Title: Field Trial to Assess the Safety of Bacille Calmette Guerin (BCG) Vaccine Administered Parenterally to Badgers

Study Protocol Number: VLAS/05/036

Study Report: Final Audited

Study Report Period: June 2006 – June 2010

Contents

Introduction

The field trial as described in Study Protocol VLAS/05/036 (Field Trial to Assess the Safety and Efficacy of Bacille Calmette Guerin (BCG) Vaccine Administered Parenterally to Badgers) had two primary objectives: a) to confirm safety in, and absence of shedding from, badgers of a commercial Bacille Calmette Guerin (BCG) vaccine when given parenterally to badgers in the field and b) to investigate the immunogenicity and efficacy of BCG in badgers. This is the final report and concerns both the safety and efficacy aspects of the field trial.

Quality Statement

This study was conducted according to the principles of Good Clinical Practice (GCP) described in VICH GL9 (GCP) guidelines implemented June 2000. This report provides a correct and faithful record of the results obtained. A final audit of this report will be completed by [redacted] and will be available in the study master file.

Signed:

[redacted], Study Investigator

Date: 21 June 2010

[redacted], Deputy Study Investigator

Date: 21 June 2010

Organisation of Study:

Study Investigator: [redacted]

Deputy Study Investigator: [redacted]. [redacted]

Field Supervisor: [redacted], Food and Environment Research Agency (Fera)

Named Veterinary Surgeon: [redacted], VLA

Veterinary Surgeons:
[redacted], VLA
[redacted], Fera

Study Monitor: [redacted], Veterinary Vaccines Consultancy

Study Auditor: [redacted], Clinical Research & Communication Ltd.

Statistical Auditor: [redacted], FIS (Fellow of the Institute of Statisticians)

TB Vaccine Study Data Group (TBVSDG) chaired by [redacted], with the following terms of reference:

To provide independent advice and guidance to the Study Sponsor, the Study Investigator and participating parties on the collection, management and analysis of data associated with the assessment of the safety and efficacy of the BCG badger vaccination field trial.

The Group will consist of key representatives from Defra, CSL[1], VLA and other organisations required to participate in the study, and other parties deemed by the Study Sponsor and Investigator to have a direct interest in the implementation of the study.

The Group will report through the Badger Vaccine Group. The Group will meet periodically during the course of the field study and its completion. It will monitor the collection and storage of data, ensure the quality of all aspects of data management, agree suitable methods of analysis, identify adequate resourcing and agree appropriate parties to undertake these tasks. It will also provide a focus for the interpretation and discussion of results arising from the data and statistical analysis.

Details of people directly involved in the study are in Appendix 1: BVS Organograms Versions 1 to 6.doc

Study Area: In Gloucestershire between 402000 in the east and 390000 in the west, 215000 in the north and 199000 in the south

Sampling facility: C/o Defra, [redacted]

Study Phase Dates: June 2006 – March 2010
Animal Test Certificate Number: Vm25830/0001
Home Office Project Licence Number: PPL 70/6415

[1] Central Science Laboratory (CSL) formed part of The Food and Environment Research Agency (Fera) on 1 April 2009.

Natural England Licence Numbers: 20061562 (from 11 Jun 06 to 29 Nov 06)
20063034 (from 30 Nov 06 to 17 Jul 07)
20072203 (from 18 Jul 07 to 6 Jun 08)
20081938 (from 7 Jun 08 to end of study)

REPORT DISTRIBUTION

Name	Location
[redacted]	Veterinary Medicines Directorate

1. Summary:

The wild badger population used in this field trial is situated in Gloucestershire and covers an area of approximately 55 km^2. Written agreement from the landowners and occupiers within the study area was sought to allow field staff access to their land. The area of signed up land for which landowner consent had been given was initially surveyed during November 2005 to February 2006 by Central Science Laboratory (CSL became part of the newly formed Food and Environment Research Agency (Fera) in April 2009) who identified 110 badger setts. Subsequent bait marking at active setts allowed Fera to determine the spatial configuration of the territories and 63 social groups were identified. The area was split into three zones for practical purposes, identified as X, Y, Z and the active badger setts in each zone were normally trapped over the course of four days, each individual sett being subjected to trapping for two consecutive nights. There were two exceptions. In 2007, the September to October trapping session was cancelled due to a Foot and Mouth outbreak. In 2008, the overnight Tuesday/Wednesday (8^{th}/9^{th} July) trapping was cancelled for animal welfare reasons as a result of the weather being very wet and windy.

As TB in badger populations is spatially and temporally aggregated (Delahay et al. 2000b), in order to account for the clustering of infection, allocation to treatment and control was conducted at a social group level with the study having a cluster-randomised design. Power analysis showed the optimum replication of treatment to control to be between 60:40 and 70:30. Allocation of social groups to treatment was carried out after the first trapping session June – July 2006 using the immunological results, culture results and group size data to randomly assign the social groups to vaccinate or control according to the 60:40 ratio while ensuring, as far as possible, approximate balance of social group sizes and TB test results between vaccinated and control social groups.

The trapping schedule for the study area was as follows:

T1 First trapping session June to July 2006
T2 Second trapping session September to November 2006
T3 Third trapping session (2 weeks after T2) September to November 2006
T4 Fourth trapping session June to July 2007
T5 Fifth trapping session September to November 2007 cancelled
T6 Sixth trapping session June to July 2008
T7 Seventh trapping session September to October 2008
T8 Eight trapping session June to July 2009
T9 Ninth trapping session September to October 2009

Badgers were transported to a Defra-owned secure site where there was a purpose built sampling facility and laboratories to process blood samples and vaccine. Badgers caught on the first day were released at the site of capture when the traps were checked on the second day and those caught on the second day were returned that afternoon. The exception was when vaccination was carried out in T2 and T3 when those badgers trapped on day 4 of the week were held overnight for temperature monitoring and observation over a 24-hour period for the purpose of gathering data on vaccine safety.

Badgers were anaesthetised, identification checked using a database developed by Centre for Epidemiology & Risk Analysis (CERA) at VLA specifically for this study, and if newly enrolled in the study identified by a unique three-digit number by microchip and tattoo. They were then examined and sampled and if allocated to a vaccine group, vaccinated with BCG Danish intramuscularly into either the left or right lumbar muscle. If allocated to a control group they were left untreated.
Clinical samples collected included tracheal aspirate, urine and faeces. If any badger had a wound or other discharge swabs were also taken. All clinical samples were submitted for

culture of *Mycobacterium bovis* at VLA Weybridge. All clinical samples from vaccinates and a selected number from control badgers to maintain blinding of the laboratory staff were sown onto selective media to culture BCG.

Blood taken for immunological testing was processed in the laboratory on site and then sent to VLA Langford Regional Laboratory or Weybridge Laboratory to be tested for interferon-gamma (IFNγ) and antibodies (Brock TB Stat-Pak Test). Results were entered onto an immunology database developed by CERA at VLA specifically for this study.

The primary objective of the trial in the first year was to assess the safety of BCG vaccination in wild badgers. Safety data continued to be collected 2007 to 2009.

Initial safety data were reported in the BVS Safety Interim Report dated 1 December 2008 (Appendix 2) and included:

1. Monitoring the body temperature by recording microchip temperatures over 24 hours post-vaccination (1, 2, 4, 6, 8, and 24 hours). Data were collected from 57 badgers. The range of temperatures recorded was between 30.2 to 40.5 ºC.

2. Two weeks later body temperatures and local reactions were measured and recorded for all re-trapped badgers. Data were collected from 48 badgers that had been vaccinated two weeks previously. Of these 5 had small intramuscular swellings and none exhibited skin ulceration.

3. The absence of shedding of BCG from vaccinated animals. All clinical samples collected were submitted for culture. 178 clinical samples were submitted from 48 badgers trapped 2 weeks after vaccinating. No BCG was cultured, with 148 samples negative and 30 samples with no result due to contamination.

4. Any badgers found dead in the study area were submitted for *post mortem* examination including examination for any local reaction at site of vaccination. At the time of the Interim Report (December 2008), 9 vaccinated badgers had been submitted: 5 with no lesions; 1 with no result as carcass autolysis was too advanced; and 3 results pending; the final results to be reported at the end of the study (now completed - see Table 1 below).

The safety data collected during the study period included:

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1. Re-examination of all vaccination sites by a veterinary surgeon. Data were collected from 265 badgers of which 22 had intramuscular swellings but none exhibited skin ulceration.

2. The absence of shedding of BCG from vaccinated badgers; 3657 clinical samples were submitted for BCG culture and no BCG was cultured.

3. *Post mortem* results of road traffic accident/found dead badgers; There were 90 badgers submitted of which 33 had been enrolled in the study, no lesions were found at the site of vaccination.

Table 1: Summary of the study totals*

Badgers trapped and identified	844
Badgers trapped and sampled (including re-traps)	1787
Badgers vaccinated (including revaccinations)	785
Re-trapped badgers and vaccinated sites re-examined	265
Badgers with intramuscular swellings at vaccination site	22
Found dead badgers enrolled in the study and submitted for *post mortem*	33
Found dead vaccinated badgers submitted for *post mortem*	18
Found dead vaccinated badgers not suitable for *post mortem*	1
Lesions found in lumbar muscle at *post mortem*	0
BCG cultured from tissue samples harvested at *post mortem*	1
BCG cultured from clinical samples	0

* **2006**: T1, T2 and T3, **2007**: T4, T5 cancelled, **2008**: T6, T7, **2009**: T8, T9

The number of adverse events resulting from injecting BCG via the intramuscular route at a dose of 2-8 x 10^6 CFU were minimal and not serious so it can be concluded that BCG is safe to use in the field.

The secondary objective of the study was to investigate the immunogenicity and efficacy of BCG in badgers. As described in N1KT Vaccination Trials – Exploration of Statistical Methods V1 (Appendix 3) the range of statistical methods to estimate the efficacy of badger TB vaccination was explored by Fera statisticians using simulated data and it was concluded that a generalised linear model (GLM) using the proportion of badgers with new TB incidence within a social group was the preferred method.

Interim (blinded) analysis was carried out after 2008 data had been collected and is reported in N1KT Badger TB Vaccine Field Trial (Interim Stats Analysis – A&B_V1) in Appendix 4. The three tests used for the analysis were IFNγ enzyme immunoassay (EIA), Brock TB Stat-Pak and *M. bovis* culture result from clinical samples.

Table 2: Study test totals of IFNγ EIA, Brock TB Stat-Pak, *M. bovis* and BCG culture

IFNγ EIA	1787
Brock TB Stat-Pak	1777
Number of clinical samples submitted for *M. bovis* culture	4852
Number of clinical samples submitted for BCG culture	3657

Vaccine efficacy in the context of BCG vaccination of badgers may be defined either as a reduction in the incidence of uninfected badgers becoming infected with *M. bovis* or a reduction in the progression/severity of TB in badgers that do. The effect of vaccination is measured with reference to a non-vaccinated control group. According to this definition it was not possible to estimate the efficacy of BCG vaccination in this study as the decision was taken not to subject study badgers to post-mortem determination of infection. However, it was possible to use the tests employed in this study (IFNγ EIA, Stat-Pak, culture) in live animals as surrogate measures of vaccine efficacy. Change of status from initial negative to

positive test incidence was measured in the two treatment groups and analysed for statistically significant differences.

The primary measure of efficacy stated in the Study Protocol was the difference between treatments in the proportion of cases of new incidence in positive outcome to any of the three tests (as the most sensitive indicator of TB available for the live animal). By this analysis there was no evidence of a statistically significant treatment effect ($P > 0.05$) although there was a reduction in incidence in the BCG vaccinated group to 31.1% cases (95% confidence interval: [22.7%, 41.0%]) from 41.5% cases (95% confidence interval: [28.0%, 56.3%]) of new incidence in control.

Possible differences between treatments in the proportion of cases of new incidence of positive outcome of each of the three tests separately were also investigated. In addition, Stat-Pak and culture were analysed together. For inclusion into this first analysis each animal had to be negative for the test(s) of interest at T1 and at the time of first capture/vaccination. However, each animal did not have to be negative to all tests to be excluded from the analysis. According to these selection criteria, this first analysis revealed no significant effect of vaccination ($P > 0.05$) for each of the tests either singly or in combination. However, in all cases there was a reduction of cases in the vaccine group compared to the control group.

Subsequent analysis addressed more directly the prophylactic (rather than therapeutic) effect of BCG vaccination by excluding from the analysis any badger positive by any of the three tests at T1 or at the time of first capture/vaccination (T2 onwards). By applying these exclusion criteria, the effect of vaccination was measured in badgers considered to be free of TB by virtue of negative results in all three tests. Analysis of the IFNγ EIA test alone still provided no conclusive ($P > 0.05$) evidence that BCG vaccination was able to prevent infection with *M. bovis*, although the trend was in that direction. In contrast, vaccination was found to have a significant effect on reducing the incidence of positivity for both Stat-Pak ($P < 0.001$) and Stat-Pak and culture combined ($P = 0.008$). Given the limitations of the study size and predicted power this was an unexpectedly clear result and is consistent with BCG vaccination of uninfected badgers reducing the progression/severity of TB in badgers.

2. Materials and methods

Appendix 5: Standard Operating Procedures List
Appendix 6: Forms List
Appendix 7: Functions and Standard Operating Lists

2.1 Materials

BCG Vaccine SSI was supplied by the Statens Serum Institut (SSI) Denmark. The vaccine, comprising an attenuated strain of *Mycobacterium bovis* (BCG), contains (according to the manufacturer's datasheet) 2.0 - 8.0 x 10^6 CFU BCG Danish strain 1331 when reconstituted in 1ml Sauton diluent, supplied with the vaccine. It is licensed for administration to humans in the UK and many other countries.

Table 3: [redacted]

[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			

The vaccine is presented in freeze-dried form in glass vials and was received by the TB Research Group at [redacted] VLA before being transported to the [redacted] facility, apart from one delivery which was sent directly to [redacted]. Upon delivery to [redacted] or [redacted] the packaging was inspected. The temperature of the thermo log (temperature recording device placed in shipment container), was noted to ensure the correct temperature was maintained throughout transport. The contents of each box of vaccine and diluent were checked and each vaccine vial was relabelled with a VLA label RP1 (stating the batch number, expiry date and Animal Test Certificate number. Each box was labelled with a VLA RP6 label. Examples of RP1 and RP6 are shown in Appendix 8. The vaccine and diluent were then stored in temperature monitored coldstores/fridges between + 2 ºC and + 8 ºC as stated in the manufacturers datasheet. Vaccine and diluent were transported from VLA to [redacted] in a portable electric cool box and temperature monitored during transit. Following a cold store breakdown containing [redacted] expiry Date [redacted] and diluent [redacted] expiry date [redacted] subsequent deliveries were separated into two locations at [redacted].

Temperatures of cold stores and fridges are monitored by a Building Management System (BMS) in Scientific Services Unit and recorded in Microbiology file MD508 for [redacted] and Scientific Services Unit registered log book SSM 0061 for [redacted]. The [redacted] has a separate BMS that monitors and records temperatures of critical equipment with log books being maintained by the building manager in the SEB administration team. At [redacted] all temperature critical equipment including the dedicated vaccine fridge were connected to a Comark IceSpy system which recorded temperature readings electronically. Temperature readings were regularly downloaded and printed then placed on file BVS 9087 "[redacted] Comark Temperature Printouts". In addition the [redacted] system was connected, via a modem to a telephone line. If any of the critical temperature equipment went out of range an alarm was sent to a 24 hour monitored mobile phone held by the Study Investigator. The vaccine fridge remained within + 2 ºC and + 8 ºC whilst it contained vaccine and diluent used in the study.

All details regarding fate of boxes and individual vials can be found in Vaccine tracking form file BVS/9017 and Vaccine log book SEB 1736.

[redacted]

Table 4: [redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

2.1.1 Preparation of Vaccine

At VLA [redacted] a number of vials from all the batches used in the field study were reconstituted and immediately sown onto modified Middlebrook 7H11 plates as per SOP BAC0108 TB: Titrating BCG vaccine.

