Animal &
Plant Health
Agency

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Animal & Plant Health Agency (APHA) report on the delivery of badger trap and test operations on chronic TB breakdown farms in Wales in 2022

Report for project TBOG0235

(Year 6)

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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 (Animal & Plant Health Agency 2018). Since 2018 test negative badgers have also been vaccinated prior to release (Animal and Plant Health Agency 2019; 2020; 2021; 2022).

In 2022 (Year 6) the programme was continued by APHA and WG contractors, using a similar protocol. This report summarises that work.

In 2022 WG made the decision that the programme of work will cease once all remaining farms have completed eight phases of trapping. The final two phases of trapping will therefore take place in 2023.

2. Preparatory phase

The farms were selected for intervention by WG in conjunction with APHA veterinary field staff. Badger activity surveys were undertaken on each farm by WG contractors prior to trap positioning and pre-baiting. 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 2022 licences were granted to undertake trap and test operations on two farms. A member of the WG TB team attended each farm during operations to audit license compliance.

Field operations involved a collaborative approach between APHA Science Directorate, Service Delivery Directorate and a WG contractor. 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. During each intervention, data was collected that could be used to monitor badger abundance, capture efficiency and diagnostic test performance.

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 was 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 or IGRA tests 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 Danish vaccination. Animals were vaccinated only once in their lifetime. 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 whole genome sequencing of any *Mycobacterium bovis* isolates obtained.
- Collation of data from laboratory tests to inform future interventions.

On one of the farms (farm 4) the intervention involved badger vaccination or removal only, no blood tests were conducted, as per an exit strategy agreed by WG and APHA case vets. On this farm, the intervention consisted of the following sequential activities:

- Badger activity survey.
- Deployment and pre-baiting of traps.
- Cage trapping and sampling trapped badgers.
    - Scanning of animals through the trap to identify individuals that had been microchipped during a previous intervention.
    - Release of animals that were captured for the first time (i.e. did not have a microchip), following BCG Danish vaccination.
    - Euthanasia by lethal injection of animals that had tested positive to the laboratory DPP or IGRA tests that were conducted following a previous intervention.
    - Release of animals that were negative to any previous laboratory-based tests, following BCG Danish vaccination.
- PM examination of test positive animal carcasses, culture of tissue samples and subsequent 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 two farms between May and October 2022. One phase of trapping occurred on Farm 4, after which this farm exited the program having completed eight phases of trapping in four years. Three phases of trapping occurred on farm 8.

4.2 Field survey and cage trapping

On each farm surveys for badger activity and cage trapping were conducted by WG contractors. Cage traps were positioned at locations where there was most badger activity and were pre-baited with peanuts for ten days before the trapping phase. Trapping took place over 2 consecutive nights for each phase.

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.

All procedures from anaesthesia through to monitoring until release were conducted by an APHA veterinarian at the trap side. 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. The tests conducted on each badger were dependent on the vaccination status of the animal (see section 4.5 below for details).

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 DPP testing

During 2022 operations, the field and laboratory DPP tests were only conducted on animals that had not been vaccinated in the previous 358 days for the following reason. The DPP measures the serological response against the protein MPB83, an immunodominant antigen, of which BCG Sofia is a known high producer. This raises the probability that animals vaccinated with BCG Sofia would produce detectable antibody titres to MPB83 leading to positivity in the DPP test. Little research has

been conducted to directly compare MPB83 positivity using the DPP test in animals vaccinated with BCG Sofia.

To date, no statistical analysis has been conducted to assess the data that has been collected since trap and test operations began for any such effects. However, given this possibility, WG decided that any animal that had been vaccinated (with either BCG Sofia or BCG Danish) in the previous 358 days would not be tested using the DPP test, in order to reduce the possibility that an animal might test positive due to previous vaccination, rather than infection with *M. bovis*.

The badger IGRA test continued to be used to test all animals. The IGRA test is used to measure cell-mediated immune responses against antigens PPD-B, PPD-A and also ESAT-6/CFP-10 *M. bovis* specific antigens. The latter antigens are not produced by BCG strains and any measured response to them is therefore considered indicative of *M. bovis* infection, regardless of vaccination status.

4.6 Vaccination and release

Captured badgers that tested negative to the field DPP and to previous laboratory tests were vaccinated by intramuscular injection with 1 ml of reconstituted BCG Danish vaccine. During 2018-2020 interventions, badgers were vaccinated with BCG Sofia because BCG Danish was not available. From 2021 onwards BCG Danish was available and was used for badger vaccination.

On farm 8, animals were vaccinated only once. Any animal that had been vaccinated during any previous capture event since 2017 was not re-vaccinated. This was facilitated by individually identifying each badger by scanning the microchip. On farm 4 only, all animals were vaccinated, whether or not they had been vaccinated previously. Since this was the last trapping phase on this farm, a second vaccination was possible without impacting future test results.

