Animal &
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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 2020

Report for project TBOG0235

(Year 4)

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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). In 2018 and 2019 (Years 2 and 3) APHA continued the trapping and testing work on persistent breakdown farms. Test positive badgers were humanely euthanased, while test negative badgers were vaccinated and released (Animal and Plant Health Agency 2019; Animal and Plant Health Agency 2020).

In 2020 (Year 4) the programme was continued by APHA and WG contractors, using a similar protocol. This report summarises that work.

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 between May and October by WG contractors. 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 2020 licences were granted to undertake trap and test operations on six farms. Licensing inspectors were unable to attend farms during trapping and testing operations due to Coronavirus restrictions.

Field operations involved a collaborative approach between APHA Science Directorate, Service Delivery Directorate and a WG contractor. One member of the WG TB team also attended each farm during operations. 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.

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 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 Sofia 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 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 farms between May and November 2020.

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 before the trapping phase. Trapping lasted between 2 and 4 days per farm.

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 2020 operations, the field and laboratory DPP tests were only conducted on animals that had not been vaccinated with BCG Sofia 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 done in this area directly comparing MPB83 positivity using the DPP test in animals vaccinated with BCG Sofia.

To date, no statistical analysis has been conducted to assess any such response in the data that has been collected since these trap and test operations began. Therefore it is not possible to conclude whether a statistically significant number of animals tested positive to the DPP in 2019 following BCG Sofia vaccination in 2018. However, given this possibility, WG decided that any animal that had been vaccinated 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 *M. bovis* infection, whether or not previous BCG vaccination has been administered.

4.6 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.

4.7 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 spoligotyping and 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 69 individual badgers were sampled in 2020 (Table 1). On four of the six farms two phases of trapping were conducted several weeks apart. Some badgers were therefore sampled more than once, resulting in 86 sampling events in 2020. (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). One phase of trapping occurred on farm 6 before it was excluded from future intervention due to the low level of badger activity. Farm 8 was then included in the programme of work by which time only one phase of trapping was possible before the end of the season.

Table 1. Number of badgers caught at six Welsh farms in 2020.

Farm	Trapping phase	No. of badgers sampled	No. of capture events of a non-target animal[1]	No. of recaptures
1	1	17	0	3
1	2	9	0	1
3	1	3	1 (Fox)	2
3	2	3	0	1
4	1	20	0	5
4	2	7	0	0
6	1	1	1 (Fox)	0
7	1	6	0	1
7	2	2	0	0
8	1	18	0	5
Total		86	2	18

[1]Non target animals were released immediately following a welfare assessment.

During initial assessment of badgers in traps, none were found in need of veterinary examination. Of 86 sampling events, there were 14 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 (9), pads bleeding split or cut (3), teeth or gums bleeding or broken (1), abrasion to the snout or chin (3). Of the remaining 72 sampling events 71 recorded no trap injuries and one omitted to record trap injuries.

Of the 86 sampling events, there were 76 instances when bite wounds were not present, nine instances when bite wounds were present, and one instance when bite wounds were not recorded. Of the nine records of bite wounds, none were fresh, two were open and old, six were healed scars, and 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 and complete results are provided in Appendix 2. During operations there were 86 sampling events, which resulted in the removal of two animals. Both were removed due to a positive test result conducted in the laboratory following a previous sampling event (one in 2019, one 2020).

Of the remaining 84 sampling events the animal was released. A total of 71 tested negative to the field DPP test and the remaining 13 were not tested with the field DPP test because they were not eligible i.e. they had been vaccinated within the previous 358 days.

Of the 84 sampling events, 71 were subsequently tested using the laboratory DPP test (13 were ineligible as described for the field DPP test) and 84 were tested using the laboratory IGRA test. A total of five animals tested positive in laboratory tests. Four tested positive to the laboratory DPP test only, one tested positive to both the laboratory DPP and IGRA B-A and C.E. Cocktail. The remaining 79 sampling events returned negative results for all laboratory blood tests that were performed.

Table 2. Summary of field and laboratory blood tests from badgers trapped at six Welsh farms in 2020.

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
1	1	17	0	16	0	1	1	12	4[1]	16	2
1	2	9	0	7	2	0	0	7	2	9	0
3	1	3	0	3	0	0	0	3	0	3	0
3	2	3	0	3	0	0	0	3	0	3	1
4	1	20	0	18	2	0	0	16	4	20	1[3]
4	2	7	0	5	1	1	1	7	0	6	0
6	1	1	0	1	0	0	0	1	0	1	0
7	1	6	0	1	5	0	0	5	1	6	0
7	2	2	0	1	1	0	0	2	0	2	0
8	1	18	0	16	2	0	0	2[2]	16	18	1
Total		**86**	**0**	**71**	**13**	**2**	**2**	**58**	**27**	**84**	**5**

[1]Of 16 animals released, 11 had been vaccinated in a previous phase, four animals were vaccinated in this phase and one animal escaped before vaccination could be administered.
[2]Some animals on farm 8 had been vaccinated as part of a private vaccination program. They could be identified by a fur clip. They were not vaccinated again, nor were they tested using the DPP test.
[3]This animal was caught in phase 2 and was removed.

5.3 *Post mortem* examination results

Two badgers were euthanased and underwent PM examination (Table 3). *M. bovis* was isolated from tissue samples from one of those animals. The isolate was subsequently characterised by spoligotyping and whole genome sequencing and was identified as genotype 9:b. 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 six Welsh farms in 2020.

