled	No. of badgers that were removed	No. of badgers positive for *M. bovis* culture	No. of badgers negative for *M. bovis* culture
1	1	35	6	1	5
2	2	17	6	0	6
3	1	17	2	0	2
3	2	5	2	0	2
4	1	29	7	0	7
4	2	16	6	3[1]	3
5	1	5	0	0	0
5	2	2	2	0	2
6	1	7	1	1	0
6	2	7	1	0	1
7	1	11	0	0	0
7	2	4	2	0	2

[1] One of these animals was positive for *M. bovis* BCG only

6. Costs

The direct cost of the preparation and delivery of the field operation in year 3 totalled £364,556 (Table 4). This covers both the field staff employed on a seasonal basis and the management team, including time dedicated to the preparation and organisation of the project ahead of the field operational phase. The staff costs, which included salaries, travel and subsistence payments accounted for the majority of the expenditure. 'Other' field costs included consumables, such as peanuts used as bait, field equipment, footwear and clothing, vehicle costs including hire costs, fuel and maintenance. 'Other' laboratory costs included consumables, such as equipment and reagents.

Table 4. Summary of costs associated with badger trap and test operations on chronic TB breakdown farms in 2019.

Activity	Cost (£)
FIELD	
Staff	220,536
Other	42,620
LABORATORY WORK, ANALYSIS AND REPORTING	
Staff	88,477
Other	12,923
TOTAL	364,556

7. The impact and effect on cattle herd breakdowns

In addition to the badger trap, test and remove operations, the chronic breakdown farms are subject to a range of other enhanced management measures. These measures aim to eliminate infection and reduce the risk of wider disease spread by identifying the possible factors contributing to the persistence of disease. They can include additional cattle movement restrictions, additional cattle testing requirements and additional biosecurity standards.

As each farm is subject to a combination of measures including badger interventions, it will be important to control for confounding effects in any analysis of the impact of specific measures. APHA have been commissioned to develop processes to gather data to achieve this, but the sample sizes required to achieve sufficient statistical power to disentangle and detect any effects may not be realised for a number of years.

8. References

Animal & Plant Health Agency (2018). Animal & Plant Health Agency (APHA) report on the delivery of badger trap and test operations on chronic TB breakdown farms in Wales in 2017 (TBOG0235). https://gov.wales/bovine-tb-badger-trapping-and-testing-chronic-tb-breakdown-farms-2017

Animal & Plant Health Agency (2019). Animal & Plant Health Agency (APHA) report on the delivery of badger trap and test operations on chronic TB breakdown farms in Wales in 2018 (TBOG0235). https://gov.wales/bovine-tb-badger-trapping-and-testing-chronic-tb-breakdown-farms-2018

Crawshaw, T. R., I. B. Griffiths, and R. S. Clifton-Hadley. 2008. Comparison of a standard and a detailed *postmortem* protocol for detecting *Mycobacterium bovis* in badgers. Veterinary Record 163: 473-477.

Welsh Government. 2017. Wales TB eradication programme delivery plan. http://gov.wales/topics/environmentcountryside/ahw/disease/bovinetuberculosis/bovinetberadication/?lang=en

APPENDIX 1. Description of blood tests

Two immunological blood tests were used for TB diagnosis in badgers. The tests each detect a different immune response and therefore may identify animals at different stages of infection. The DPP test was undertaken on whole blood in the field to provide a rapid result so that animals could be identified for release (negative) or euthanasia (positive). The DPP test (on serum) and IGRA were conducted subsequently in APHA laboratories so as to inform future field operations.

The DPP® VetTB (Chembio) is a serological lateral-flow assay that detects antibody responses against antigen targets MPB83 and ESAT6/CFP10 independently. A positive response to MPB83 is indicated by a line on band 1, and a positive response to ESAT6/CFP10 is indicated by a line on band 2 of the lateral flow device. During the DPP validation process for badger blood and serum in 2017, it was demonstrated that only band 1 was consistently diagnostically informative. As a result only band 1 was used for TB diagnosis.