At the [redacted] facility vaccine was reconstituted prior to inoculation into the badgers according to SOP BAC0094: Administration of BCG Vaccine to Badgers. Each vial was reconstituted in 1ml of Sauton diluent and mixed by gently inverting the vial. The in use shelf-life of reconstituted vaccine is 4 hours and this was not exceeded.

Following SOP BAC0108: Titrating BCG for Badger Vaccine Study, samples of residual vaccine from between 2-5 vials were cultured for viable counts as well as a vial reconstituted at the beginning of the day, not used for vaccination, to confirm the titre of the vaccine. These titrations were carried out within 4 hours of the first vial being reconstituted except for one which was carried out 4 hours and 5 minutes later.

2.2 Methods

2.2.1 Social Group Identification

Initial surveying of the study area was carried out from November 2005 to February 2006 following Standard Operating Procedure CSLWP026 - Surveying for badger activity (Badger vaccine study only) and then each December to January period for the duration of the study.

The purposes of surveying for badger activity are as follows:

- To identify and record setts and other signs of badger activity
- To provide data to inform the delineation of badger social group territories
- To finalise the boundaries of study areas
- To identify areas where traps may be sited
- To update data held on the database as necessary

The principal aim of surveying was to provide information on badger distribution and density in the study area. Individual survey data including the map reference location of all setts (active and inactive), latrines, badger runs and other mammal burrows were recorded on form BVS 2 Badger Sett Survey and mapped onto a 1:10,000 map for the study area. The presence of other mammal burrows such as rabbit warrens or fox earths was recorded as they may be enlarged and used by badgers at a later date. Other relevant data recorded included sett activity scores (number of well used, partially used and disused holes) and presence of latrines on or near the sett.

The bait marking method (described in detail in Delahay et al., 2000a) is a method of determining the territorial organisation of badger populations. This is achieved by feeding palatable bait (peanuts and syrup) laced with an indigestible plastic marker at badger setts. A different colour/type of plastic marker is fed at each sett and the subsequent distribution of markers in badger latrines indicates where territorial boundaries lie. Bait marking was conducted during February, March and April when territorial activity is considered to be at its peak, following Standard Operating procedure CSL WP017 – Bait Marking (Badger Vaccine Only). Social group boundaries were delineated using the method described in Delahay *et al*. (2000a). The master bait marking map was used in the process and Fera staff worked systematically through the master map using the following three main criteria to determine the presence of discrete groups:

1. ***Presence of shared boundary latrines***. Determined by returns from two setts being found in latrines between each sett. This is the least subjective method for determining discrete social groups.

2. ***Field signs such as boundary runs (well-used badger paths occurring between adjacent setts***). These were used to help determine whether two adjacent setts represented discrete social groups in the absence of shared boundary latrines.

3. ***Distance between the outermost returns from one sett and the outermost returns from an adjacent sett***. When there were no shared boundary latrines and no boundary runs identified, the distance between the outermost returns of two adjacent setts was used to help determine whether they represented discrete social groups. In some cases it may also be possible to determine that a sett that was not included in the bait marking survey represents a discrete social group due to a combination of the following factors: 1) distance from the sett to the outermost returns of adjacent setts; 2) activity at the sett that was not included; 3) activity at adjacent setts.

The following criterion was added 2007 onwards.

4. ***Levels of activity at a fed sett***. If a social group could not be determined by any of the above three criteria, the level of activity at a fed sett, determined during sett checks prior to feeding, was used to indicate whether a sett represented a discrete social group. Indicators of activity were based on the presence and number of active holes and the presence of latrines around the main sett.

2.2.2. Treatment Allocation

Allocation of social groups to treatment (vaccinate or control) in year one was carried out after the first trapping session in June and July 2006, employing stratified randomisation of social groups using their immunological and culture results together with group size data to assign the treatments. The original intention was to allocate treatments according to a 50:50 ratio, but following agreement at a TBVSDG Meeting on 5th October 2006 the vaccine:control ratio was changed to 60:40. This is documented in Study Protocol Amendment 2. The change was made following consideration of the distribution of the numbers of badgers found positive in social groups identified for the treatment allocation process. Analyses at CSL (Fera) showed that precision and power of the study were best over a range of allocations between 60:40 and 70:30 and the adjustment would provide protection against the risk of ending up with too few vaccinated animals due to difficulties with field work such as low trapping efficiency.

The vaccine allocation protocol, including the randomisation procedure, was carried out by a Fera statistician (the only Fera staff member not blinded to laboratory results). The aim of the randomisation at baseline was to allocate vaccine:control as close to, but not exceeding a ratio of 60:40. Randomisation was stratified according to: (i) estimated group size (i.e. the number of animals trapped at baseline); and (ii) estimated group prevalence (considering both the number of IFNγ positive and culture positive results). These were considered to be major sources of variation *a priori.* Before randomisation, the 56 groups for which information was available were allocated to one of 12 sub-groups (see Table 5) based on the two stratification measures above. The groups were sampled at random (initially 50:50) from each of these sub-groupings. This resulted in an allocation of 28 vaccinates and 28 controls. The allocation of groups was accepted if it met the following criteria: (i) the number of animals allocated to the vaccine group was between 45% and 55% of the total population; (ii) the number of groups with at least one culture positive result was evenly split (i.e. 7:7 as there were 14 such groups identified), and (iii) the difference in occurrence of IFNγ animals in the two groups was not greater than 5%. If these criteria were not met, the procedure was repeated as many times as was necessary to achieve a satisfactory allocation.

In order to get close to the desired ratio of 60:40 it was necessary to then re-allocate five groups from control to vaccine. This was done by ranking the initial 28 controls into four sub-groups based on IFNγpositive results and sampling five groups such that all of these four sub-groups were represented at least once. Of the remaining seven groups for which no information was available (due to no animals being caught in these groups during the initial trapping operation), four were randomly allocated to vaccine and three to control. In total, 37 groups were allocated to vaccine and 26 to control.

Table 5: Sub-groups of the 56 social groups where badgers were trapped in June or July 2006, stratified by estimated IFNγ prevalence and estimated group size

Size Category	% group γ-prevalence values (total number of social groups in brackets)	Number allocated to control (total number of groups in brackets)
Low (1-3 animals)	0(10)	5(10)
Low	33(2), 50(3)	3(5)
Low	100(12)	6(12)
Low Medium (4-5 animals)	0(4)	2(4)
Low Medium	25(2), 40(1)	1(3)
Low Medium	75(2)	1(2)
High Medium (6-11 animals)	0(5)	3(5)
High Medium	10(1), 11(1), 14(1), 17(2), 29(1), 38(1)	3(7)
High Medium	50(1), 60(1)	1(2)
High Medium	67(1), 100(1)	1(2)
High (12-17 animals)	18(1), 21(1)	1(2)
High	31(1), 47(1)	1(2)

In years two (2007) and four (2009) a modified protocol was followed to account for additional groups identified during bait marking of that year, but for which there was no information on test positive animals or group size. Because the prevalence of test positive animals appeared to be aggregated and differed between the three trapping zones, trap zone was used as a crude proxy for disease prevalence, and recorded activity levels at setts were used as a proxy for group size. In year three (2008) all new groups identified during the Spring bait marking were allocated to controls in order to attempt to maintain the desired 60:40 ratio, as agreed with the TBVSDG and Defra. This decision is documented in Study Protocol Amendment 8.

2.2.3. Trapping and Transportation

Badgers were trapped in cages located around the entrances to the setts, then transferred in the field into holding cages, which had an identifying strip of tape with the sett name written on it. They were then transported to the sampling facility according to SOP CSL WP001 in vehicles customised with built-in ventilation. Flexible screens were placed in the vehicles to segregate holding cages containing badgers from different social groups. All vehicles, cages and screens were cleaned and disinfected according to SOP CSL WP005.

On arrival at the sampling facility the badgers were weighed in their cages and the social group and weight written on the identifying strip of tape with the sett name. They were then arranged on holding racks in the holding area according to social group (SOP CSLWP001) and left in the

darkened holding room to settle down. At the start of the sampling session badgers were inspected by a veterinary surgeon then sequentially anaesthetised by intra-muscular injection (SOP CSL WP003) using a volume appropriate for the weight of the badger, estimated from the weight supplied on the cage.

2.2.4 Examination, Sampling and Vaccination

All animal procedures in this Study were covered under the Animals (Scientific Procedures) Act 1986, by Home Office Project Licence Number PPL 70/6415.

Examination

Once anaesthetised, each new badger was identified by inserting a programmed microchip, which has a unique three-digit number in the format of BVS -VNNN. The calibrated microchip (manufactured and supplied by Plexx BV, Einsteinweg 13A, 6662 PW ELST, The Netherlands) was placed subcutaneously between the shoulders (SOP BAC 0095). The microchip, which also measures temperature, was read using a Plexx reader. The ID and measured temperature were recorded on the Data Capture Form (BA596). For badgers trapped on the fourth day of T2 and T3 a Temperature Monitoring form (BA599) was started. Adhesive labels with the unique alphanumeric number, the date and sample identifiers were printed from a purpose written database for use on the Data Capture Form (BA596) and sample containers by the recorder of data throughout the examination, sampling and vaccination procedure (SOP BAC0102).

Badgers were then weighed and their length measured before being moved into the sampling room. On arrival in the sampling room they were placed on the tattooing table, the microchip number was checked again using a Plexx reader, and the digits from the unique alphanumeric identifier was tattooed on the abdomen (SOP BAC0105).
If the badger was allocated to a vaccinate group, a horizontal line was tattooed above the first digit to indicate vaccination in 2006, above second digit for 2007, above third digit for 2008 and under first digit for 2009.

The badgers were then moved onto a sampling table and a sampler carried out a clinical examination following SOP BAC0102 and appendices with the following data recorded on the Data Capture Form (BA596) by a recorder (a person different from the sampler in each case):

1. Rectal temperature using an electronic thermometer
2. Age
3. Tooth wear
4. Sex
5. Reproductive status
6. Body condition
7. Eye condition
8. State of mucous membranes
9. Any wounds or swellings and whether wounds attributable to trap injuries
10. Any fractures

Sampling

Samples were then taken following SOP BAC0104 and identified using the pre-printed unique adhesive labels.

1. Blood taken into vacutainer tubes of heparin and SST. The volume permitted to be taken according to weight and sampling interval is listed in tables in Appendix 1 of SOP BAC0104. The samples were then transferred to the [redacted] for processing.

2. Tracheal Aspirate using a catheter to collect a sputum sample. The sputum sample was then flushed into a tube containing culture medium and the catheter cut three times and left in the sample tube.

3. Urine collected by manual expression or catheter.

4. Wounds/other discharges swabs taken, or if encapsulated abscess, needle aspirate undertaken.

5. Faeces: a Microlax enema was administered at the end of the procedure and samples collected later in the holding area from the holding rack under the badger.

All clinical samples were transferred to the laboratory at the sampling facility for packing then forwarded to [redacted] VLA ([redacted]) by courier or a staff member.

Vaccination

If the badger was allocated to be vaccinated, vaccination was carried out according to SOP BAC0094: Administration of BCG Vaccine to Badgers, by one of three veterinary surgeons. For first time vaccinates a shaved area approximately 5x5 cm over the left lumbar muscle was cleaned with sterile water and a digital photograph taken of the exposed skin. Intramuscular injection of the BCG vaccine was then carried out. For those badgers vaccinated previously, the skin above the lumbar muscle used (i.e. left or right or both) was shaved, examined by a veterinary surgeon, cleaned, a digital photograph taken and intramuscular injection of BCG carried out. The left lumbar muscle was used unless an intramuscular swelling was found there on examination, in which case the right lumbar muscle was used. Only one badger BVS-V393 had swellings in both lumbar muscles. In this case, a veterinary decision was made to inject vaccine into the right lumbar muscle to the right of the palpated swelling.

2.2.5 Monitoring and examination of vaccination site.

All badgers in the holding area were monitored when they were being anaesthetised and during recovery by the Named Veterinary Surgeon or the Study Investigator. In the interests of animal welfare, the holding area has climate control to regulate the temperature, and water and Lectade were provided for badgers to drink after recovery and overnight. Infra red lamps were used as required and under direction of a veterinary surgeon during recovery.

Body Temperature Monitoring

On return to the holding area all badgers trapped on day 4 in trapping sessions T2 and T3 had their body temperatures taken by a nominated staff member using the Plexx reader following SOP BAC0095 and the data recorded on Temperature Monitoring Form (BA599) by a different nominated staff member according to SOP BAC0102.
The time and microchip temperature were recorded on the Temperature Monitoring form in 24 hour format as follows:

+1hr, +2hrs, +4hrs, +6hrs, +8hrs, and +24hrs after the time of first examination (for control unvaccinated badgers) or after the time of vaccination (for the vaccinated badgers). Temperatures were then taken at the appropriate time.

As well as the body temperature being recorded any clinical observations were noted.

Re-examination of vaccination site

All vaccinated badgers re-trapped in each trapping session had their vaccination site re-examined by a veterinary surgeon following SOP BAC0102: Recording data on the Badger Form BA595, with the previously shaved area over the left and/or right lumbar muscle being shaved again prior to examination. A digital photograph of all vaccination sites was taken. Examination of the skin aspect and palpation of the lumbar muscle was carried out and any observations noted. When any swelling was palpated this was measured and recorded using a digital calliper and a digital photograph.

2.2.6 Found Dead Badger Examination

Any reports of dead badgers within the study area received by Fera field staff were investigated and all badgers found were collected and taken to VLA [redacted] Regional Laboratory for *post mortem* examination following the standard operating procedure VISI 224: PM RTA Badgers. All badgers that died or were euthanized on welfare grounds after being anaesthetised for sampling were also submitted to [redacted].
Tissue samples including lymph nodes, any suspect TB lesion found in other tissues and bite wound samples were collected and cultured following the standard operating procedure BA030.A: Mycobacteria Isolation from Badger Tissues and deviation STX D0001/06.

Post mortem examination also included examination of lumbar muscles of vaccinated badgers and tissues samples taken from any lesion and sent for histopathology examination.

2.2.7 Immunological testing

The two immunology tests carried out and reported in this report were:

1. Badger interferon-gamma (IFNγ) enzyme immunoassay (EIA): an assay based on measuring cell-mediated immunity (CMI) to *M. bovis* infection in whole-blood samples.
2. Brock TB Stat-Pak (Chembio Diagnostic Systems Inc.): a lateral-flow test designed to detect serum antibodies to *M. bovis.*

Blood taken into vacutainer tubes of heparin and SST were processed on the same day as collection in the fully equipped laboratory located at the sampling facility. SOP BAC0156: Use of multiple antigens to produce supernatants and cells for the badger IFNγ EIA were followed for blood collected in heparin tubes. Blood collected in the SST tubes was processed following SOP BAC0109: TB Separation and Storage of Badger Serum from SST Tubes, to provide aliquots of serum for using in the Brock TB Stat-Pak test.

The supernatants produced by SOP BAC0156 were used to test for badger IFNγ following SOP BAC0251: TB Enzyme Immunoassay of badger IFN-gamma. Testing was carried out at the VLA [redacted]. Quantitative data from the IFNγ EIA (in the form of optical density units) were converted into binary data (pos/neg result) on the basis of a test cut-off determined during the development of the test and reported in Dalley et al., 2008. Guidelines regarding the criteria for test validity and test interpretation were prepared and are presented in Appendix 9: Test Validity Criteria and Test Interpretation Guidelines into the badger IFN-γ ELISA SOP BAC0251.

SOP SE.180: Lateral flow (Stat-Pak) serology test for TB was followed using serum harvested to provide the serology results with the testing being carried out at [redacted] Regional Laboratory.