Animals were then 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 trap, before being released at the point of capture.

On the second day 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.

4.7 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 whole genome sequencing. 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 46 individual badgers were sampled in 2022 (Table 1). On farm 4 one phase of trapping was conducted, while on the other farm three phases of trapping were conducted several weeks apart. Some badgers were therefore sampled more than once, resulting in 58 sampling events in 2022. (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 five Welsh farms in 2022.

Farm	Trapping phase	No. of badger sampling events	No. of capture events of a non-target animal[2]	No. of recaptures
4[1]	1	10	0	3
8	1	19	0	2
8	2	21	0	2
8	3	8	0	1
Total		58	0	8

[1]On this farm intervention involved badger vaccination or removal of animals that tested positive to a previous laboratory test only, no blood tests were conducted.

[2]Non target animals are released immediately following a welfare assessment.

During initial assessment of badgers in traps, none were found in need of veterinary examination. Of the 48 sampling events when the animal was anaesthetised enabling close inspection (i.e. excluding 10 events on Farm 4 when animals were vaccinated and released only), there were 4 instances when animals had minor injuries that were likely to have arisen while being in the trap. The injuries reported were: broken or bleeding claws and quick (3) and abrasion to the snout or chin (1). Of the remaining 44 sampling events 42 recorded no trap injuries and two omitted to record trap injuries.

Of the 48 sampling events, there were 44 instances when no bite wounds were present, 3 instances when bite wounds were present, and 1 instance when bite wounds were not recorded. Of the 3 records of bite wounds, one badger had one fresh bite wound, one had a few healed bite wounds, while the condition of the bite wound on the remaining badger was not recorded. All individuals were considered 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 and complete results are provided in Appendix 2.

Operations resulted in the euthanasia of four animals. One was removed due to a positive test result conducted in the laboratory following a previous sampling event and 3 were removed following a positive field DPP test. Subsequently, 1 of the 3 tested positive to at least one of the laboratory blood tests.

In each of the remaining 44 sampling events the animal was released. Laboratory test results for those animals are as follows:

- One animal tested positive to a laboratory test in phase 1, however when it was trapped during phase 2 it was released for welfare reasons because it was lactating. It was trapped again in phase 3 at which time it was euthanased (as described above).
- The laboratory DPP test was not conducted for eight sampling events because the animal was not eligible i.e. it had been vaccinated within the previous 358 days.
- Laboratory tests for three sampling events were incomplete because a sufficient blood sample could not be obtained.

Table 2. Summary of field and laboratory blood tests from badgers trapped at five Welsh farms in 2021.

Farm	Phase	No. badgers	No. positive to field DPP test	No. negative to field DPP test	No. ineligible for DPP test	No. positive to previous laboratory tests	No. removed	No. previously vaccinated	No. vaccinated	No. released	No. positive to subsequent laboratory tests
4[1]	1	10	NA	NA	NA	0	0	8	10	10	NA
8	1	19	2	13	4	0	2	11	7	17	1
8	2	21	0	18	2	1[2]	0	8	13	21	0[3]
8	3	8	1	4	2	1	2	5	3	6	0
Total		**58**	**3**	**35**	**8**	**2**[4]	**4**	**32**	**33**	**54**	**1**

[1]On this farm intervention involved badger vaccination or removal only, no blood tests were conducted.
[2]This animal was trapped, however it was not euthanased on welfare grounds because it was lactating.
[3] Three animals did not have a laboratory DPP or IGRA test because a blood sample could not be obtained.
[4]One animal was positive to previous laboratory tests. It was trapped in phase 2 and released on welfare grounds. It was trapped again in phase 3 and was euthanased.

5.3 *Post mortem* examination results

Four badgers were euthanased and underwent PM examination (Table 3). *M. bovis* was isolated from tissue samples from three of those animals. Two isolate were identified as genotype B6-14, a clade ID was not obtained for the other. 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* examination results from badgers trapped at five Welsh farms in 2021.

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
4[1]	1	10	0	NA	NA
8	1	19	2	2	0
8	2	21	0	NA	NA
8	3	8	2	1	1
Total		58	4	3	1

[1]On this farm intervention involved badger vaccination or removal only, no blood tests were conducted.

6. Costs

The direct cost of the preparation and delivery of the field operation totalled £103,939.80 (Table 4). This covered 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 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 from April 2022 to March 2023.

Activity	Cost (£)
FIELD	
Staff[1]	£72,687.80
Other	£1,258
LABORATORY WORK, ANALYSIS AND REPORTING	
Staff	£28,052
Other	£1.942
TOTAL	£103,939.80

[1]WG contractor costs=£69,016.80; APHA costs=£3,671

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 and biosecurity requirements.

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 has been commissioned to develop approaches to gather the data necessary for such an analysis, 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

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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 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 was validated 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

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APPENDIX 2. Results of field and laboratory blood tests and *post mortem* examination from badgers trapped at six Welsh farms in 2022. See page 16 for explanation of abbreviations.