The DPP can be conducted (with different protocols) on whole blood or serum samples. It has recently replaced the validated STAT-PAK®_TB (Chambers *et al.*, 2008) with apparently similar test performance. In the field the test was used to provide a rapid (within 30 minutes) qualitative assessment (positive or negative) on a sample of whole blood. Subsequently, under laboratory conditions, the test was performed on serum.

The IGRA detects the *in-vitro* cell mediated response in whole-blood. It requires a larger blood volume, more sophisticated laboratory facilities and takes longer to complete than serological assays. Samples also need to be subjected to the first stage of the process (T cell stimulation) within 7 hours of collection. The second stage of the test involves detection of IFN-γ in supernatants (which can be stored frozen until required). The test is expected to detect infected animals at an earlier stage of infection than serological tests and to be more sensitive. The IGRA measures the net response to bovine tuberculin minus avian tuberculin (PPD-B-PPD-A, referred to as B-A response), and to the DIVA antigens CFP-10/ESAT-6 protein cocktail antigens (referred to as C.E. Cocktail). The B-A response is an attempt to control for the occurrence of some shared antigens in both *M. bovis* (PPD-B) and environmental bacteria such as *Mycobacterium avium* (PPD-A). Hence the PPD-A response is subtracted from the response to PPD-B in order to avoid concluding that an animal is positive where both are high owing to infection with environmental mycobacteria. Furthermore, since a positive result to this test could indicate infection with *M. bovis*, and/or that the animal had been vaccinated with BCG, the C.E. Cocktail was also used. The C. E. Cocktail indicates infection with *M. bovis* only, not BCG, although it tends to be less sensitive than the B-A test. By conducting both B-A and C. E. Cocktail parts of the test, we provide a DIVA test (Differentiating Infected from Vaccinated). A positive B-A response, combined with a negative C. E. Cocktail response indicates that the badger has been vaccinated, but that it is not infected with *M. bovis*. Use of both tests in combination will allow us to differentiate vaccinated from infected badgers during future interventions. Cut-off points are defined for each antigen. To date the test has only been used for research purposes in badgers (Dalley *et al*., 2008; Carter *et al*., 2012).

These two blood tests have been used in parallel (Chambers *et al*., 2011; Carter *et al*., 2012) and continue to be used in the 'Test and vaccinate or remove' (TVR) study in Northern Ireland. Given their different performances and the different immune responses they measure, it is expected that they will occasionally provide discordant results in individual badgers. In particular, badgers that are negative by DPP could be positive by IGRA, due to the higher sensitivity of the latter test and the earlier development in the infected host of a cellular response relative to a serological (antibody) response. The scenario of a positive DPP result and a negative IGRA result should be less frequent because serological responses tend to become stronger as the disease progresses, while at the same

time strong cellular immune responses are also generally stimulated (Buzdugan *et al*., 2017). However, IGRA results are known to fluctuate over time in infected animals, possibly in response to the multiplication of mycobacteria which may not be constant, even when large lesions have developed (Tomlinson *et al*., 2015). The classic cellular anergy reported in cattle in the latest stages of the disease may also occur in badgers.

Neither of the tests used has perfect sensitivity and/or specificity and so it is expected that they will only detect a percentage of truly infected animals (sensitivity) and will report false positive results for some truly negative animals (specificity).

The IGRA has a published sensitivity of 80.9% (95% CI: 66.7 to 90.9) and specificity of 93.6% (95% CI: 89.1 to 96.7) (Dalley *et al.*, 2008). The DPP has been estimated to have a sensitivity with serum of 55.3% (95% CI: 38.3 to 71.4) and a specificity of 97.5% (95% CI: 86.6 to 99.9) when interpreting band 1 only. With whole blood (interpreting band 1 only) the sensitivity is 52.5% (95% CI: 36.1 to 68.5) and specificity is 97.5% (95% CI: 86.6 to 99.9). The DPP test was signed off as an APHA validated test in February 2018 and the badger IGRA in June 2018. Validation provides confidence in the performance characteristics of the test, including (importantly) its limitations. Validation of a test allows APHA to create a test code and to provide the test to commercial and government customers as a service. Results of the DPP validation are expected to be submitted to a peer reviewed scientific journal in the future.