2.2.8 Bacteriology

The clinical samples collected and forwarded to VLA [redacted] ([redacted]) were sown onto selective medium following SOP BAC0096: TB Processing and culturing badger clinical samples for the vaccine study, in order to establish whether *M. bovis* and/or BCG were being excreted. The detection of *M. bovis* in clinical samples was pathognomonic for TB, whereas the detection of BCG in clinical samples was required to assess the safety of the vaccine. Since BCG is derived from *M. bovis* and both bacteria could be present in the same clinical sample, a culture method using cycloserine was deployed. At the correct concentration cycloserine inhibits the growth of *M. bovis,* but permits growth of BCG. Formal differentiation between the two bacteria was achieved using spoligotyping and variable number tandem repeat (VNTR) molecular typing methods on colonies growing on plates containing cycloserine, according to SOPs CBU 0245: Spoligotyping, BAC 0046: VNTR Fragment Generation and BAC0084: VNTR Running Polyacrylamide Gels.

The overall culture result assigned to a badger at any one sampling point was based on the following rules:

- Positive: Any sample positive for *Mycobacterium bovis*
- Negative: Minimum of one sample negative with no positive samples for *Mycobacterium bovis*
- No result: All samples contaminated

3. Statistical methods

3.1 Design Principles

At the outset of the design process, a comprehensive literature search of approaches taken for vaccine evaluation in clinical science was carried out and relevant papers reviewed including EMEA/CVMP Note for Guidance 852/99 - FINAL “Field Trials with Veterinary Vaccines” and the EMEA/CVMP Guideline 816/00 – FINAL “Guideline on Statistical Principles for Veterinary Clinical Trials”. Consideration was given to the particular ecology and behaviour of badgers, which are social animals living in defined territories with much higher contact rates within than between groups. Past observations at Fera’s badger study site, Woodchester Park, show social groups persisting over decades with characteristic levels of TB prevalence; often high prevalence next to low prevalence persisting over time implying substantially lower rates of transmission between than within groups. Thus, the chosen cluster-randomised design was the most appropriate, with stratification to ensure approximate balance of TB prevalence levels between control and vaccinated groups and even distribution of social group size. This design is appropriate for both the primary purpose of confirming field safety and the secondary exploration of efficacy in the field.

3.2 Power Analysis

To determine the optimum allocation of treatment and control for both primary and secondary purposes within the achievable and affordable total sample size, it was important to undertake a power analysis. For the evaluation of field efficacy, it was proposed to use a battery of three tests: IFNγ EIA, Brock TB Stat-Pak serology, and culture of clinical samples. The test results would be presented as binary (0/1) data. All three tests have different levels of sensitivity and specificity (see section 4.7).The proposed primary variable for the assessment of efficacy was the combination (positive for any) of all 3 tests, as this was postulated to be the most sensitive measure of any likely prophylactic effect of vaccine. The resulting binary data would not be normally distributed, so a simple power analysis based on assumed normality was invalid. Furthermore, the study was to be a multi-year trial on a wild species under field conditions with correspondingly less control over recruitment and follow-up than in a conventional veterinary clinical trial on domesticated animals. The complexity of this situation meant that a simulation approach had to be adopted, taking into account the ecology of badgers, the known epidemiology of TB in badgers, trapping efficiency, and the levels of sensitivity and specificity of the proposed tests. The size of the field study was also limited by cost and logistic considerations. A team at CSL/Fera used an approach based on the Receiver Operating Curves of the various interferon and serology tests under consideration but did not consider intra-group clustering or go beyond one year of vaccination (300 badgers in 1 population for 1 year). A second team at the University of Newcastle used an existing validated simulation model of the epidemiology of TB in badgers, which used information on social groups and differential between and within group disease transmission (500 badgers in 50 social groups for 2 years + 1 observation only year). This model was later developed further for the evaluation of methods for statistical analysis and options for continuation of the study into additional years. A full description of the model is at Appendix 10: Newcastle Simulation model description. These approaches were informed by the best available information on key parameters from literature and in-house research. The CSL/Fera approach predicted that power would be low for this sample size but was not able to estimate overall power for the multi-year study. The Newcastle approach provided estimates of power between 50% and 80% under a range of design options; it also clearly demonstrated a substantial (10-20%) increase in power from use of the cluster-randomised approach. Both approaches demonstrated that optimum power would be expected with more than 50% of animals / social groups allocated to the vaccination treatment. Reports of the two power studies are in Appendix 11, [redacted], Fera, August 2005: Assignment of optimum proportion of subjects to treatment group in order to maximise efficacy, Appendix 12, Roy Macarthur, Fera, April 2006: Examination of effect of combination of TB tests, and Appendix 13: Immunisation methods. The final choice of a ratio of 60% vaccinated to 40% control was made by the TBVSDG taking into consideration the results of the power analyses, the ecology and behaviour of badgers, and the field logistics. This allocation was considered to give the best all round compromise and also to satisfy the power requirements for the safety evaluation, which was the primary purpose of the study. With hindsight, it can be seen that the design chosen was robust and has performed much as expected.

3.3 Statistical Analysis Plan

The original study protocol proposed four methods that could be used to analyse the data. Further evaluation of analysis plans was undertaken in early 2008 and reported in N1KT Vaccination Trials - Exploration of Statistical Methods V1 (Appendix 3), which describes a range of statistical methods to estimate the efficacy of badger TB vaccination and investigates various potential scenarios for the protocol after the end of 2008 assuming a range of possible levels of efficacy. Simulated data, provided by the University of Newcastle using their validated epidemiological model (Appendix 10), were used to compare analysis methods and scenarios using the most up-to-date parameter values available.

A variety of methods were explored and their advantages and disadvantages were presented and summarised in the report (Appendix 3). Methods were assessed according to the simplicity and plausibility of their assumptions, and their ability to recover a known signal in the simulated data with sufficient precision. A Generalised Linear Model (GLM) using the proportion of badgers with new TB incidence within a social group was the preferred method. This had many advantages over other methods:
1. It accounts for the effect of clustering of badgers within social groups
2. Interpretation of the results can be made readily on the original scale
3. It is less complex than Generalised Estimating Equation (GEE) or Generalised Linear Mixed Models (GLMM) alternatives and likely to present fewer computational problems (e.g. more likely to converge to a solution if the data contain lots of missing observations as expected).
4. It is possible to estimate, and adjust for, any possible over-dispersion (i.e. variance greater than predicted by theory) of the data.
Furthermore, the GLM method had the advantage that it was pre-specified by the study design (i.e. design based) and not dependent on any model building using data from the field measurements; thus it is entirely free of any subjective bias by a statistical analyst. This analysis choice was made before any field data were seen by the statistical analysis team.

When applied to the simulated data, the GLM method shows increased precision with increasing efficacy level, and with additional duration of the trial due to added data. It also shows an increase in vaccine effect with trial duration for high efficacy levels. This is mainly due to the cumulative effect of the vaccine over time becoming more evident. This does not appear to be the case for the low (35% and 50%) simulated efficacy levels, as the effect is not sufficient to be sustained and is lost in the “noise” (random variability).

The objective of this exploratory work was two-fold: first to investigate statistical methods to assess the efficacy of badger TB vaccination and, second, to investigate various potential scenarios for the extension of the protocol after the end of 2008. In order to achieve the latter, data incorporating the spread and detection of TB in badgers under several scenarios were simulated.
Five scenarios were investigated and are ordered by perceived extra effort:
1. Protocol to the end of 2008, not including observation in year 2009.
2. As protocol (i.e. scenario 1 plus observation in year 2009).
3. Protocol to the end of 2008 plus applying vaccine to treated group in 2009.
4. Protocol to the end of 2008 plus applying vaccine to both control and treated groups in 2009.
5. Protocol to 2008 plus applying vaccine to the treated group in 2009 and 2010.
Each scenario was simulated five times for 5 different vaccine efficacy levels; 35%, 50%, 70%, 85%, 100%. Assessments of the statistical methods were made using scenario 2 (i.e. the current protocol). Full details and results are in the in N1KT Vaccination Trials - Exploration of Statistical Methods V1 (Appendix 3).

A standard operating procedure documents the process that was followed; final version called “Management of the Analysis of the BVS Field Trial” Fera reference SIS101 (Appendix 14). This SOP defined rules for the selection of data that could be used to calculate the variables to be analysed: only animals that could be observed to change test status within the vaccination phase of the trial were eligible (negative on first encounter and trapped twice or more); any animals in control social groups that merged with vaccinated groups would be regarded as new to the trial and their history prior to vaccination ignored.

4. Results

4.1 Social Group Identification

Bait marking surveys were carried out annually 2006 to 2009 and changes to the social group configuration from 2006 and subsequent years were identified which included new, merging and splitting groups. Allocation of newly identified groups to treatment was completed prior to the first trapping session each year. Whilst bait marking and surveying give an indication of badger activity and define social groups, actual numbers of badgers that would be caught once trapping started was difficult to predict. Study Protocol Amendment 5 dated 21 March 2007 documents the increase in numbers of badgers to be captured from a predicted 500 to 1000. The sett locations and bait marking returns were plotted onto master maps each year and the social groups were allocated at meetings held each May, prior to the start of that year's trapping sessions.

At a meeting held at Woodchester Park 26th May 2006 and documented in Report No. CSLWP/BVS/001 "Report documenting the rationale for the allocation of social groups in the Badger Vaccine Study" the discrete social groups in the study area were identified.

In 2007 and reported in CSLWP/BVS/003, 81 social groups were identified, including 17 new social groups, two groups that had merged and two groups that had split into two. The increase in social group numbers was due to more landowners and occupiers within the study area agreeing to allow access to their land. At a meeting of the TB Vaccine Study Data Group (TBVSDG) on 23rd May 2007 it was agreed that it was logistically and scientifically possible to add the new groups to the study and that the extra badgers recruited would be of benefit to the study.

Report number CSLWP/BVS/004 describes all the setts trapped during 2006 and 2007. In 2007, trapping as per protocol was completed in June and July but due to an outbreak of Foot and Mouth disease in summer 2007 the trapping session September to November was initially delayed then cancelled. This is documented in Study Protocol Amendment 7 and in Study Deviation Number 2. The impact on the study was unknown at this stage but since no trapping had occurred in this session the data collection would remain balanced and the integrity of the experimental design should not have been compromised.

In 2008 and reported in CSLWP/BVS/005, 81 social groups were identified which included five new groups and a number of groups which had split and merged. A number of the merged groups consisted of groups with differing original treatment allocation and Study Amendment 8 documents the decision to allocate these groups to be vaccinated and the new groups as controls to maintain the 60:40 ratio of vaccinates and controls. The impact on the study was considered low as various data sets would be considered at the time of analysis to ensure reported findings are robust. Report number CSL/WP/BVS/006 describes all the setts trapped during 2006, 2007 and 2008.

In 2009 and reported in CSL/WP/BVS/007, 92 active social groups were identified with the social group configuration changing again since 2008. Report number CSL/WP/BVS/008 documents all trapped setts and allocated treatments 2006 to 2009.

4.2 Social Group Treatment Allocation

In 2006, 63 social groups were identified. Following the first trapping session (T1) immunological, culture data and social group size were analysed, and 37 social groups were allocated to be vaccinated with 26 allocated to be controls.

Table 6: Social Groups and treatment allocation in 2006

Social Group Name	Treatment Allocation
Alpaca	Control
Bear	Control
Beaver	Control
Beluga	Vaccine
Bison	Vaccine
Boar	Control
Brandts	Control
Chamois	Vaccine
Chipmunk	Vaccine
Coypu	Control
Daubentons	Vaccine
Dexter	Vaccine
Dolphin	Control
Donkey	Vaccine
Dormouse	Vaccine
Genet	Vaccine
Goat	Vaccine
Hare	Vaccine
Harvest	Control
Hedgehog	Vaccine
Horse	Vaccine
Horseshoe	Vaccine
Humpback	Control
Ibex	Control
Jackal	Vaccine
Jersey	Vaccine
Killer	Vaccine
Lama	Control
Lemming	Vaccine
Long Eared	Vaccine
Lynx	Vaccine
Marten	Vaccine
Mink	Vaccine
Mole	Vaccine
Mongoose	Vaccine
Mouflon	Control
Mouse	Vaccine
Muntjac	Vaccine
Natterers	Vaccine
Noctule	Vaccine
Otter	Vaccine
Pip	Control
Polecat	Control

Pony	Vaccine
Porcupine	Control
Porpoise	Vaccine
Pygmy	Vaccine
Reindeer	Control
Roe	Control
Ruddy	Control
Seal	Vaccine
Serotine	Control
Shrew	Control
Sika	Vaccine
Squirrel	Vaccine
Stoat	Control
Vole	Control
Wallaby	Control
Walrus	Vaccine
Weasel	Control
Whiskers	Control
Wolf	Vaccine
Wolverine	Control

At the meeting on 17th May 2007 the rationale for naming previous 2006 social groups that had either merged or split was discussed and it was agreed that in the instance of two groups merging that the new social group name would become a double-barrelled name of the previous two social groups. This decision was made to allow conversion back to the original names if the groups split again in future years. One instance of social groups combining was observed, between Marten and Horse (becoming Marten-Horse). Where social groups split to form two separate groups, the original social group name was retained and differentiated with a unique letter. This would identify each group as separate but would allow the original name to be re-established if the groups merged again in the future. Two cases of social groups separating were observed: Wolverine (becoming Wolverine-A and Wolverine-B) and Squirrel (becoming Squirrel-A and Squirrel-B).

Table 7: Social Groups and treatment allocation in 2007 for new, merged and split groups

Social Group Name	Treatment Allocation	Comment
Aardvark	Vaccine	New group
Bandicoot	Vaccine	New group
Bonobo	Control	New group
Bottlenose	Vaccine	New group
Chimpanzee	Control	New group
Dingo	Vaccine	New group
Dog	Vaccine	New group
Elephant	Control	New group
Fox	Vaccine	New group
Jaguar	Vaccine	New group
Kangaroo	Control	New group
Panda	Vaccine	New group
Rabbit	Control	New group
Rhino	Vaccine	New group
Warthog	Vaccine	New group
Whale	Control	New group
Wombat	Vaccine	New group

Marten-Horse	Vaccine	Merged group
Wolverine-A	Control	Split Group
Wolverine-B	Control	Split Group
Squirrel-A	Vaccine	Split Group
Squirrel-B	Vaccine	Split Group
Sika A	Vaccine	Split Group
Sika B	Vaccine	Split Group

In 2008 the changes to the social group configuration are documented in Report CSLWP/BVS/005 "Confirmation of existing social groups and allocation of new groups in the Badger Vaccine Study from the 2008 bait marking data". A meeting was held with Fera staff, study sponsor, study monitor, study investigator, VLA data management staff and the chairman of the TBVSDG on 29th May 2008 to discuss and agree the allocation of merged groups that contained both vaccine and control allocated groups as well as the inclusion of 5 newly identified groups. The study protocol stated that individual badgers would remain in their initial group allocation and be treated accordingly throughout the trial even if they were subsequently trapped in a social group allocated to alternative treatment but there was no statement on social groups merging containing groups of both treatments. Study Protocol Amendment 8 documents that merged groups consisting of both treatments will be allocated to be vaccinated and the new groups identified will be allocated to be controls. The reason for the change was by vaccinating all the badgers in the merged groups, more data would be collected on vaccinates and allocation of the new groups to controls would maintain the 60:40 ratio.

Table 8: Social Groups and treatment allocation in 2008 for new, merged and split groups

Social Group Name	Original Treatment Allocation/New Group	Treatment Allocation
Kangaroo-Whiskers	Both controls	Control
Mole-Beaver-Brandts	Mole: Vaccine Beaver: Control Brandts: Control	Vaccine
Beluga-Weasel	Beluga: Vaccine Weasel: Control	Vaccine
Coypu-Genet	Coypu: Control Genet: Vaccine	Vaccine
Dormouse-Dog	Dormouse: Vaccine Dog: Control	Vaccine
Armadillo	New group	Control
Caribou	New group	Control
Cheetah	New group	Control
Potto	New group	Control
Zebra	New group	Control

In 2009 the changes to the social group configuration are documented in Report CSLWP/BVS/007 "Confirmation of existing social groups and allocation of new groups in the Badger Vaccine Study from the 2008 bait marking data" with 92 social groups being identified. A meeting was held at Woodchester Park 14th May 2009 with Fera staff, study monitor, the chairman of the TBVSDG, study auditors and study investigator to discuss the further changes to the social group configuration identified in 2009 which included merged groups, split groups, previously merged groups now split and merged with other groups, previously merged groups now split and new groups.

