



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 2018**

## **Report for project TBOG0235**

### **(Year 2)**

The Animal and Plant Health Agency is an Executive Agency of the Department for Environment, Food and Rural Affairs working to safeguard animal and plant health for the benefit of people, the environment and the economy.

Mae'r Asiantaeth Iechyd Anifeiliaid a Phlanhigion yn un o Asiantaethau Gweithredol Adran yr Amgylchedd, Bwyd a Materion Gwledig sy'n gweithio i ddiogelu iechyd anifeiliaid a phlanhigion er budd pobl, yr amgylchedd a'r economi.

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## **Report for project TBOG0235 (Year 2)**

### **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.

### **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 June and November. 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. The Office of the Chief Veterinary Officer applied for the licences in 2018. Licences were granted to undertake trap and test operations on seven farms. One farm withdrew before trapping operations began and the licence was subsequently revoked. Licensing inspectors attended all farms during trapping and testing operations.

Interventions were carried out between May and November 2018. During each intervention, data was collected that could be used to monitor badger abundance, capture efficiency, diagnostic test performance and potentially, to detect any signs of perturbation.

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, tissue culture, as well as hair sample genotyping.

Each intervention consisted of the following sequential activities:

- Badger activity survey.
- Pre-treatment hair trapping to estimate abundance.
- 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 during a previous intervention (either in 2017 or 2018).
  - BCG Sofia vaccination if not already vaccinated and release of animals negative to the field DPP test.
- PM examination of test positive animal carcasses, culture of tissue samples and subsequent spoligotyping and whole genome sequencing of isolates.
- Post-treatment hair trapping to monitor for any evidence of changes in abundance or disruption to normal patterns of behaviour.
- 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 2018.

### **4.2 Field survey, hair trapping and cage trapping**

Each farm was surveyed for badger activity by appropriately skilled field staff. Following surveys for badger activity, hair traps were set for 14 days before cage traps were deployed. Hair traps were deployed on badger runs, at setts and elsewhere on the farm. Hair traps were checked each day and any hairs present were collected and sent to the APHA laboratory for genotype analysis.

Following hair trapping, cage traps were positioned at locations where there was most badger activity. Cage traps were pre-baited with peanuts before the trapping phase. Trapping lasted between 2 and 4 days per farm. Captured badgers were anaesthetised, blood samples taken for diagnostic testing (see below) and a hair sample was taken for genotyping. Following cage trapping, hair traps were reinstalled at the same locations as previously, and hair was again collected every day for a further 14 days, before being dispatched to the laboratory for genotype analysis.

The genotype data from collected hairs will be used to attempt to estimate the proportion of badgers using the farm land that were trapped and tested. It may also provide data to enable assessment of whether the cage trapping operations caused any disruption to normal patterns of behaviour. Analysis of genotype data will follow a methodology that was developed for estimating badger abundance (Frantz *et al.*, 2004; Pope *et al.*, 2007; Scheppers *et al.*, 2007). Results are contingent on the collection and genotyping of sufficient number of hair samples.

### **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 5-10 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. A hair sample (approximately 10 hairs) was taken for genotype analysis, 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.

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 badgers from infected badgers during future interventions.

#### **4.5 Vaccination and release**

Badgers that tested negative on the field DPP were vaccinated by intramuscular injection with 1 ml of reconstituted BCG Sofia vaccine. Animals were vaccinated only once during the intervention, thus any animal that was sampled subsequently did not receive additional vaccine. This was facilitated by marking each captured animal that was destined for release, by cutting a small area of hair on the rump and spraying it with coloured stock mark. 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 animals were recognised by the temporary coloured stock mark and fur clip, and were individually identified by scanning the microchip through the cage. If possible, a small hair sample was taken for genotypic matching, and the animals were 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 119 individual badgers were sampled in 2018 (Table 1). On some farms (1, 3 and 4) two phases of trapping were conducted several weeks apart. Some badgers were therefore sampled more than once, resulting in 165 sampling events in 2018. (Note, 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 2018**

Farm		No. of badgers sampled for the first time in 2018 <sup>1</sup>	No. of badgers sampled in phase 2 that were previously sampled in phase 1	Total badgers sampled in phase 2 (sampled for the first time + previously sampled in phase 1).	No. of non-target animals <sup>2</sup>	No. of recaptures <sup>3</sup>
1	Phase 1	30	NA	NA	1	8
1	Phase 2	12	13	25	0	2
2		11	NA	NA	0	2
3	Phase 1	13	NA	NA	1	5
3	Phase 2	2	6	8	0	4
4	Phase 1	36	NA	NA	1	13
4	Phase 2	5	26	31	0	8
5		7	NA	NA	0	3
6		4	NA	NA	0	1

<sup>1</sup>A total of 119 badgers were sampled for the first time in 2018. This column total is 120 animals, because 1 animal was sampled on two different farms.

<sup>2</sup>Non target animals: 3 foxes. Non target animals were released immediately following a welfare assessment.