Appendix 1 References

Buzdugan, S. N., M. A. Chambers, R. J. Delahay, and J. A. Drewe. 2017. Quantitative interferon-gamma responses predict future disease progression in badgers naturally infected with *Mycobacterium bovis*. Epidemiology and Infection 145: 3204-3213.

Carter, S. P., M. A. Chambers, S. P. Rushton, M. D. F. Shirley, P. Schuchert, S. Pietravalle, A. Murray, F. Rogers, G. Gettinby, G. C. Smith, R. J. Delahay, R. G. Hewinson, and R. A. McDonald. 2012. BCG vaccination reduces risk of tuberculosis infection in vaccinated badgers and unvaccinated badger cubs. Plos One 7:e49833.

Chambers, M. A., T. Crawshaw, S. Waterhouse, R. Delahay, R. G. Hewinson, and K. P. Lyashchenko. 2008. Validation of the BrockTB stat-pak assay for detection of tuberculosis in Eurasian badgers (*Meles meles*) and influence of disease severity on diagnostic accuracy. Journal of Clinical Microbiology 46: 1498-1500.

Chambers, M. A., F. Rogers, R. J. Delahay, S. Lesellier, R. Ashford, D. Dalley, S. Gowtage, D. Dave, S. Palmer, J. Brewer, T. Crawshaw, R. Clifton-Hadley, S. Carter, C. Cheeseman, C. Hanks, A. Murray, K. Palphramand, S. Pietravalle, G. C. Smith, A. Tomlinson, N. J. Walker, G. J. Wilson, L. A. L. Corner, S. P. Rushton, M. D. F. Shirley, G. Gettinby, R. A. McDonald, and R. G. Hewinson. 2011. Bacillus Calmette-Guerin vaccination reduces the severity and progression of tuberculosis in badgers. Proceedings of the Royal Society B-Biological Sciences 278: 1913-1920.

Dalley, D., D. Dave, S. Lesellier, S. Palmer, T. Crawshaw, R. G. Hewinson, and M. Chambers. 2008. Development and evaluation of a gamma-interferon assay for tuberculosis in badgers (*Meles meles*). Tuberculosis 88: 235-243.

Tomlinson, A. J., M. A. Chambers, R. A. McDonald, and R. J. Delahay. 2015. Association of quantitative interferon-gamma responses with the progression of naturally acquired *Mycobacterium bovis* infection in wild European badgers (*Meles meles*). Immunology 144: 263-270.

APPENDIX 2. Results of field and laboratory blood tests and *post mortem* examination from badgers trapped at six Welsh farms in 2019

Page 1 of 7

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
1	1	1	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	2	22/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	3	23/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	4	22/07/2019	NEG	POS	NEG	NEG	Y	N			
1	1	5	24/07/2019	POS	POS	NEG	NEG	N	N	POS	9	9:b
1	1	6	22/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	7	22/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	8	22/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	1	9	22/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	1	10	22/07/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
1	1	11	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	12	22/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	13	22/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	14	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	15	22/07/2019	NEG	POS	NEG	NEG	Y	N			
1	1	16	24/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	1	17	24/07/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
1	1	18	22/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	1	19	22/07/2019	NEG	POS	NEG	NEG	N	PV			
1	1	20	22/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	1	21	23/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	23	22/07/2019	NEG	NEG	NEG	NEG	Y	PV			

Appendix 2 continued, Page 2 of 7.