The chairman of the TBVSDG by email 25 May 2009 to the study investigator supported the recommendations which were based on the previous agreed practice of a control split becoming

two controls, a vaccine split becoming two vaccines, two controls merging to become one control, two vaccines merging to become a single vaccine, and a control and vaccine merging to become a vaccine group. The change is documented in Study Protocol Amendment 12.

Tables 9 to 12 detail the treatment allocations for 2009:

Table 9: Merged groups treatment allocation 2009		
Social Group Name	**2009 Allocation**	**Comments**
Squirrel AB	**VACCINE**	2006 original group Squirrel 2007 split into Squirrel A and Squirrel B. 2008 remained Squirrel A and Squirrel B discrete groups Original 2006 allocation: Vaccine
Fox-Wolf	**VACCINE**	2006 original group Wolf. Fox not identified until 2007 2007 Both Fox and Wolf remained discrete groups 2008 Both Fox and Wolf remained discrete groups Original 2006 allocation Wolf: Vaccine 2007 allocation Fox: Vaccine
Potto-Reindeer	**CONTROL**	2006 original group Reindeer. Potto not identified until 2008 2007 Reindeer remained discrete group 2008 Both Reindeer and Potto discrete groups Original 2006 allocation Reindeer: Control 2008 allocation Potto: Control
Lemming-Vole	**VACCINE**	2006 - 2008 both discrete groups Original 2006 allocation for Lemming: Vaccine Original 2006 allocation for Vole: Control

Table 10: Split groups treatment allocation 2009		
Social Group Name	**2009 Allocation**	**Comments**
Shrew A	**CONTROL**	2006-2008 remained as a discrete group. Original 2006 allocation for Shrew: Control
Shrew B	**CONTROL**	
Shrew C	**CONTROL**	Parsons sett originally in Alpaca in 2006 and 2007 then in 2008 within Shrew's territory. Documented in report CSLWPBVS006. Original 2006 allocation for Alpaca: Control Original 2006 allocation for Shrew: Control
Kangeroo1	**CONTROL**	2006 original group Whiskers, Kangaroo no activity in 2006 2007 Whiskers discrete group, Kangaroo discrete group 2008 Merged into Kangaroo-Whiskers Original 2006 allocation for Whiskers: Control Original 2007 allocation for Kangaroo: Control
Whiskers1	**CONTROL**	
PorpoiseA	**VACCINE**	2006-2008 Porpoise remained as discrete group Original 2006 allocation for Porpoise: Vaccine
PorpoiseB	**VACCINE**	

Table 11: Previously merged groups now split and merged with other groups treatment allocation 2009		
Social Group Name	**2009 Allocation**	**Comments**
Beluga1	**VACCINE**	2006 Beluga and Weasel discrete groups. Rabbit not identified until 2007. 2007 Beluga, Weasel and Rabbit discrete groups 2008 Beluga and Weasel merged. Rabbit remains discrete group. 2009 Beluga-Weasel split into Beluga1 with Rabbit merging with Weasel becoming Rabbit-Weasel1 Original 2006 allocation for Beluga: Vaccine Original 2006 allocation for Weasel: Control 2007 allocation for Rabbit: Control 2008 Beluga-Weasel allocation: Vaccine
Rabbit-Weasel1	**VACCINE**	
Marten-Horse-Mole1	**VACCINE**	2006 Marten, Horse and Mole discrete groups. 2007 Marten and Horse merge to Marten-Horse, Mole remains as discrete group 2008 Marten-Horse remains as merged discrete group. Mole merged with Beaver and Brandts to form Mole-Beaver-Brandts 2009 Mole splits from Mole-Beaver-Brandts and merges with Marten-Horse Original 2006 allocation for Marten: Vaccine Original 2006 allocation for Horse: Vaccine Original 2006 allocation for Mole: Vaccine 2007 and 2008 allocation for Marten-Horse: Vaccine 2008 allocation for Mole-Beaver-Brandts: Vaccine
Beaver1	**VACCINE**	2006 and 2007 Mole, Beaver and Brandts discrete groups 2008 Mole, Beaver and Brandts merge 2009 Mole splits from Mole-Beaver-Brandts and merges with Marten-Horse to form Marten-Horse-Mole1. Beaver and Brandts also split to form discrete groups Beaver1 and Brandts1. Original 2006 allocation for Beaver: Control Original 2006 allocation for Brandts: Control Original 2006 allocation for Mole: Vaccine 2008 allocation for Mole-Beaver-Brandts: Vaccine
Brandts1	**VACCINE**	

Table 12: Previously merged groups now split treatment allocation		
Social Group Name	**2009 Allocation**	**Comments**
Genet1	**VACCINE**	2006 and 2007 Coypu and Genet discrete groups 2008 Coypu and Genet merge 2009 Genet splits to Genet1. Coypu splits into two discrete groups Coypu1A and Coypu1B Original 2006 allocation for Genet: Vaccine Original 2006 allocation for Coypu: Control 2008 allocation for Coypu-Genet: Vaccine
Coypu1A	**VACCINE**	
Coypu1B	**VACCINE**	

The 7 newly identified groups in 2009 were randomly assigned to vaccine and control in the ratio of 60:40 respectively. Table 13 below details the treatment allocation.

Table 13: New groups 2009 treatment allocation

Social Group Name	2009 Allocation
Anteater	Vaccine
Ferret	Control
Lion	Vaccine
Meerkat	Control
Ocelot	Vaccine
Tapir	Control
Tiger	Vaccine

Table 14 below summarises the group numbers and their allocation to treatment.

Table 14: Study social groups and allocated treatment

Year	Vaccine	Control	Total
2006	37	26	63
2007	47	34	81
2008	48	33	81
2009	56	36	92

Table 15 below summarises the group numbers and allocation to treatment where badgers were trapped during the study.

Table 15: Group numbers and allocated treatment of badgers trapped during the study

Year	Vaccine	Control	Total
2006	34	23	57
2007	43	32	75
2008	44	30	74
2009	53	32	85

4.3 Vaccination

[redacted].

Table 16: [redacted]

Date Titrated	Box and Vial Number	Vaccine Batch Number	Result
[redacted]			

At [redacted] there were 281 vials titrated and the results were recorded on BA 600 Vaccine Titration Worksheet. Time from reconstitution to plating ranged between 36 minutes and 4 hours 5 minutes.

The number of controls titrated on the same day as badgers were vaccinated was 89 with 15 results lower than 2.0×10^6 and 2 having higher results than 8.0×10^6. There were 3 titrations with no results due to contamination.

The number of vials containing residual vaccine and titrated on the days badgers were vaccinated was 192 with 18 results lower than 2.0×10^6 and 18 higher than 8.0×10^6. One titration had no result due to contamination.

The full data are presented Appendix 15: Control and Residue Titration Results.

The titrations carried out in VLAS/05/036 with control and residual vaccine were carried out as an extra level of checking that the vaccine being injected into the study badgers was viable. It is unknown if the technique used during the study is the same as used by the manufacturer SSI and given the fact that the product is a GMP manufactured vaccine there is no reason to believe that any vaccine batch was out of specification considering the low sample volumes and the inherent variation expected with a viability count.

The volume of vaccine administered to each badger was recorded on data capture forms BA596 and ranged between 0.80 to 1 ml.

4.4 Body Temperature Monitoring

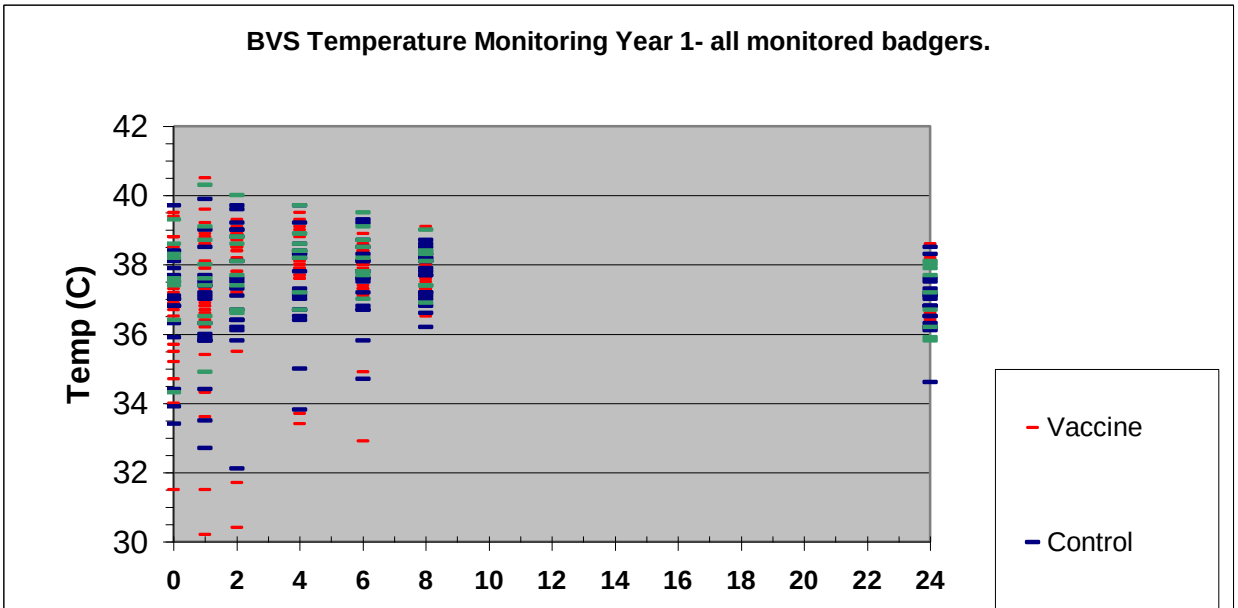
Table 17 below is a summary of badgers that were temperature monitored for 24 hours in 2006.

Table 17: 24 hour chip temperature monitoring 2006

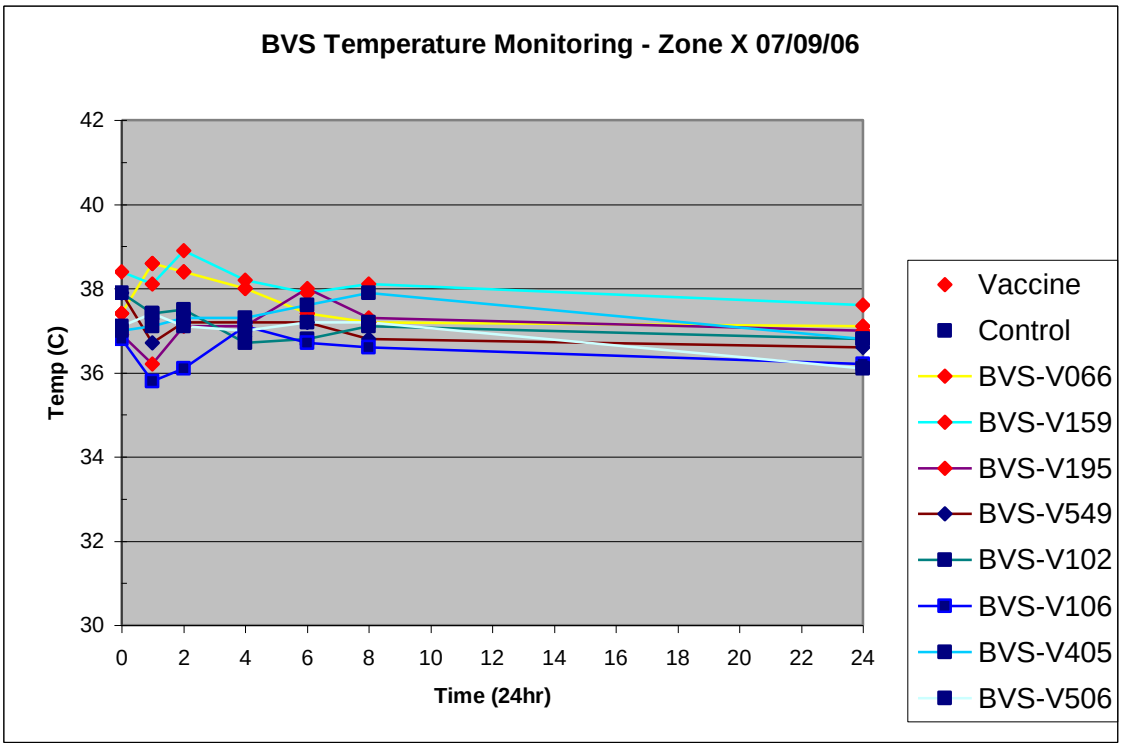
Directly after Vaccination	Unvaccinated	Vaccinated two weeks previously	Monitored twice
29	19	7	2

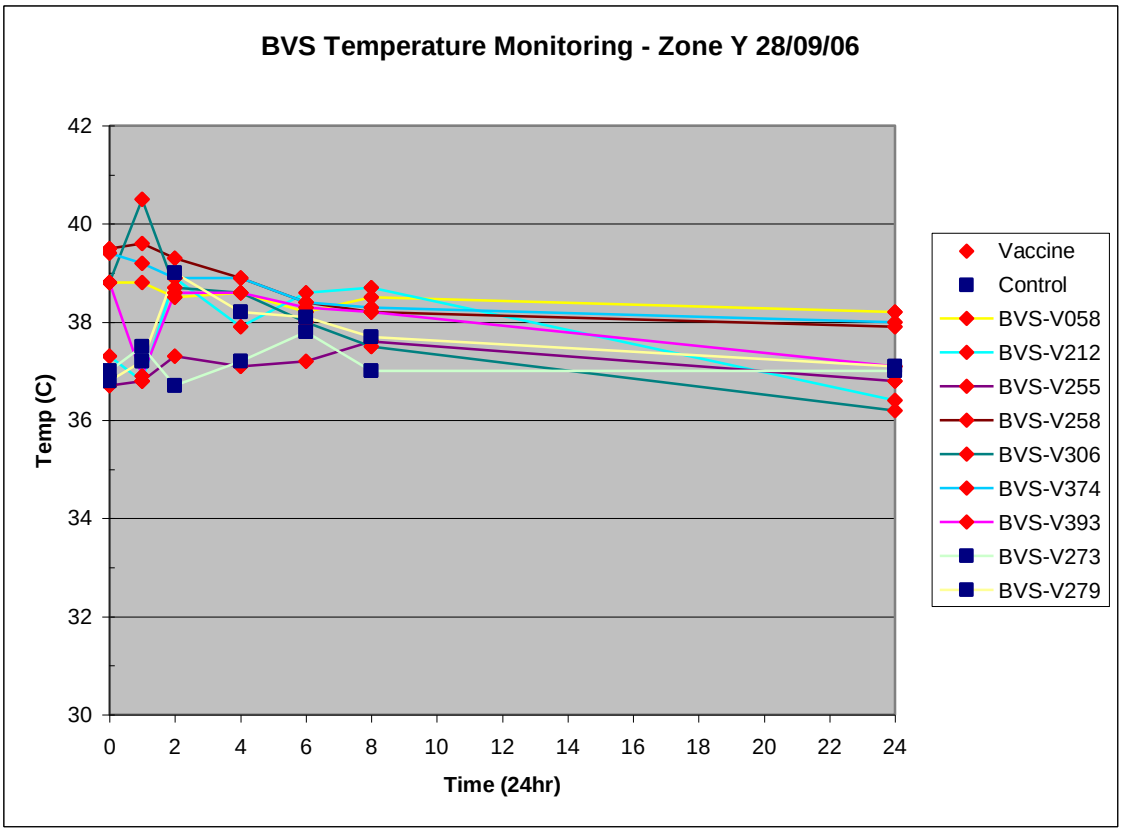
As rectal temperature can only be measured under anaesthesia it was not possible to obtain this measurement frequently over a 24 hour period. Instead, readings from the subcutaneous Plexx microchips were used as these could be obtained from conscious animals with little to no disturbance. The correlation between Plexx temperature and rectal temperature for all badgers measured in 2006 is shown in Appendix 16. The Plexx microchip provides a reliable surrogate reading of the animal's core temperature.