<sup>3</sup>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 165 sampling events, there were 22 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 (10), pads (2), knuckles (2), mouth or teeth (2), snout (3) forehead or neck (5). The only other injuries observed were old or healed bite wounds. Of the 165 sampling events, there were 44 instances when the animals had bite wounds, 39 of which were old or healed, five of which were fresh. All of the individuals were considered to be fit and healthy for sampling and for release if returning a negative DPP test result.

### 5.2 Summary of badger sampling and diagnostic test results

Results of blood tests are summarised in Table 2. Occasionally a blood test result was unavailable if sufficient blood could not be taken to complete the test. This occurred if the animals recovered from anaesthesia before sampling could be completed.

During operations there were 165 sampling events which resulted in the removal of 26 animals; 22 due to a positive field DPP result and four due to having returned positive laboratory results at a previous sampling event. All four positive laboratory results occurred following sampling in 2018. None of the animals that were caught in 2017 that tested negative to the field DPP test but subsequently positive to laboratory tests were recaptured in 2018. Of the 22 field DPP positive

animals from 2018, 19 subsequently tested positive to the laboratory DPP test, four tested positive to the IGRA (B-A) test and two were positive to the IGRA (C.E. Cocktail). Two of the 22 animals tested negative to all subsequent blood tests, while one tested negative to all subsequent blood tests except the laboratory DPP, which could not be completed because a blood sample was not available.

Of the 165 sampling events, on 139 occasions the animal was released (137 tested negative to the field DPP, while two could not be tested because it was not possible to obtain a blood sample). Subsequently, 25 animals tested positive to at least one of the laboratory tests (23 tested positive to the laboratory DPP, three tested positive to the IGRA (B-A) test and three tested positive to the IGRA (C. E. Cocktail). A total of 112 of the 137 sampling events returned negative results for all subsequent laboratory blood tests (all blood tests were completed for 105 events, partial blood tests were completed for seven events because sufficient blood was not available to complete all tests).

**Table 2 Summary of field and laboratory blood tests from badgers trapped at six Welsh farms in 2018 (continued on next page).**

Farm			Vaccinated in a previous phase of trapping	Laboratory DPP		IGRA: B-A		IGRA: C. E. Cocktail	
				POS	NEG	POS	NEG	POS	NEG
<b>Farm 1 Phase 1</b>									
No. badgers	30	→	0						
No. positive to field DPP	2	→	0	2	0	1	1	1	1
No. negative to field DPP	28 <sup>1</sup>	→	0	4	23	0	28	0	27
No. positive on previous laboratory test	0		-						
No. vaccinated	27 <sup>2</sup>								
No. released	28								
<b>Farm 1 Phase 2</b>									
No. badgers	25	→	11						
No. positive to field DPP	7	→	5	7	0	1	6	1	6
No. negative to field DPP	16	→	4	3	13	0	16	0	16
No. positive on previous laboratory test	2	→	2						
No. vaccinated	12								
No. released	16								
<b>Farm 2</b>									
No. badgers	11	→	0						
No. positive to field DPP	2	→	0	2	0	2	0	0	2
No. negative to field DPP	9	→	0	2	7	1	8	1	8
No. positive on previous laboratory test	0		-						
No. vaccinated	9								
No. released	9								
<b>Farm 3 Phase 1</b>									
No. badgers	13	→	0						
No. positive to field DPP	1	→	0	0	1	0	1	0	1
No. negative to field DPP	12	→	0	1	11	1	11	1	11
No. positive on previous laboratory test	0		-						
No. vaccinated	12								
No. released	12								
<b>Farm 3 Phase 2</b>									
No. badgers	8	→	5						
No. positive to field DPP	1 <sup>3</sup>	→	1	-	-	0	1	0	1
No. negative to field DPP	7 <sup>4</sup>	→	4	2	4	0	6	0	6
No. positive on previous laboratory test	0		-						
No. vaccinated	3								
No. released	7								



**Table 2 continued from previous page.**

Farm			Vaccinated in a previous phase of trapping	Laboratory DPP		IGRA: B-A		IGRA: C. E. Cocktail	
				POS	NEG	POS	NEG	POS	NEG
<b>Farm 4 Phase 1</b>									
No. badgers	36		0						
No. positive to field DPP	1	→	0	1	0	0	1	0	1
No. negative to field DPP	33 <sup>5</sup>	→	0	2	29	1	31	0	30
No. positive on previous laboratory test	0								
No. vaccinated	35								
No. released	35								
<b>Farm 4 Phase 2</b>									
No. badgers	31	→	25						
No. positive to field DPP	7	→	6	6	1	0	7	0	7
No. negative to field DPP	22	→	17	9	13	0	22	0	22
No. positive on previous laboratory test	2	→	2						
No. vaccinated	4 <sup>6</sup>								
No. released	22								
<b>Farm 5</b>									
No. badgers	7	→	1 <sup>7</sup>						
No. positive to field DPP	1	→	1	1	0	0	1	0	1
No. negative to field DPP	6	→	0	0	6	0	6	1	5
No. positive on previous laboratory test	0		-						
No. vaccinated	6								
No. released	6								
<b>Farm 6</b>									
No. badgers	4	→	0						
No. positive to field DPP	0	→	-	-	-	-	-	-	-
No. negative to field DPP	4	→	0	0	4	0	4	0	4
No. positive on previous laboratory test	0								
No. vaccinated	4								
No. released	4								

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 two animals tested positive to the field DPP. Of these, none were previously vaccinated (Column 2). Subsequently two tested positive to the laboratory DPP (column 3). One tested positive and one negative to the IGRA (B-A) (column 4). One tested positive and one negative to the IGRA (C. E. Cocktail), (column 5).