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
1	1	24	25/07/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
1	1	25	22/07/2019	NEG	NEG	NEG	NEG	Y	PV			
1	1	26	22/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	1	30	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	31	25/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	32	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	33	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	34	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	35	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	36	24/07/2019	NEG	NEG	NEG	NEG	Y	N			
1	1	38	24/07/2019	POS	POS	NEG	NEG	N	N	NEG	NA	NA
1	1	91	24/07/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
1	1	92	25/07/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	1	10/10/2019	POS	NEG	NEG	NEG	N	PV	NEG	NA	NA
1	2	2	08/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	6	08/10/2019	POS	POS	POS	NEG	N	PV	NEG	NA	NA
1	2	7	08/10/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
1	2	8	08/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	14	10/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	15	08/10/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
1	2	18	08/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	22	08/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	26	08/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	27	10/10/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
1	2	28	10/10/2019	POS	POS	NEG	NEG	N	N	NEG	NA	NA

Appendix 2 continued, Page 3 of 7.

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
1	2	29	11/10/2019	NEG	NEG	NEG	NEG	Y	N			
1	2	30	10/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	32	11/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	36	10/10/2019	NEG	NEG	NEG	NEG	N	PV			
1	2	37	08/10/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	39	08/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	40	09/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	41	09/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	42	10/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	43	09/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	44	08/07/2019	NEG	POS	NEG	NEG	N	PV			
3	1	45	08/07/2019	NEG	NEG	NEG	NEG	N	PV			
3	1	46	08/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	47	09/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	48	08/07/2019	POS	POS	NEG	NEG	N	N	NEG	NA	NA
3	1	49	08/07/2019	NEG	NEG	NEG	NEG	N	PV			
3	1	50	08/07/2019	NEG	NEG	NEG	NEG	N	PV			
3	1	51	08/07/2019	NEG	NEG	NEG	NEG	N	PV			
3	1	52	08/07/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
3	1	53	08/07/2019	NEG	POS	NEG	NEG	N	PV			
3	1	54	08/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	1	55	09/07/2019	NEG	NEG	NEG	NEG	Y	N			
3	2	45	23/09/2019	NEG	NEG	NEG	NEG	N	PV			
3	2	46	23/09/2019	NEG	NEG	NEG	NEG	N	PV			
3	2	51	23/09/2019	NEG	NEG	NEG	NEG	N	PV			

Appendix 2 continued, Page 4 of 7.

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
3	2	53	25/09/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
3	2	54	23/09/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
4	1	56	24/06/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
4	1	57	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	58	24/06/2019	POS	NEG	NEG	NEG	N	PV	NEG	NA	NA
4	1	59	25/06/2019	O	O	O	O	N	PV			
4	1	60	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	61	26/06/2019	NEG	POS	NEG	NEG	N	PV			
4	1	62	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	63	26/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	65	24/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	66	24/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	67	24/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	68	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	69	26/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	70	27/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	71	24/06/2019	NEG	NEG	NEG	NEG	Y	PV			
4	1	72	24/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	73	24/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	1	74	24/06/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
4	1	75	24/06/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
4	1	76	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	77	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	78	24/06/2019	NEG	NEG	NEG	NEG	N	N			
4	1	79	24/06/2019	NEG	NEG	NEG	NEG	N	PV			

Appendix 2 continued, Page 5 of 7.

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
4	1	80	24/06/2019	NEG	NEG	NEG	NEG	N	PV			
4	1	81	25/06/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
4	1	82	24/06/2019	NEG	POS	NEG	NEG	N	PV			
4	1	83	27/06/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
4	1	85	24/06/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
4	1	88	24/06/2019	NEG	NEG	NEG	NEG	Y	N			
4	2	59	09/09/2019	NEG	NEG	NEG	NEG	N	PV			
4	2	64	09/09/2019	NEG	NEG	NEG	NEG	Y	N			
4	2	65	09/09/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
4	2	66	10/09/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
4	2	67	09/09/2019	NEG	POS	NEG	NEG	N	PV			
4	2	68	09/09/2019	NEG	NEG	NEG	NEG	N	PV			
4	2	71	09/09/2019	NEG	NEG	NEG	NEG	N	PV			
4	2	72	09/09/2019	NEG	POS	NEG	NEG	N	PV			
4	2	73	09/09/2019	NEG	NEG	NEG	NEG	N	PV			
4	2	82	09/09/2019	LABPOS	NA	NA	NA	N	PV	POS	9	9:c
4	2	84	11/09/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
4	2	86	11/09/2019	NEG	NEG	NEG	NEG	Y	N			
4	2	87	09/09/2019	POS	POS	POS	NEG	N	N	POS	9	9:b
4	2	88	09/09/2019	POS	POS	NEG	NEG	N	PV	POS	1	NA
4	2	89	11/09/2019	NEG	NEG	NEG	NEG	Y	N			
4	2	90	11/09/2019	NEG	NEG	NEG	NEG	Y	N			
5	1	91	06/06/2019	NEG	NEG	NEG	NEG	Y	N			
5	1	93	03/06/2019	NEG	NEG	NEG	NEG	Y	N			
5	1	94	04/06/2019	POS*	POS	NEG	NEG	N	N			