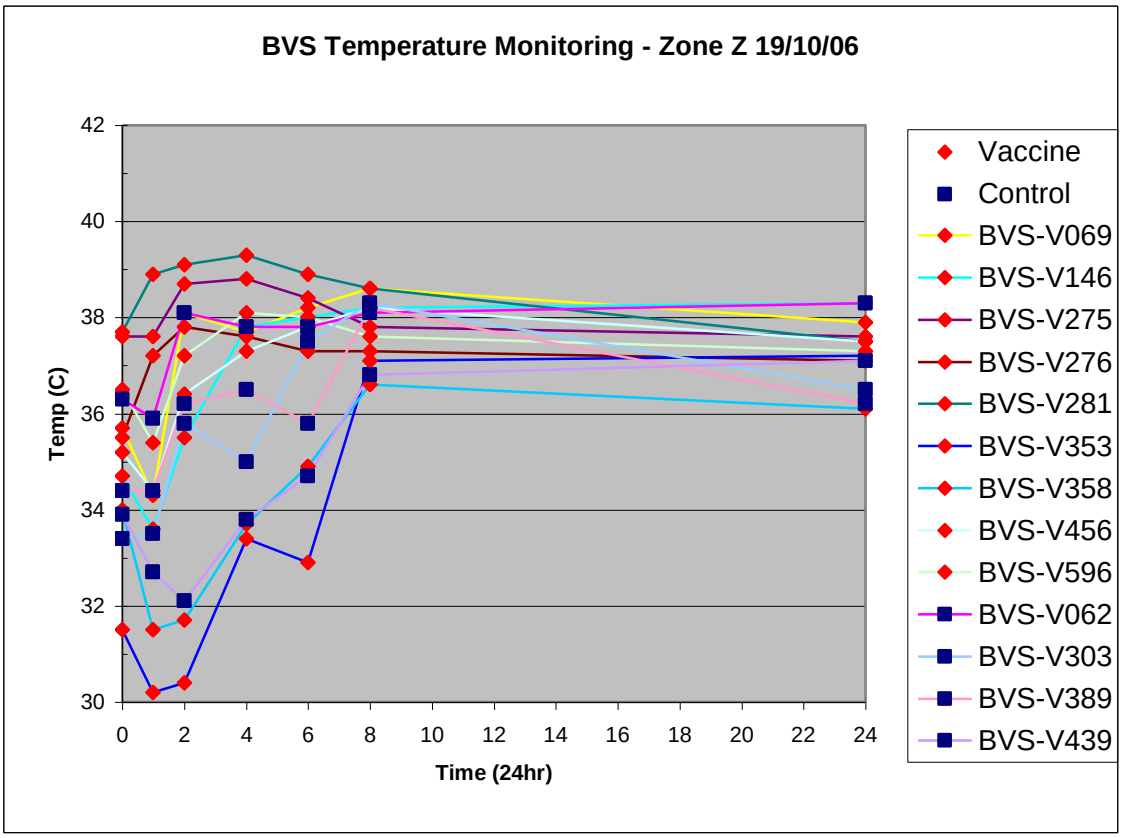
The following graphs display chip temperatures recorded from the 24 hour monitored badgers in 2006.



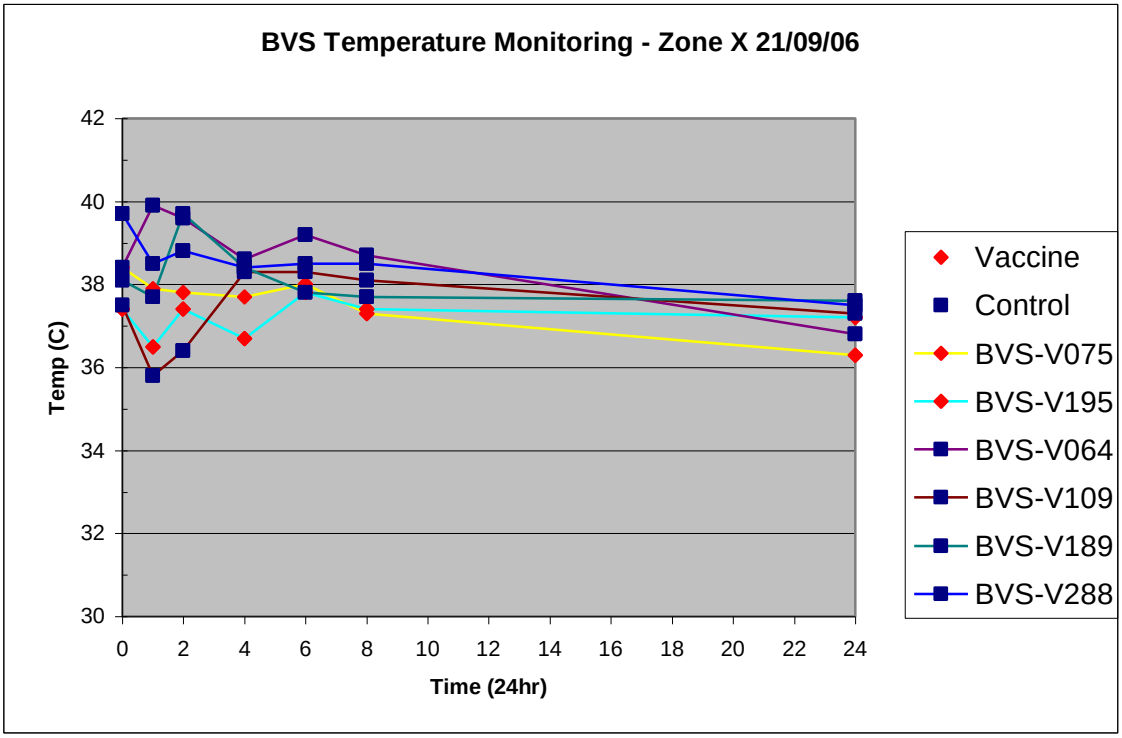
Temp ^{0}C	Vaccine	Vacc/Retrap	Control
Median	37.80	38.00	37.20
Mean	37.46	37.83	37.18
Std Dev	1.55	1.13	1.40

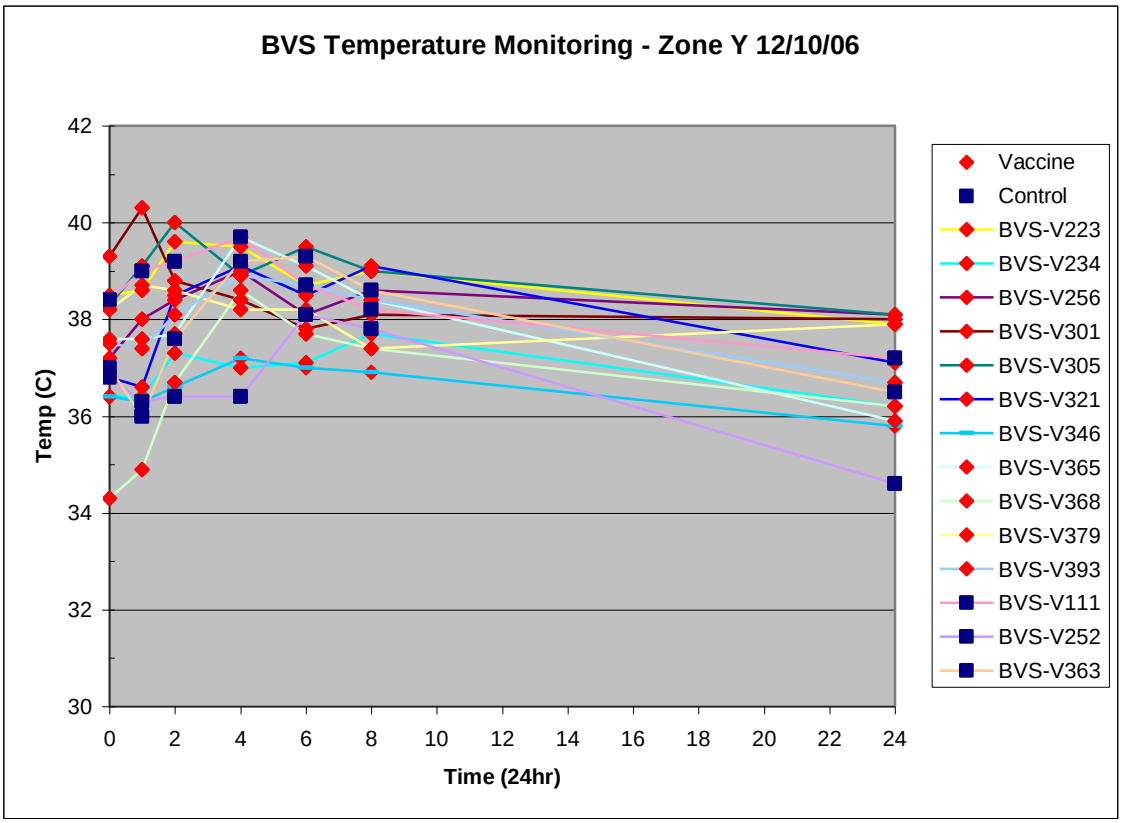

BVS Temperature Monitoring - Zone X 07/09/06
Temp (C)
42
40
38
36
34
32
30
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (24hr)
Vaccine
Control
BVS-V066
BVS-V159
BVS-V195
BVS-V549
BVS-V102
BVS-V106
BVS-V405
BVS-V506

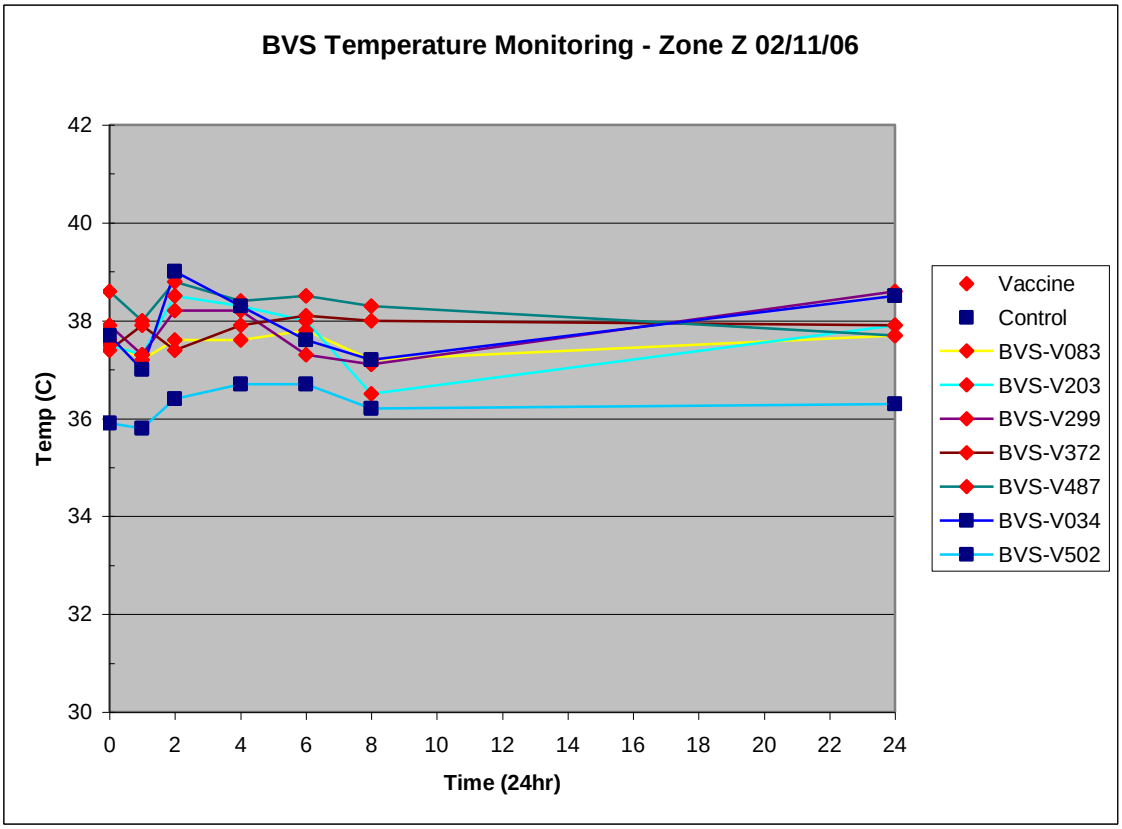

BVS Temperature Monitoring - Zone Y 28/09/06
Temp (C)
42
40
38
36
34
32
30
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (24hr)
Vaccine
Control
BVS-V058
BVS-V212
BVS-V255
BVS-V258
BVS-V306
BVS-V374
BVS-V393
BVS-V273
BVS-V279



Week 19/10/06 was very wet and all the badgers were muddy necessitating the need to wash them prior to sampling. Increased use of infrared lamps in the holding area occurred during recovery after anaesthesia.




BVS Temperature Monitoring - Zone Y 12/10/06
Temp (C)
Time (24hr)
Vaccine
Control
BVS-V223
BVS-V234
BVS-V256
BVS-V301
BVS-V305
BVS-V321
BVS-V346
BVS-V365
BVS-V368
BVS-V379
BVS-V393
BVS-V111
BVS-V252
BVS-V363


BVS Temperature Monitoring - Zone Z 02/11/06
Temp (C)
Time (24hr)
Vaccine
Control
BVS-V083
BVS-V203
BVS-V299
BVS-V372
BVS-V487
BVS-V034
BVS-V502

4.5 Immunological and Culture Status of Temperature Monitored Badgers

Table 18 below details the immunological and culture status of temperature monitored badgers in 2006.

Table 18: Detailed temperature monitored badgers - immunological and culture status

Microchip Number	Detailed Temperature Monitoring Date	Vaccination Date /Control	Sample Results		
			IFNγ	Brock TB Stat-Pak	Culture (*M. bovis*)[1]
BVS -V066	07/09/06	07/09/06	Positive	Negative	Negative
BVS -V159	07/09/06	07/09/06	Positive	Negative	Negative
BVS -V195	07/09/06	07/09/06	Positive	Negative	Negative
BVS -V549	07/09/06	07/09/06	Negative	Negative	Negative/Contaminated
BVS -V102	07/09/06	Control	Positive	Negative	Negative
BVS -V106	07/09/06	Control	Positive	Negative	Negative
BVS -V405	07/09/06	Control	Positive	Negative	Positive
BVS -V506	07/09/06	Control	Negative	Negative	Negative
BVS -V075	21/09/06	21/09/06	Positive	Negative	Negative
BVS -V195	21/09/06	07/09/06	Positive	Negative	Negative
BVS -V064	21/09/06	Control	Negative	Negative	Negative
BVS -V109	21/09/06	Control	Negative	Negative	Negative
BVS -V189	21/09/06	Control	Positive	Negative	Negative
BVS -V288	21/09/06	Control	Negative	Negative	Negative/Contaminated
BVS -V058	28/09/06	28/09/06	Negative	Negative	Negative
BVS -V212	28/09/06	28/09/06	Negative	Negative	Negative
BVS -V255	28/09/06	28/09/06	Negative	Negative	Negative
BVS -V258	28/09/06	28/09/06	Positive	Negative	Negative
BVS -V306	28/09/06	28/09/06	Positive	Negative	Negative
BVS -V374	28/09/06	28/09/06	Negative	Negative	Negative
BVS -V393	28/09/06	28/09/06	Negative	Negative	Negative
BVS -V273	28/09/06	Control	Positive	Negative	Negative
BVS -V279	28/09/06	Control	Negative	Negative	Negative
BVS -V223	12/10/06	12/10/06	Positive	Negative	Negative
BVS -V234	12/10/06	12/10/06	Positive	Negative	Positive
BVS -V256	12/10/06	12/10/06	Negative	Negative	Negative
BVS -V301	12/10/06	27/09/06	Negative	Negative	Negative
BVS -V305	12/10/06	27/09/06	Negative	Negative	Negative
BVS -V321	12/10/06	12/10/06	Positive	Positive	Positive
BVS -V346	12/10/06	27/09/06	Positive	Negative	Positive
BVS -V365	12/10/06	27/09/06	Negative	Negative	Negative
BVS -V368	12/10/06	27/09/06	Positive	Positive	Positive
BVS -V379	12/10/06	27/09/06	Positive	Negative	Positive
BVS -V393	12/10/06	28/09/06	Positive	Negative	Negative/Contaminated
BVS -V111	12/10/06	Control	Negative	Positive	Negative
BVS -V252	12/10/06	Control	Negative	Positive	Positive
BVS -V363	12/10/06	Control	Positive	Negative	Positive
BVS -V069	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V146	19/10/06	19/10/06	Positive	Positive	Negative
BVS -V275	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V276	19/10/06	19/10/06	Negative	Negative	Negative

BVS -V281	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V353	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V358	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V456	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V596	19/10/06	19/10/06	Negative	Negative	Negative
BVS -V062	19/10/06	Control	Negative	Negative	Negative
BVS -V303	19/10/06	Control	Negative	Negative	Negative
BVS -V389	19/10/06	Control	Negative	Negative	Negative
BVS -V439	19/10/06	Control	Negative	Negative	Negative
BVS -V083	2/11/06	2/11/06	Positive	Negative	Negative
BVS -V203	2/11/06	2/11/06	Negative	Negative	Negative/Contaminated
BVS -V299	2/11/06	2/11/06	Negative	Negative	Negative
BVS -V372	2/11/06	2/11/06	Negative	Negative	Negative/Contaminated
BVS -V487	2/11/06	18/10/06	Negative	Negative	Negative/Contaminated
BVS -V034	2/11/06	Control	Negative	Negative	Negative/Contaminated
BVS -V502	2/11/06	Control	Negative	Negative	Negative/Contaminated

[1]Positive = *M. bovis* cultured from one or more samples; Negative = no *M. bovis* cultured from all samples; Negative/Contaminated = all samples negative/no result due to contamination or insufficient sample.