Farm[1]	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	Genotype
4	1	257	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	62	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	235	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	71	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	59	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	277	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	258	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
4	1	X	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	N		
4	1	X	20/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	N		
4	1	252	21/06/2022	NA-VO	NA-VO	NA-VO	NA-VO	Y	PV		
8	1	217	17/05/2022	NEG	NEG	NEG	POS	N	PV		
8	1	219	17/05/2022	POS	POS	NEG	NEG	N	PV	M. bovis	No clade ID
8	1	279	18/05/2022	NEG	NEG	NEG	NEG	Y	N		
8	1	280	17/05/2022	NEG	NEG	NEG	NEG	Y	N		
8	1	224	17/05/2022	NEG	NEG	NEG	NEG	N	PV		
8	1	227	17/05/2022	NEG	NEG	NEG	NEG	N	PV		
8	1	231	18/05/2022	NEG	NEG	NEG	NEG	N	PV		
8	1	240	17/05/2022	NEG	NEG	NEG	NEG	N	PV		
8	1	241	18/05/2022	NEG	NEG	NEG	NEG	N	PV		
8	1	255	17/05/2022	RV	RV	NEG	NEG	N	PV		
8	1	262	17/05/2022	RV	RV	NEG	NEG	N	PV		
8	1	264	17/05/2022	RV	RV	NEG	NEG	N	PV		
8	1	271	17/05/2022	RV	RV	POS[3]	NEG	N	PV		
8	1	302	17/05/2022	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	Genotype
8	1	281	17/05/2022	NEG	NEG	NEG	NEG	Y	N		
8	1	297	17/05/2022	POS	NEG	NEG	NEG	N	N	M. bovis	B6-14
8	1	298	17/05/2022	NEG	NEG	NEG	NEG	Y	N		
8	1	299	17/05/2022	NEG	NEG	NEG	NEG	Y	N		
8	1	300	17/05/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	217[2]	25/07/2022	NA-LP	NA-LP	NA-LP	NA-LP	N	PV		
8	2	279	25/07/2022	RV	RV	NEG	NEG	N	PV		
8	2	224	25/07/2022	NEG	NEG	NEG	NEG	N	PV		
8	2	240	26/07/2022	NEG	NEG	NEG	NEG	N	PV		
8	2	255	25/07/2022	NEG	NEG	NEG	NEG	N	PV		
8	2	256	25/07/2022	NEG	NEG	NEG	NEG	N	PV		
8	2	262	25/07/2022	NEG	NEG	NEG	NEG	N	PV		
8	2	282	25/07/2022	NEG	O	O	O	Y	N		
8	2	284	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	286	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	287	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	288	25/07/2022	NEG	O	O	O	Y	N		
8	2	289	25/07/2022	NEG	O	O	O	Y	N		
8	2	290	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	291	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	292	26/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	293	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	294	25/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	295	26/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	301	26/07/2022	NEG	NEG	NEG	NEG	Y	N		
8	2	281	25/07/2022	RV	RV	POS[3]	NEG	N	PV		

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	Genotype
8	3	217	10/10/2022	NA-LP	NA-LP	NA-LP	NA-LP	N	PV	NEG	NA
8	3	227	10/10/2022	POS	NEG	NEG	NEG	N	PV	M. bovis	B6-14
8	3	262	10/10/2022	NEG	NEG	NEG	NEG	N	PV		
8	3	283	10/10/2022	NEG	NEG	NEG	NEG	Y	N		
8	3	285	10/10/2022	NEG	NEG	NEG	NEG	Y	N		
8	3	291	10/10/2022	RV	RV	NEG	NEG	N	PV		
8	3	293	10/10/2022	RV	RV	NEG	NEG	N	PV		
8	3	296	10/10/2022	NEG	NEG	NEG	NEG	Y	N		

[1]Farm 4 operations involved badger vaccination and release, or removal of animals that tested positive to a previous laboratory test only, no blood tests were conducted.
[2]This animal tested positive to a previous laboratory test, however it was not euthanased for welfare reasons. It was subsequently trapped in phase 3 at which time it was euthanased.
[3]An animal positive to the IGRA B-A test and negative to the C. E. Cocktail indicates that the badger was not infected with *M. bovis* and had been vaccinated.

Abbreviations:
Animal ID:
X – animal was not given an identification microchip because this phase of trapping was the last at this location

Field DPP; Lab DPP; IGRA B-A; IGRA C. E. Cocktail:
NEG = Negative.
POS = Positive.
NA-LP = Not applicable because the animal tested positive to a lab test at a previous sampling event.
NA-VO = Not applicable because the animal was trapped on Farm 4 where the intervention was vaccination only.
RV = The DPP test was not conducted because the animals was vaccinated within the previous 358 days.
O = A sufficient blood sample could not be taken to conduct this test.

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 genotype is NA.