Appendix 2 continued, Page 6 of 7.

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
5	1	95	03/06/2019	NEG	NEG	NEG	NEG	Y	N			
5	1	96	03/06/2019	NEG	NEG	NEG	NEG	N	PV			
5	2	92	19/08/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
5	2	103	19/08/2019	POS	POS	NEG	NEG	N	PV	NEG	NA	NA
6	1	92	06/06/2019	NEG	POS	NEG	NEG	N	PV			
6	1	97	03/06/2019	POS	POS	NEG	NEG	N	N	POS	17	17:a
6	1	98	03/06/2019	NEG	POS	NEG	NEG	Y	N			
6	1	99	06/06/2019	NEG	NEG	NEG	NEG	N	PV			
6	1	103	06/06/2019	NEG	NEG	NEG	NEG	Y	N			
6	1	104	06/06/2019	NEG	NEG	NEG	NEG	N	PV			
6	1	105	06/06/2019	NEG	NEG	NEG	NEG	Y	N			
6	2	98	19/08/2019	LABPOS	NA	NA	NA	N	PV	NEG	NA	NA
6	2	99	19/08/2019	NEG	NEG	NEG	NEG	N	PV			
6	2	100	20/08/2019	NEG	NEG	NEG	NEG	Y	N			
6	2	101	19/08/2019	NEG	NEG	NEG	NEG	Y	N			
6	2	102	19/08/2019	NEG	NEG	NEG	NEG	Y	N			
6	2	104	19/08/2019	NEG	NEG	NEG	NEG	N	PV			
6	2	106	20/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	107	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	108	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	109	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	110	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	111	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	112	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	113	05/08/2019	NEG	NEG	NEG	NEG	Y	N			

Farm	Trapping phase	Animal ID	Date	Field DPP	Lab DPP	IGRA B-A	IGRA C. E. Cocktail	Vaccine administered on this date?	Vaccinated on a previous date?	Culture	Spoligotype	Genotype
7	1	114	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	115	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	116	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	1	117	05/08/2019	NEG	NEG	NEG	NEG	Y	N			
7	2	107	21/10/2019	POS	NEG	NEG	NEG	N	PV	NEG	NA	NA
7	2	109	21/10/2019	NEG	POS	NEG	NEG	N	PV			
7	2	113	21/10/2019	NEG	NEG	NEG	NEG	N	PV			
7	2	116	21/10/2019	POS	NEG	NEG	NEG	N	PV	NEG	NA	NA

Abbreviations:
Field DPP; Lab DPP; IGRA B-A; IGRA C. E. Cocktail:
NEG = Negative.
POS = Positive.
LABPOS = Animal tested positive to a lab test at a previous sampling event.
NA = Test was not conducted because the animal tested positive to a lab test at a previous sampling event.
O = A sufficient blood sample could not be taken to conduct this test.
* = The animal was positive to the field DPP, but was released for welfare reasons because it was a lactating female.

Vaccine administered on this date:
N = No.
Y = Yes.

Vaccinated on a previous date:
N = No.
PV = Vaccinated during a previous trapping phase.

Culture; spoligotype; genotype (data entered if an animal was euthanased):
NEG = Negative.
POS = Positive.
NA = Not applicable. If a culture result was negative then spoligotype and genotype = NA.