4.6 Re examination of Vaccination Site

A total of 265 vaccinated badgers were re-trapped after being vaccinated and 22 badgers were found to have intramuscular swellings but there were no associated skin lesions.

Additional data are presented in:

- Vaccination Site Examination Report (Appendix 17)
- Digital photographs of badgers with swellings in (Appendix 18)

Table 19 shows when intramuscular swellings were observed, their size and location, in relation to the TB status of the badger. For the sake of analysis, a badger is considered to be TB positive when it is positive by one or more of the three tests on any one occasion. On this basis, there were 14 badgers considered to TB positive before or at the time of vaccination preceding detection of an intramuscular swelling. A further eight badgers in which an intramuscular swelling was observed were deemed to be TB negative at or before the time of vaccination preceding the swelling.

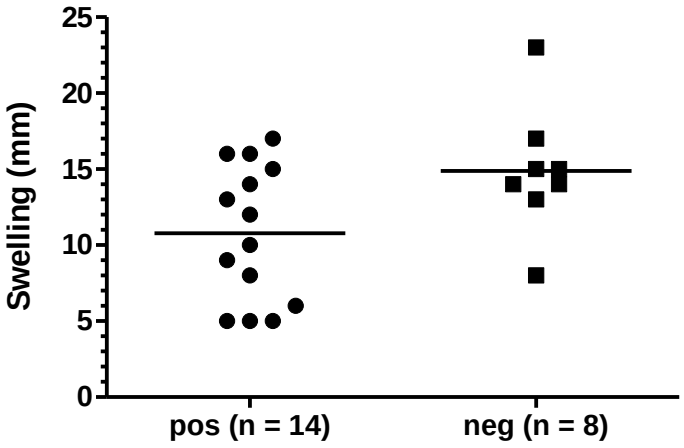
To determine if intramuscular swelling was exacerbated by vaccinating animals with pre-existing infection the maximum swelling size for each badger is shown in the graph below as a function of TB status. The mean maximum swelling size was 11mm in the TB positive group and 15mm in the TB negative group. This difference was statistically significant ($p = 0.048$, Unpaired t test). Sustained swelling (i.e. present at repeat sampling sessions) was rare (n = 3/22); with 2 animals in the TB positive group and 1 animal in TB negative group.

Table 19: Immunology and culture results of badgers with intramuscular swellings at the site of vaccination

Age	Sex	Found Dead	Last Trap	Test	T1	T2	T3	T3 Comments	T4	T4 Comments	T5	T6	T6 Comments	T7	T7 Comments	T8	T8 Comments	T9	T9 Comments
Cub	Male		07/10/2009	Gamma_IFN_ELISA		v			v			v				v			16mm swelling left lumbar
				Stat-pak		v			v			v				v			
				MbovisResult		v			v			v				v			
Adult	Male		24/06/2008	Gamma_IFN_ELISA		v						v	23mm swelling left lumbar						
				Stat-pak		v						v							
				MbovisResult		v						v							
Adult	Male	17/09/2007 09:50:00 ()	09/07/2007	Gamma_IFN_ELISA		v		15mm swelling left lumbar	v										
				Stat-pak		v			v										
				MbovisResult		v			v										
Adult	Male		05/10/2009	Gamma_IFN_ELISA										v		v			5mm swelling left lumbar
				Stat-pak										v		v			
				MbovisResult										v		v			
Adult	Female		25/06/2009	Gamma_IFN_ELISA								v				v	16mm swelling left lumbar		
				Stat-pak								v				v			
				MbovisResult								v				v			
Adult	Female		25/06/2007	Gamma_IFN_ELISA			v		v	15mm swelling left lumbar									
				Stat-pak			v		v										
				MbovisResult			v		v										
Adult	Female		05/10/2009	Gamma_IFN_ELISA		v			v			v				v	13mm swelling left lumbar		
				Stat-pak		v			v			v				v			
				MbovisResult		v			v			v				v			
Adult	Female		23/06/2009	Gamma_IFN_ELISA		v								v		v	14mm swelling left lumbar		
				Stat-pak		v								v		v			
				MbovisResult		v								v		v			
Cub	Female		25/06/2008	Gamma_IFN_ELISA			v		v	15mm swelling left lumbar		v	7mm swelling left lumbar						
				Stat-pak			v		v			v							
				MbovisResult			v		v			v							
Adult	Male		05/10/2009	Gamma_IFN_ELISA			v		v			v				v	12mm swelling left lumbar		
				Stat-pak			v		v			v				v			
				MbovisResult			v		v			v				v			
Adult	Female		05/10/2009	Gamma_IFN_ELISA		v		8mm swelling left lumbar				v				v			
				Stat-pak		v						v				v			
				MbovisResult		v						v				v			
Adult	Male		05/10/2009	Gamma_IFN_ELISA										v		v	14mm swelling left lumbar		3mm swelling left lumbar
				Stat-pak										v		v			
				MbovisResult										v		v			
Adult	Male		05/10/2009	Gamma_IFN_ELISA								v				v			5mm swelling left lumbar
				Stat-pak								v				v			
				MbovisResult								v				v			
Adult	Male	17/03/2008 12:30:00 (MIXED)	09/07/2007	Gamma_IFN_ELISA		v		17mm swelling left lumbar	v										
				Stat-pak		v			v										
				MbovisResult		v			v										
Adult	Female		12/10/2006	Gamma_IFN_ELISA		v		13mm swelling left lumbar											
				Stat-pak		v													
				MbovisResult		v													
Adult	Male		06/10/2009	Gamma_IFN_ELISA		v								v		v			9mm swelling left lumbar
				Stat-pak		v								v		v			
				MbovisResult		v								v		v			
Adult	Female		24/06/2009	Gamma_IFN_ELISA		v			v	17mm swelling left lumbar		v	11mm swelling left lumbar		No swellings	v	11mm swelling left lumbar		
				Stat-pak		v			v			v	9mm swelling right lumbar			v	13mm swelling right lumbar		
				MbovisResult		v			v			v				v			
Cub	Female		05/10/2009	Gamma_IFN_ELISA					v							v			5mm swelling left lumbar
				Stat-pak					v							v			
				MbovisResult					v							v			
Cub	Female		08/07/2009	Gamma_IFN_ELISA		v			v			v	14mm swelling left lumbar			v			
				Stat-pak		v			v			v				v			
				MbovisResult		v			v			v				v			
Cub	Female		09/07/2007	Gamma_IFN_ELISA		v		6mm swelling left lumbar	v										
				Stat-pak		v			v										
				MbovisResult		v			v										
Cub	Male		05/10/2009	Gamma_IFN_ELISA								v				v			8mm swelling left lumbar
				Stat-pak								v				v			
				MbovisResult								v				v			
Adult	Female		07/10/2009	Gamma_IFN_ELISA										v		v			10mm swelling left lumbar
				Stat-pak										v		v			
				MbovisResult										v		v			

Positive
Negative
No Result (Culture), No Result/Not Done (Immunology)

Maximum swelling size for each badger as a function of TB status



On the basis of these results, there was no evidence that vaccinating badgers with pre-existing infection resulted in greater intramuscular swelling compared with TB negative individuals. In fact, the evidence pointed to the opposite conclusion.

4.7 Immunology Results

Two immunological tests were applied in this study: the IFNγ EIA and the Brock TB Stat-Pak Test (Chembio Diagnostic Systems, Inc.). The first test measures the production of IFNγ following stimulation of whole heparinised blood with bovine and avian tuberculins and has an estimated sensitivity of 80.9% and an estimated specificity of 93.6% (Dalley et al., 2008). The Brock TB Stat-Pak is a lateral flow assay to test for the presence of antibodies in serum to *M. bovis* antigen MPB83. It has an estimated sensitivity of 49.2% and an estimated specificity of 93.1% based on a study of 1464 badgers naturally infected with *M. bovis* as determined by culture (Chambers et al., 2008). Sensitivity of the Stat-Pak varies according to disease severity, such that sensitivity was found to be 34.4% in infected badgers with no visible lesions at *post mortem*, 66.1% in infected badgers with visible lesions at *post mortem*, 41.7% in infected badgers that excrete *M. bovis*; rising to 78.1% in so-called 'Super-Excretor' badgers (Chambers et al., 2008).

Appendix 19 summarises the results of the two tests alongside the culture result for each animal at each occasion of trapping. The following summary data were extracted from the underlying data (Table 20).

Table 20: Number of individual badgers per study year with missing or negative test results

Year	Number of individual badgers caught	Number with missing test results[1]	Negative for both immunological tests and culture[2]	Remainder[3]
2006	337	25	175	137
2007	311	11	219	81
2008	296	6	177	113
2009	370	12	222	136

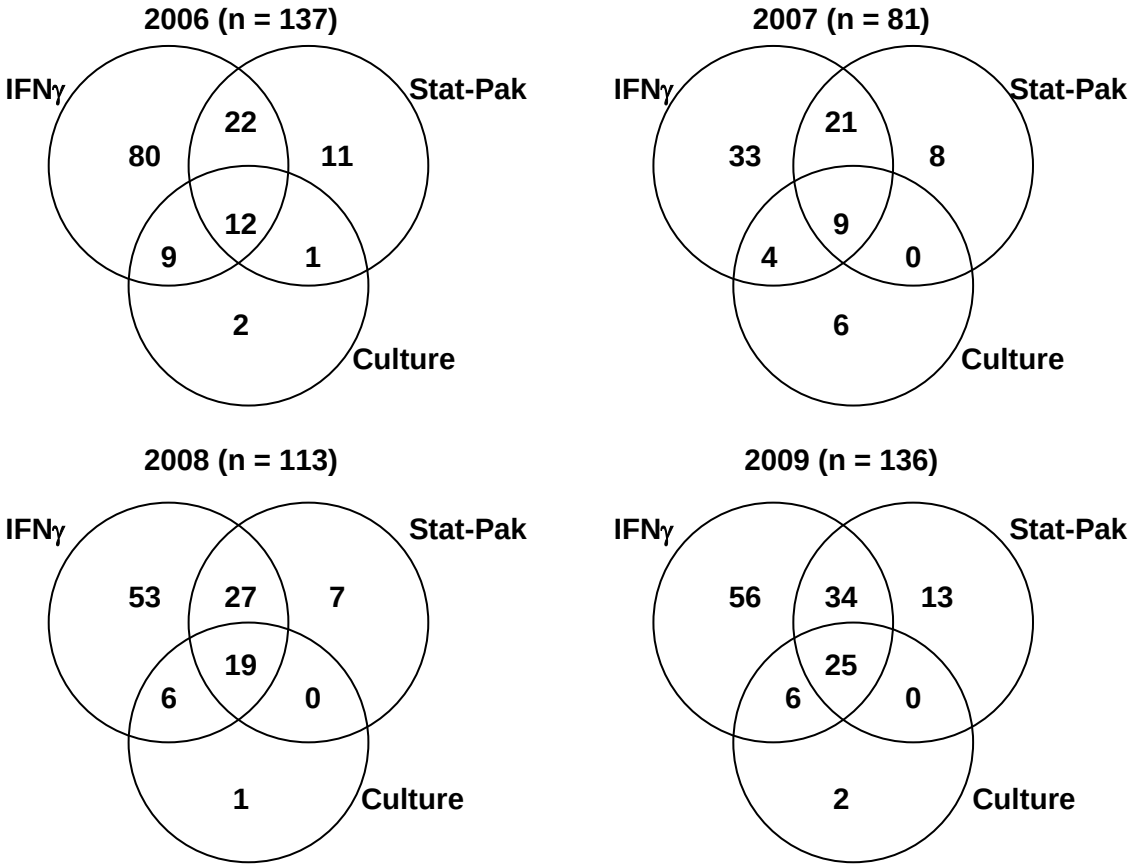
[1]Table 21 shows the breakdown of missing tests results per year.

Table 21: Number of missing test results per year

	Number of missing test results				
Year	**IFNγ EIA**	**Brock TB Stat-Pak**	**Culture**	**Total**	**Number of badgers affected**
2006	19	5	1	**25**	25
2007	11	1	0	**12**	11
2008	4	3	0	**7**	6
2009	11	1	0	**12**	12
Total	**45**	**10**	**1**	**56**	**54**

[2]Each of the badgers in Table 21 that had one or more missing test results for that year (either due to contaminated culture, insufficient blood taken, or failed immunological test result) were excluded from the count of badgers that were negative for both immunological tests and culture.

[3]The remaining badgers were positive for one or more test in each of the years indicated. The intersections of circles in the following Venn diagrams indicate the number of animals positive in two or more tests that year.



More positive results were obtained with the IFNγ EIA than the Brock TB Stat-Pak, as anticipated by the higher sensitivity for the IFNγ EIA at equivalent specificity to the Brock TB Stat-Pak.

Fewer animals testing positive were observed in 2007 due to the fact that only one trapping session was conducted that year.

The dose of vaccine used in this study was chosen as it was demonstrated to generate post-vaccination IFNγ responses measurably by EIA (see Appendix 20). It would not be expected to generate a detectable antibody response to MPB83 as the vaccine strain is a constitutively low expresser of this antigen (Charlet et al., 2005). The influence of vaccination on IFNγ EIA result could only be addressed by analysing the data from 2006. It would not be possible to conclude anything by comparing data in other years, since the minimum time elapsing between sampling events is two months. This is time for an immune-negative, vaccinated badger to be exposed to *M. bovis* and develop immunity before it is next sampled. Therefore, a positive IFNγ EIA test result following vaccination could simply be due to exposure to *M. bovis*. In contrast, the time interval between T2 and T3 was only two weeks. Whilst it is feasible that a badger could be exposed to *M. bovis* and develop immunity within these two weeks it is unlikely. A quarter of all vaccinated badgers negative by IFNγ EIA at T2 were IFNγ EIA positive at T3 (Table 22).

Table 22: Number of badgers changing or retaining IFNγ EIA test status between time points T2 and T3 as a function of treatment

	T2(neg) -> T3(pos)	T2(neg) -> T3(neg)	Total
Vaccinated (at T2)	8	24	32
Controls (not vaccinated)	0	20	17

These differences are statistically significant (p = 0.017, Fisher's Exact Test), representing an odds ratio of 14 for a change in IFNγ EIA status from negative at T2 to positive at T3 (two weeks apart) as a consequence of being in the vaccinated group. Whilst this does not prove the IFNγ positivity at T3 is a consequence of vaccination, it does support the notion that the vaccine is stimulating a peripheral IFNγ response in wild vaccinated badgers, akin to that seen in captive animals.