<sup>1</sup>Blood sample from 1 field DPP negative animal was not available to complete the laboratory DPP or the IGRA C.E. Cocktail

<sup>2</sup>One animal recovered and was released before vaccination could be administered.

<sup>3</sup>Blood sample from 1 field DPP positive animal was not available to complete the laboratory DPP.

<sup>4</sup>Blood sample from 1 field DPP negative animal was not available to complete the laboratory DPP or the IGRA tests.

<sup>5</sup>Blood samples were not available to complete any blood tests for 2 animals. In addition, only partial blood samples were available for 5 animals, therefore 2 laboratory DPP, 1 IGRA B-A, and 3 IGRA C. E. Cocktail could not be completed.

<sup>6</sup>One animal recovered and was released before vaccination could be administered.

<sup>7</sup>One animal was caught during intervention on another farm, and therefore had been vaccinated previously.

### 5.3 Post mortem results

A total of 26 badgers were euthanased during the operations and all were submitted for PM examination (Table 3). *M. bovis* was isolated from tissue samples from 11 animals, and was not isolated from the remaining 15 animals. Isolates were characterised by spoligotyping and whole genome sequencing (WGS). Eight of the *M. bovis* cultures were identified as spoligotype 9, one was spoligotype 17, and two were spoligotype 1 (BCG).

**Table 3 Summary of Post mortem results from badgers trapped at six Welsh farms in 2018.**

Farm	Phase	No. of badgers sampled	No. of badgers that were removed	No. of badgers positive for <i>M. bovis</i> culture	No. of badgers negative for <i>M. bovis</i> culture
1	Phase 1	30	2	1	1
1	Phase 2	25	9	5	4
2		11	2	0	2
3	Phase 1	13	1	0	1
3	Phase 2	8	1	0	1
4	Phase 1	36	1	1	0
4	Phase 2	31	9	3	6
5		7	1	1	0
6		4	0	NA	NA

### 5.4 Hair trapping and sampling

The collection of hair samples was undertaken on six farms, prior to, during and post cage trapping during phase 1 only. The number of hair traps set on each farm ranged between 22 and 37 (Table 4). Prior to the deployment of badger traps the hair traps yielded between 6 and 49 hair samples per farm. During the post cage trapping phase the hair traps yielded between 2 and 24 hair samples. Hair samples were also collected directly from animals that were sampled, as well as from recaptured animals, giving a total of 125 samples. Laboratory processing of hair samples for genotyping and subsequent genotype data analysis will be completed in 2019. Any results obtained relating to trapping efficiency, target population size and any patterns consistent with perturbation will be available when all samples have been analysed.

**Table 4 Number of hair traps set and hair samples collected in 2018.**

Farm	No. hair traps set	Number of hair samples collected from: <sup>1</sup>			
		Pre cage trapping	Post cage trapping	Cage trapped animals phase 1	Cage trapped animals phase 2
1	36	31	18	36	25
2	32	49	24	11	NA
3	22	6	NA	18	12
4	24	30	20	45	38
5	26	18	2	10	NA
6	32	11	6	5	NA

<sup>1</sup>Total number of hair samples collected from cage trapped animals, does not reconcile with numbers of animals caught in table 1, because it was not always possible to obtain a hair sample from a recaptured animal.

## 6. Costs

A breakdown of the costs directly incurred in the preparation and delivery of the field operation in year 2 totalled £395,802.10 (Table 5).

The costs cover both the field staff employed on a seasonal basis and the management team, including their 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 5. Summary of costs associated with badger trap and test operations on chronic TB breakdown farms in 2018.**

Activity	Cost (£)
<b>FIELD</b>	
Staff	202,623.45
Other	43,331.96
<b>SCIENCE</b>	
Staff	109,501.79
Other	40,344.90
<b>TOTAL</b>	<b>395,802.10</b>

## 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. These measures 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/docs/drah/publications/180712-delivery-of-badger-trap-and-test-operations-2017-report-en.pdf>

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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 used during intervention

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 also 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 samples or on 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, both qualitative and quantitative (using an electronic reader) assessments were made on serum.

The IGRA detects the *in-vitro* cell mediated response in whole-blood. It requires larger blood sample volumes, 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- $\gamma$  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), and to the DIVA antigens CFP-10/ESAT-6 protein cocktail antigens (to allow animals that have been vaccinated to be distinguished from animals that are infected). 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 2019.

## Appendix 1 References

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