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	1	0	1
4	2	17	1	1	0

6. Costs

The direct cost of the preparation and delivery of the field operation from April to December 2020 totalled £88,710 (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 2020 to March 2021

Activity	Costs (£)
FIELD	
Staff[1]	151,121
Other	3,948
LABORATORY WORK, ANALYSIS AND REPORTING	
Staff	51,459
Other	2,121
TOTAL	208,619

[1]WG contractor costs=£103,231; APHA costs=£47,860

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 has 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

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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 2020

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	4	21/07/2020	NA-LP	NA-LP	NA-LP	NA-LP	N	Y	NEG	NA	NA
1	1	9	20/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	13	20/07/2020	NEG	POS	NEG	NEG	N	Y			
1	1	14	27/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	182	20/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	20	20/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	21	20/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	195	27/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	23	20/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	30	27/07/2020	NEG	POS	NEG	NEG	N	Y			
1	1	223	20/07/2020	NEG	NEG	NEG	NEG	Y	N			
1	1	226	20/07/2020	NEG	NEG	NEG	NEG	Y	N			
1	1	32	27/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	229	27/07/2020	NEG	NEG	NEG	NEG	Y	N			
1	1	36	27/07/2020	NEG	NEG	NEG	NEG	N	Y			
1	1	242	20/07/2020	NEG	NEG	NEG	NEG	N (escaped)	N			
1	1	243	27/07/2020	NEG	NEG	NEG	NEG	Y	N			
1	2	8	06/10/2020	NEG	NEG	NEG	NEG	N	Y			
1	2	14	07/10/2020	NEG	NEG	NEG	NEG	N	Y			
1	2	16	07/10/2020	NEG	NEG	NEG	NEG	N	Y			
1	2	182	06/10/2020	NEG	NEG	NEG	NEG	N	Y			
1	2	195	07/10/2020	NEG	NEG	NEG	NEG	N	Y			

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	223	05/10/2020	RV	RV	NEG	NEG	N	Y			
1	2	228	05/10/2020	NEG	NEG	NEG	NEG	Y	N			
1	2	243	07/10/2020	RV	RV	NEG	NEG	N	Y			
1	2	245	08/10/2020	NEG	NEG	NEG	NEG	Y	N			
3	1	45	06/07/2020	NEG	NEG	NEG	NEG	N	Y			
3	1	46	06/07/2020	NEG	NEG	NEG	NEG	N	Y			
3	1	51	06/07/2020	NEG	NEG	NEG	NEG	N	Y			
3	2	45	21/09/2020	NEG	POS	NEG	NEG	N	Y			
3	2	46	21/09/2020	NEG	NEG	NEG	NEG	N	Y			
3	2	51	21/09/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	57	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	121	24/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	59	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	60	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	62	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	67	23/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	68	23/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	69	24/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	70	25/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	73	22/06/2020	NEG	POS	POS	POS	N	Y			
4	1	77	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	142	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	86	24/06/2020	RV	RV	NEG	NEG	N	Y			
4	1	232	22/06/2020	NEG	NEG	NEG	NEG	Y	N			
4	1	89	24/06/2020	RV	RV	NEG	NEG	N	Y			

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	235	23/06/2020	NEG	NEG	NEG	NEG	Y	N			
4	1	238	22/06/2020	NEG	NEG	NEG	NEG	Y	N			
4	1	239	23/06/2020	NEG	NEG	NEG	NEG	Y	N			
4	1	71	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	1	76	22/06/2020	NEG	NEG	NEG	NEG	N	Y			
4	2	59	08/09/2020	NEG	NEG	NEG	NEG	N	Y			
4	2	60	07/09/2020	NEG	NEG	NEG	NEG	N	Y			
4	2	62	08/09/2020	NEG	NEG	NEG	NEG	N	Y			
4	2	73	07/09/2020	NA-LP	NA-LP	NA-LP	NA-LP	N	Y	POS	9	9:b
4	2	235	07/09/2020	RV	RV	NEG	NEG	N	Y			
4	2	71	08/09/2020	NEG	NEG	NEG	NEG	N	Y			
4	2	76	07/09/2020	NEG	NEG	NEG	NEG	N	Y			
6	1	99	03/08/2020	NEG	NEG	NEG	NEG	N	Y			
7	1	108	15/06/2020	RV	RV	NEG	NEG	N	Y			
7	1	109	15/06/2020	RV	RV	NEG	NEG	N	Y			
7	1	111	16/06/2020	RV	RV	NEG	NEG	N	Y			
7	1	114	15/06/2020	RV	RV	NEG	NEG	N	Y			
7	1	115	15/06/2020	RV	RV	POS[1]	NEG	N	Y			
7	1	234	15/06/2020	NEG	NEG	NEG	NEG	Y	N			
7	2	109	01/09/2020	NEG	NEG	NEG	NEG	N	Y			
7	2	234	01/09/2020	RV	RV	NEG	NEG	N	Y			
8	1	216	14/09/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	217	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	218	22/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	219	19/10/2020	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
8	1	220	21/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	221	19/10/2020	NEG	POS	NEG	NEG	Y	N			
8	1	222	21/10/2020	RV	RV	NEG	NEG	N	Y			
8	1	224	19/10/2020	RV	RV	NEG	NEG	N	Y			
8	1	225	22/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	227	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	230	14/09/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	231	21/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	233	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	236	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	237	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	240	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	241	19/10/2020	NEG	NEG	NEG	NEG	Y	N			
8	1	244	14/09/2020	NEG	NEG	NEG	NEG	Y	N			

[1]This animal was positive to the IGRA B-A test and negative to the C. E. Cocktail. This indicates that the badger was not infected with *M. bovis* and had been vaccinated.

Abbreviations:

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.
RV = The DPP test was not conducted because the animals was vaccinated within the previous 358 days.

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 are NA.