The effect of vaccination on the incidence of immunological test positivity as a surrogate measure of vaccine efficacy was evaluated and is reported in Appendix 21 (N1KT Badger TB Vaccine Field Trial Final Stats Analysis 2, version 1 revision 2, 16 March 2010). To control for the confounding influence of vaccination on the IFNγ EIA in the short-term (~2 weeks), an *a priori* decision was made to exclude T3 from the analysis of incidence of new positive test outcomes. Table 22 provides *a posteriori* justification of that decision.

4.8 Culture Results

Total clinical samples taken from anaesthetized badgers with results are summarized below in Table 23.

Table 23: Summary of clinical samples taken and their results

Medium	Sample Type	Result	Sample Count
7H11	Faeces	M.bovis	28
7H11	Faeces	NEGATIVE	1244
7H11	Faeces	NO RESULT[1]	10
7H11	Other[2]	M.bovis	9
7H11	Other	NEGATIVE	15
7H11	Tracheal Aspirate	M.bovis	71
7H11	Tracheal Aspirate	NEGATIVE	1670
7H11	Tracheal Aspirate	NO RESULT	46
7H11	Urine	M.bovis	41
7H11	Urine	NEGATIVE	1558
7H11	Urine	NO RESULT	19
7H11	Wound Swab	M.bovis	40
7H11	Wound Swab	NEGATIVE	103
7H11+Cy	Faeces	NEGATIVE	716
7H11+Cy	Faeces	NO RESULT	8
7H11+Cy	Other	M.bovis	1
7H11+Cy	Other	NEGATIVE	10
7H11+Cy	Tracheal Aspirate	M.bovis	1
7H11+Cy	Tracheal Aspirate	NEGATIVE	951
7H11+Cy	Tracheal Aspirate	NO RESULT	47
7H11+Cy	Tracheal Aspirate + Cycloserine	NEGATIVE	899
7H11+Cy	Tracheal Aspirate + Cycloserine	NO RESULT	27
7H11+Cy	Urine	NEGATIVE	888
7H11+Cy	Urine	NO RESULT	19
7H11+Cy	Wound Swab	NEGATIVE	90

[1]NO RESULT due to contamination of plates.
[2]Other included aspirates from non discharging abscesses.

The individual animal sample results are given in Appendix 22: Study Culture Results.

The incidence of *M. bovis* culture positive badgers by year in relation to the immunological test results is shown in the Venn diagrams in the preceding section. The occurrence of contamination on some plates resulted in NO RESULT being reported for some clinical samples (see Table 23 above). Despite this, only one badger (2006, T3: V389) could not be given an overall culture result as a result was not available for every clinical sample taken. In all other cases, an overall *M. bovis* result could be assigned based on the individual results from each clinical sample (see Appendix 19). The fewest positive results were obtained for culture in comparison with the IFNγ EIA and Brock TB Stat-Pak, as anticipated by the infrequent nature of *M. bovis* excretion among infected animals (Chambers et al., 2002). An estimate of the sensitivity of culture in this study was calculated as follows. Table 24 gives the number of positive IFNγ EIA, Brock TB Stat-Pak and culture results for each year.

Table 24: Number of positive IFNγ EIA, Brock TB Stat-Pak and culture results by year

Year	IFNγ EIA	Brock TB Stat-Pak	Culture
2006	123	46	24
2007	67	38	19
2008	105	53	24
2009	121	72	33
Total	**416**	**209**	**100**
Published sensitivity	80.9%	49.2%	Unknown[1]

[1]On the basis of the reported sensitivities for the two immunological tests and the total number of positive tests results, the sensitivity of culture is estimated to be in the range of 19.5-23.5%. The ratio of positive tests results is therefore in the order of 4:2:1. The sensitivity of culture is approximately a quarter of that of the IFNγ EIA and half that of the Brock TB Stat-Pak.

The effect of vaccination on the incidence of culture positivity as a surrogate measure of vaccine efficacy was evaluated and is reported in Appendix 21: N1KT Badger TB Vaccine Field Trial_Final Stats Analysis 2, version 1 revision 2, 16 March 2010.

4.9 Road Traffic Accident/Found Dead badger results

There were 90 badgers submitted for *post mortem* from 25/06/2006 to 21/10/2009 of which 33 badgers had been enrolled into the study (Table 25). One was not suitable for *post mortem* due to time from death to collection and submission and was too decomposed.

Table 25: Allocation to treatment of 33 study badgers submitted for post-mortem examination

Enrolled Study Badgers	Vaccinated	Unvaccinated
33	18	15

Of the 18 vaccinated badgers enrolled in the study and submitted for *post mortem*, one was not subjected to *post* mortem examination due to the carcass being collected a number of days after death and autolysis was too advanced. Two badgers had lesions identified in the lumbar muscle area but they were not associated with a vaccination reaction.

Data are presented in Appendix 23: Study Badgers Post Mortem and Culture Results, and are summarised in Table 26.

Table 26: Summary of post-mortem findings

Enrolled Study Badgers*	Visible Lesion M.bovis +ve[1]	Visible Lesion M.bovis -ve[1]	Non Visible Lesion M.bovis +ve[1]	Non Visible Lesion M.bovis -ve[1]	Non Visible Lesion BCG +ve[1]
Vaccinated	4	0	3	9	1
Unvaccinated	2	0	3	10	0

*32 badgers examined
[1]Tissues culture positive or negative

There were 5 badgers enrolled in the study that died whilst under anaesthetic or were euthanized on welfare grounds. Table 27 below documents the details.

Table 27: Details of badgers that died while under anaesthetic or were euthanized

Date	Badger ID	Age	Sex	Vaccine/Control Allocation	Comments
26/06/06	BVS-V364	Adult	Female	N/A	Died during anaesthetic recovery in T1 before treatment allocation decided. *Post mortem* examination indicated most likely cause of death to be acute heart failure.
07/0708	BVS-V895	Adult	Male	Vaccine	Euthanasia carried out by named veterinary surgeon after examination under anaesthetic and prior to vaccination on welfare grounds. It was extremely emaciated and had a number of bite wounds. It had not been trapped or vaccinated previously. At *post mortem* examination the badger had visible TB lesions and *M. bovis* was cultured from the lymph nodes.
08/07/09	BVS-V678	Adult	Male	Control	Died during anaesthetic recovery. At *post mortem* it was found to have a heavy ectoparasite burden, empty stomach and full bladder. Veterinary opinion was that it has a possible concurrent viral infection which predisposed it to the post sampling anaesthetic death. There were no visible TB lesions nor was *M. bovis* cultured from the lymph nodes.
08/07/09	BVS-V551	Adult	Female	Control	Sudden death whilst being sampled. At *post mortem* it was found to have very poor dentition and impacted rectum which may have led to circulatory failure. There were no visible TB lesions nor was *M. bovis* cultured from the lymph nodes.
06/10/09	BVS-V093	Adult	Male	Vaccine	Heart and breathing stopped during sampling procedure. Badger resuscitated but euthanized by named veterinary surgeon on welfare grounds as not recovering from anaesthetic after 4 hours of monitoring and deemed unfit to release. Badger had been trapped 7 times and vaccinated twice (23/06/2008 and 22/06/2009). At *post mortem* examination the badger had visible TB lesions and *M. bovis* was cultured from the lymph nodes.

4.10 Recommendations regarding culling of badgers at end of study

As per the study protocol a review point was scheduled for November/December 2008 in order to review data and make a decision on the need for further live capture and/or *post mortem* assessment of pathology in 2009. The review was completed at a meeting of the TBVSDG on 3rd December 2008 where the interim analysis report by Fera (Appendix 4) and a paper by [redacted] "Scientific case for post mortem analysis of animals in the Badger Vaccine Study" (Appendix 24) were discussed. At this meeting it was agreed that there was insufficient scientific grounds to justify culling badgers in 2009 and the decision was made that the study would continue in 2009 with the schedule being the same as in 2008, i.e. two trapping sessions where badgers in the whole study area are trapped, sampled and if allocated to a vaccine group being vaccinated in June-July 2009. For the September-October trapping session, badgers would be sampled and any badger allocated to a vaccine group that was NOT vaccinated in the previous trapping session vaccinated. One of the objectives in the study protocol was to collect data in such a way as to increase the opportunity to detect efficacy of BCG in wild badgers. Investigated and documented in N1KT Vaccination Trials - Exploration of Statistical Methods V1 (Appendix 3) were various potential scenarios for the extension of the protocol after the end of 2008. The report concluded *"increased trial duration would improve the accuracy with which the vaccine effect can be estimated and that this pattern is consistent across a wide range of efficacy levels*" and *"Further, the increased duration of the trial would allow increased accuracy of the estimation of the treatment effect (i.e. greater power)."* Continuing to run vaccinate and control groups in 2009 would allow further data to be collected, which when added to the data collected to date, would increase the opportunity to detect efficacy in the field by increasing the statistical power of the study. The additional data would also help to compensate for the missing T5 data in 2007, which would otherwise have reduced the power of the study. Study Protocol Amendment 11 documenting the change to vaccinating badgers in the vaccinate groups in 2009 was agreed and signed by the sponsor. Defra requested that the decision on culling should be revisited in 2009.

At a meeting of the TBVSDG on 24th February 2010 a paper by [redacted] "Decision to cull at the end of the BVS - revised v2_12_Jan_10" was discussed. This document underwent several iterations following input from members of the TBVSDG, resulting in the final version presented in Appendix 25. This document was presented and discussed at a Vaccine Programme Advisory Group meeting on 27th April 2010.

It should be noted that at the time the Study Protocol was written it was not envisaged that vaccine efficacy data could be obtained experimentally. However, these data were obtained subsequently through completion of two studies conducted at the VLA between November 2006 and March 2009. The data from these were submitted to the VMD as part of the Marketing Authorisation application for BadgerBCG. For this reason, obtaining evidence of vaccine efficacy was no longer reliant on this study.

4.11 Statistical Analyses

Interim Statistical Analysis

As specified in the study protocol and planned at the TBVSDG 02/09/2008, interim statistical analysis was undertaken in late 2008 (IFNγ EIA and Stat-Pak data) to early 2009 (culture data added); the blinding of treatment codes was maintained. The agreed method of Generalized Linear Model (GLM) (McCullagh and Nelder 1992) was the method used on an interim dataset (Appendix 26: Excluded Badgers for Interim Report) supplied by the VLA, which contained the full test history of all badgers eligible for analysis. Eligibility for analysis was defined in advance in the Standard Operating Procedure for the statistical analysis. Some pre-selection of data was performed at the VLA (exclusion of T1 & T3, exclusion of

individual data prior to the date they entered the trial as vaccinates for control animals in social groups that later joined vaccinated groups) and a dataset passed to CSL/Fera. Further filtering of data was performed at CSL/Fera to exclude animals positive at first capture and captured only once, as these cannot be used to calculate rates of new incidence in positive test results. Any animal with missing test data were also excluded. Once observed to have given a positive test result, the status of an animal was taken to be positive for the remainder of its observed history, thus assuming any further negative test results were false and due to imperfect test sensitivity rather than a real change in underlying status. The unit of analysis was the badger social group and data analysed were the number of new incidences of positive test outcomes within each social group. The distribution of the data was therefore binomial (number of new incidences / total eligible social group size: the total number of animals test negative at initial capture) and the link function used was the logit link: transformed probability of positive test result, p, as log odds ratio = log (p/(1-p)).

Five outcomes were investigated in turn:
1. New positive incidences for IFNγ EIA
2. New positive incidences for Stat-Pak
3. New positive incidences for Culture
4. New positive incidences for either the Stat-Pak or Culture
5. New positive incidences for either of the above three tests.

Part A of the Interim Analysis (IFNγ and Stat-Pak only), was considered confidentially by the statistical and science members of the project team. An agreed text recommending that the study should continue was reported to the TBVSDG on 03/12/2008. Prior to this meeting, parameters in the Newcastle University Simulation model were updated to reflect more accurately the size and design of the field study and the latest information on test performance (using data from contemporaneous studies employing the same test methods at Woodchester Park). The model was “tuned” to mimic the observed results of the serology (Stat-Pak) test and used to evaluate options for continuation of the study. Results from these simulations were summarised and presented orally to the TBVSDG on 03/12/2008 to aid decision making. These results showed that continuing the vaccination protocol for one or more years would very likely increase the chance of finding a statistically significant effect on serological (Stat-Pak) tests, although not very likely on the primary efficacy variable (all three tests combined).

N1KT Badger TB Vaccine Field Trial _Interim Stats Analysis_A& B_ V1 (Appendix 4) documents the statistical methods and results from the full interim analysis including culture data. The analysis and report was thoroughly checked by the statistical team according to the SOP SIS101 and independently audited by the external statistical auditor. Peer review was provided by other members of the TBVSDG. The analysis and report passed all checks at that time. Following later revision of the analysis procedure (see below) these interim analyses were repeated in the same way as the final analyses performed. A revised interim analysis report is presented in N1KT Badger TB Vaccine Field Trial (Interim Stats Analysis 2) V2.doc (Appendix 27).

Final Statistical Analysis

Following completion of the field work and associated laboratory tests, checked data were passed to the statistical analysis team at Fera on 20/01/2010 (Appendix 28: Excluded Badgers for Final Report. The agreed data filtering and GLM analyses were carried out, checked and reported to the TBVSDG on 17/02/2010. Quality control on this occasion also included an independently programmed analysis in R, carried out by the Newcastle University team using code developed for the analysis of simulated data for study extension options modified to accept the observed field data. All checks indicated the data were

correctly pre-processed and the GLM analyses were correct. Peer review by the TBVSDG highlighted an area of ambiguity in the pre-filtering of data that affected only the secondary variables for analysis – the primary variable (combination of all three test results), being unaffected. The issue was whether for analysis of single test results, data should be filtered using only that test (called the "per test filter") as was done in the interim analysis reported in 2009, or whether a pre-filter condition that only animals negative for all three tests simultaneously at first capture (called the "all test filter") should be applied across all analyses including those for single tests. This rule was also applied to the analysis of Stat-Pak + Culture. It was agreed at the TBVSDG meeting on 17/02/2010 that the application of the "all test filter" across all analyses made more 'biological sense' and would give a better indication of the prophylactic efficacy of the vaccine by including only those badgers considered to be free of TB preceding vaccination. The request for further analysis according to the "all test filter" criterion was made prior to generation or sight of any results from such an analysis. These additional analyses were then performed, checked and presented to the TBVSDG on 24/02/2010. Given the intention of the "all test filter" to only pass for analysis those badgers considered to be free of TB preceding vaccination, a further minor amendment to the process was made whereby T1 data were considered for the purpose of pre-filtering only: any animal that was positive at T1 and subsequently negative at first capture from T2 onwards would now be excluded from the analyses. This is consistent with the treatment of the rest of the data, where a negative following a positive test result is assumed false. Nine animals were identified as falling in this category. The changes were documented on the SOP SIS101 (Appendix 14) and, following receipt of a revised data set now including T1 data, the analyses were repeated, checked and a revised report issued on 16/03/2010 (Appendix 21). Appendix 29 N1KT Badger TB Vaccine Field Trial _Final Stats Analysis 2 Additional Tables & Figures Version 1 dated 31 March 2010 contains the tables and figures summarising the data used in the final statistical analysis.

4.12 Unblinding of treatment allocation

On 24th March 2010 [redacted] was advised of the treatment allocation, as agreed by the chairman of the TBVSDG, to allow him to complete the final draft report conclusions and discussion. On 14th April 2010 the following people were sent a draft copy of this report for comment: [redacted]. This effectively unblinded them to treatment allocation.

5. Discussion

5.1 Safety

Safety data collected in 2006 are documented in an interim study report dated 1 December 2008, Appendix 2: BVS Safety Interim Report. A summary of safety data collected 2006 to 2008 is documented in Appendix 30: Update for VMD Dossier of Safety Data from BVS April_2009.

In 2006 on day 4 of the two vaccination sessions T2 and T3, body temperatures were measured from the microchip placed subcutaneously. The range of temperatures recorded was between 30.2 to 40.5 ºC. Given their subcutaneous location it is likely that the Plexx microchips are more responsive to reductions in local skin temperatures (see Appendix 16). There are few studies recording rectal or subcutaneous temperatures to establish the normal range for wild badgers (Davison et al., 2004, McLaren et al., 2005). This range will be dependent on the unknown health status and pre-anaesthetic stress, therefore pyrexia is difficult to assess. Only 3 of the vaccinated badgers monitored for 24 hours had a

temperature recorded as 40ºC or higher: values of 40.0ºC, 40.3ºC and 40.5ºC were recorded within 2 hours of vaccination and two of the badgers in question were observed to be very active. At 24 hours the temperatures were 38.1ºC, 38.0ºC and 36.2ºC, respectively. The badger with the highest temperature (40.5ºC) tested positive to IFNγ and negative to both Brock TB Stat-Pak and *M. bovis* culture**.** The other two badgers tested negative for both IFNγ and Brock TB Stat-Pak and were *M. bovis* culture negative.

This supports the conclusion that BCG vaccine does not cause a discernable hyperthermia after administration to badgers.

All badgers were monitored on recovery from anaesthesia and were not released at site of capture unless a release form (BA606) was signed by a veterinary surgeon. There were no adverse reactions noted prior to release. It was noted on clinical observation during recovery and prior to release that vaccinated badgers behaved indistinguishably from control animals.

Reactions at the site of injection were considered mild and veterinary opinion was that the impact on the individuals re examined was minimal. No skin ulceration was observed in any of the badgers re-examined two weeks post-vaccination that would indicate local reaction following inoculation with BCG. Of 142 badgers vaccinated in 2006, 48 badgers were trapped 2 weeks later and 5 had intramuscular swellings. A total of 265 badgers vaccinated during the study were re-trapped and 22 badgers were found to have intramuscular swellings but there were no associated skin lesions. The swelling size, which included skin thickness, ranged between 5 to 23mm.

18 vaccinated badgers were submitted for *post mortem* examination, having been found dead in the study area, died during sampling or euthanized on welfare grounds. There were no lesions at vaccination sites associated with administration of BCG vaccine. BCG was cultured from a sample of pooled lymph nodes from one cub vaccinated 12/06/2007 and found dead 03/09/2007 but there were no macroscopic lesions identified. In total, 3657 clinical samples were submitted for BCG culture and no BCG was grown.

This supports the conclusion that BCG vaccine is safe to wild badgers and is not excreted when administered intramuscularly at a dose of 2-8 x 10^6 CFU.

5.2 Efficacy

Vaccine efficacy in the context of BCG vaccination of badgers may be defined either as a reduction in the incidence of uninfected badgers becoming infected with *M. bovis* or a reduction in the progression/severity of TB in badgers that do. The effect of vaccination is measured with reference to a non-vaccinated control group. According to this definition it was not possible to estimate the efficacy of BCG vaccination in this study as the decision was taken not to subject study badgers to post-mortem determination of infection. However, it is possible to use the tests employed in this study (IFNγ EIA, Stat-Pak, culture) in live animals as surrogate measures of vaccine efficacy.

The IFNγ EIA test measures the production of IFNγ following stimulation of whole heparinised blood with bovine and avian tuberculins and has an estimated sensitivity of 80.9% and an estimated specificity of 93.6% (Dalley et al., 2008). It is the most sensitive test currently available for detecting infection with *M. bovis* in the live animal but the dose of BCG used in this study is able to generate a response measurable by the IFNγ EIA. To control for the confounding influence of vaccination on the IFNγ EIA result in the short-term (~2 weeks), data from T3 were excluded from the analysis. However, at other time points post-vaccination a positive IFNγ EIA result could still be due to vaccination rather than infection with *M. bovis*. As such, new incident cases of IFNγ EIA positivity in the vaccinated

group would be expected to arise from vaccination as well as genuine *M. bovis* infection. The effect of vaccination on reducing the incidence of *M. bovis* infection is therefore likely to be greater than reported in this study.

The Brock TB Stat-Pak is a lateral flow assay that detects the presence of antibodies in serum to *M. bovis* antigen MPB83. It has an estimated sensitivity of 49.2% and an estimated specificity of 93.1% (Chambers et al., 2008). Sensitivity of the Stat-Pak varies according to disease severity, such that sensitivity was found to be 34.4% in infected badgers with no visible lesions at *post mortem*, 66.1% in infected badgers with visible lesions at post-mortem, 41.7% in infected badgers that excrete *M. bovis*; rising to 78.1% in so-called 'Super-Excretor' badgers (Chambers et al., 2008). Vaccination with BCG does not generate a positive Brock TB Stat-Pak result because the vaccine strain is a constitutively low expresser of the principle target antigen in the test (MPB83) (Charlet et al., 2005).

Isolation of *M. bovis* by culture is pathognomonic for TB in a badger. However, excretion of *M. bovis* from tuberculous badgers has been shown to be both relatively insensitive and intermittent (Chambers et al., 2002). Therefore, whilst regarded as 100% specific, it is the least sensitive of the assays used to detect TB in the live badger.

Analysis of positive test incidence in the two treatment groups is presented in Appendix 21. The primary measure of efficacy stated in the Protocol was the difference between treatments in the proportion of cases of new incidence in positive outcome to any of the three tests. Using all three tests to define a TB case is the most sensitive method available for the live animal although we cannot exclude the possibility that a positive IFNγ response in the vaccine treatment group was induced by BCG vaccination. For all three tests combined there was a reduction from 41.5% cases (95% confidence interval: 28.0%, 56.3%) of new incidence in the unvaccinated group down to 31.1% cases (95% confidence interval: 22.7%, 41.0%) of new incidence in the BCG vaccinated group. **In this analysis there was no evidence of a statistically significant treatment effect (P > 0.05) although there was a reduction in incidence in the BCG vaccinated group**.

The final analysis also investigated possible differences between treatments in the proportion of cases of new incidence of positive outcome of each of the three tests separately. In addition, Stat-Pak and culture were analysed together. This represented an exploratory approach to assess the efficacy of the vaccine based on different scientific hypotheses for how efficacy can be measured. For admission into the analysis each animal had to be negative for the test(s) of interest at T1 (if captured then) and at the time of first capture/vaccination from T2 onwards. However, each animal did not have to be negative to all tests to be excluded from the analysis. Thus, for example, an IFNγ EIA positive animal was included in the analysis if it was negative to Stat-Pak and/or culture. The analyses shown in the main part of the report at Appendix 27 reveal no significant effect of vaccination (P > 0.05) for each of the tests either singly or in combination. However, in all cases there was a reduction of cases in the vaccine group compared to the control group. For the IFNγ EIA there was a reduction from 35.0% cases (95% confidence interval: 22.6%, 49.8%) of new incidence in the control group down to 31.1% cases (95% confidence interval: 23.1%, 40.4%) of new incidence in the vaccinated group. For the Stat-Pak test there was a reduction from 18.2% cases (95% confidence interval: 11.2%, 28.3%) of new incidence in the control group down to 10.5% cases (95% confidence interval: 6.6%, 16.1%) of new incidence in the vaccinated group. For the culture test there was a reduction from 9.6% cases (95% confidence interval: 5.2%, 17.1%) of new incidence in the control group down to 8.1% cases (95% confidence interval: 5.2%, 12.5%) of new incidence in the vaccinated group. For the Stat-Pak and Culture tests combined there was a reduction from 21.4% cases (95% confidence interval: 13.4%, 32.5%) of new incidence in the control group down to 14.0% cases (95% confidence interval: 9.3%, 20.6%) of new incidence in the vaccinated group. **In this analysis there was no evidence of a statistically significant treatment**

effect (P > 0.05) although there was a reduction in new incidence in all cases in the BCG vaccinated group.

This observation is in contrast to the findings presented in Appendix B of the same report. Here an "all test" exclusion criterion was used such that any badger positive by any of the three tests at T1 or at the time of first capture/vaccination (T2 onwards) was excluded from the analysis. This analysis addresses more directly the prophylactic effect of BCG vaccination since the effect of vaccination is measured in badgers considered to be free of TB by virtue of negative results in all three tests. Whilst this does not rule out infection completely, it is the best measure of TB status in the live animal. As the combination of all three tests would not be 100% sensitive, some badgers regarded as TB-free by this criterion would actually harbour infection. This would have the effect of reducing the measure of vaccine efficacy. Against this background, the incidence of IFNγ EIA positivity was reduced by vaccination from 35.0% cases (95% confidence interval: 23.0%, 49.3%) to 28.5% cases (95% confidence interval: 20.8%, 37.7%) but it was not significant at the 5% level.

The analysis presented currently for the IFNγ EIA test alone provided **no conclusive (P < 0.05) evidence that BCG vaccination was able to prevent infection with *M. bovis*, although the trend was in that direction.** However, failure to detect a significant effect of vaccination in this respect could be a consequence of: (1) 'false positive' test results among vaccinates arising directly from the vaccine itself; (2) inclusion of animals with TB arising from 'false negative' results in all three tests.

In contrast, vaccination was found to have a significant effect on reducing the incidence of positivity for both Stat-Pak or Stat-Pak and culture combined. The incidence of Stat-Pak positivity was reduced by vaccination from 17.1% cases (95% confidence interval: 10.8%, 25.9%) to 4.4% cases (95% confidence interval: 2.4%, 8.2%), which was significant statistically (P < 0.001). The incidence of Stat-Pak and culture combined positivity was reduced by vaccination from 21.7% cases (95% confidence interval: 13.5%, 32.9%) to 8.3% cases (95% confidence interval: 4.9%, 13.9%), which was also significant statistically (P = 0.008). As the likelihood of a positive Stat-Pak result or excretion of *M. bovis* increases with disease progression/severity (Chambers et al., 2008; Gallagher & Clifton-Hadley 2000) **this study provides evidence consistent with the progression/severity of TB being significantly reduced in BCG vaccinated badgers after they become infected.**

These observations are consistent with the reported effect of BCG vaccination in naïve individuals in that its effect is more frequently expressed in terms of reducing disease progression rather than preventing infection *per se* (reviewed in Suazo et al., 2003).

It is beyond the scope of this study and report to comment with any certainty on the potential for IM BCG vaccination to break the onward transmission of *M. bovis* to susceptible individuals. However, a vaccine that can reduce the likelihood of an animal progressing to later stages of disease where *M. bovis* is excreted is likely to have this beneficial effect.

6. Conclusions

The data presented in this report support the conclusion that BCG vaccine is safe to use in wild badgers in the field when administered via the intramuscular route confirming the observations in the GLP Safety study. No BCG has been cultured from clinical samples submitted in the study and this supports the conclusion that when BCG is administered by the intramuscular route it is not excreted.

By use of three tests employed in live animals (IFNγ EIA, Stat-Pak, culture) as surrogate measures of vaccine efficacy, the following conclusion were drawn from this study based on the analytical approach outlined in the Study Protocol and Appendix 21:

- This study provides evidence consistent with the progression/severity of TB being significantly reduced in BCG vaccinated badgers after they become infected.

- The positive effect of BCG vaccination on preventing infection was too small to reach statistical significance in a study of this size. 'False positive' test results among vaccinates arising directly from the vaccine itself and inclusion of animals with TB arising from 'false negative' test results mean the effect of vaccination on reducing the incidence of *M. bovis* infection is therefore likely to be greater than reported in this study.

7. Administration and Compliance

Location of study documentation: Current and Final

Appendix 31: Badger Vaccine Study Files documents the list of files containing the study documentation. The files will be held in [redacted], VLA, [redacted] until the final audit by [redacted] has been completed and then they will be moved to the VLA archive.

Project Meetings
Regular meetings were held with key staff members to discuss the management of the day to day running of the study. The sponsor, study monitor and study auditor attended several meetings. Agenda and minutes are held on file BVS 9020.

TB Vaccine Study Data Group (TBVSDG)
There were 17 (up until February 2010) TBVSDG Meetings to discuss data issues. Agenda and minutes held on file BVS 9122.

Staff Training

Staff who worked in the study are listed in Appendix 1: BVS Organograms Versions 1 to 6.

The Home Office issued an Animals (Scientific Procedures) Act 1986 Project Licence Number: PPL 70/6415 to permit the programme of scientific procedures in the study. All staff members anaesthetising, sampling or vaccinating badgers, being regulated procedures under the act, held Home Office Personal Licences for the duration of the study. Details of the licence and copies of personal licences are held on File SEB1267: BVS A(SP)A Paperwork Project OM0023. Annual unannounced visits were made by Home Office inspectors to inspect work being carried out and documentation on files.

Study File BVS 9014 contains details on staff training and holds A(SP)A PIL competency registers, training summary of laboratory staff at the [redacted] laboratory and VLA [redacted], signature lists for risk assessments, details of "dry run" trainings following standard operating procedures used at the sampling and laboratory facility at [redacted], laboratory at VLA [redacted] and bacteriology laboratory at VLA [redacted]. Date of training, details of training and trainer, names of trainees and any observers were recorded.

Monitor and Auditor Visits

Appendix 32 documents the monitor and auditors visits. A copy of the final audit report by [redacted] will be added to the study documentation on its completion and receipt by the study investigator.

Data Management

A database was specifically developed for this study and is managed by the Data Systems workgroup. The workgroup is in the department of Centre for Epidemiology and Risk Analysis (CERA) at VLA.

Details of data management are held on file: CER1491: BVS System Specification and contains the following information:

1. BVS System Specification: summary of database specification
2. Report on Version changes to BA596 data capture form
3. Data standard operating procedures
4. Data management users: documents overall data flow and reports generated
5. Data Provision Service: documents types of data which can be obtained from the system
6. System Management: documents archiving and security user permissions
7. BVS Hardware: hardware used including chip information
8. Quality Control and Validation Procedures: including quality control reports
9. Software Incident Reporting and Change Management: Bugs found and resulting actions
10. Reporting to External Researchers: procedures to be followed on request for data
11. Database Dictionary

8. Acknowledgements

The authors would like to thank the valuable contribution of all personnel, past and present, involved in this study. Their names are listed in Appendix 1: BVS Organograms Versions 1 to 6.

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List of Appendices

Number	Title
1	BVS Organograms Versions 1 to 6
2	BVS Safety Interim Report
3	N1KT Vaccination Trials - Exploration of Statistical Methods V1
4	N1KT Badger TB Vaccine Field Trial (Interim Stats Analysis A & B) V1
5	Standard Operating Procedures List
6	Forms List
7	Functions and Standard Operating Procedures Lists
8	Examples of RP1 and RP6
9	Test Validity Criteria and Test Interpretation Guidelines into the badger IFN-gamma ELISA SOP BAC0251
10	Newcastle Simulation model description
11	Assignment of optimum proportion of subjects to treatment group in order to maximise efficacy
12	Examination of effect of combination of TB tests
13	Immunisation methods
14	Management of the Analysis of the BVS V2.2
15	Control and Residue Titration Results
16	Correlation of rectal and chip temperatures
17	Vaccination Site Examination Report
18	Digital Photographs of badgers vaccination sites with intramuscular swellings
19	Excel Report Summary_All
20	Report - amended 19/12/05
21	N1KT Badger TB Vaccine Field Trial Final Stats Analysis 2_ V1
22	Study Culture Results
23	Study Badgers Post Mortem and Culture Results
24	Scientific case for post mortem analysis of animals in the Badger Vaccine Study [redacted]
25	Decision to cull at the end of the BVS - final assessment
26	Excluded Badgers Report For Interim Report
27	N1KT Badger TB Vaccine Field Trial _Interim Stats Analysis 2 V2
28	Excluded Badgers Report For Final Report
29	N1KT Badger TB Vaccine Field Trial Final Stats Analysis 2 Additional Tables & Figures_ V1
30	Update for VMD Dossier of Safety Data from BVS April 2009
31	Badger Vaccine Study Files
32	BVS Monitor and Auditors Visits