THE DESIGN OF A FIELD TRIAL TO TEST AND VALIDATE THE PERFORMANCE OF CATTLE BCG VACCINE AND ASSOCIATED DIVA DIAGNOSTIC TEST IN ENGLAND AND WALES

DEFRA PROJECT SE3287

Short Title: Feasibility study into testing and validating cattle BCG vaccine and DIVA

Sponsor:
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Welsh Government

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### Glossary

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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</thead>
<tbody>
<tr>
<td>AFU</td>
<td>Approved Finishing Unit*</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse Event(s)</td>
</tr>
<tr>
<td>AHVLA</td>
<td>Animal Health and Veterinary Laboratories Agency</td>
</tr>
<tr>
<td>APHA</td>
<td>Animals and Plant Health Agency (formerly AHVLA)</td>
</tr>
<tr>
<td>ARV</td>
<td>Attack Rate in Vaccinated</td>
</tr>
<tr>
<td>ARU</td>
<td>Attack Rate in Unvaccinated</td>
</tr>
<tr>
<td>A(SP)A</td>
<td>Animals (Scientific Procedures) Act 1986</td>
</tr>
<tr>
<td>ATC</td>
<td>Animal Test Certificate</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette-Guérin vaccine strain of <em>Mycobacterium bovis</em>. &quot;Danish&quot; BCG strain 1331</td>
</tr>
<tr>
<td>BioSS</td>
<td>Biomathematics &amp; Statistics Scotland</td>
</tr>
<tr>
<td>BS</td>
<td>Blood Sample</td>
</tr>
<tr>
<td>bTB</td>
<td>Bovine tuberculosis</td>
</tr>
<tr>
<td>ca.</td>
<td>circa</td>
</tr>
<tr>
<td>CE</td>
<td>Clinical Examination</td>
</tr>
<tr>
<td>Consortium</td>
<td>Group led by Triveritas**</td>
</tr>
<tr>
<td>CP</td>
<td>Control Product (non-immunological vaccine placebo)</td>
</tr>
<tr>
<td>CRO</td>
<td>Contract Research Organisation</td>
</tr>
<tr>
<td>DDU</td>
<td>Disease Dynamics Unit</td>
</tr>
<tr>
<td>Defra</td>
<td>Department for Environment Food and Rural Affairs</td>
</tr>
<tr>
<td>DIVA</td>
<td>Differentiate Infected from Vaccinated Animals</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>GCPv</td>
<td>Veterinary Good Clinical Practice</td>
</tr>
<tr>
<td>GLP</td>
<td>Good Laboratory Practice</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>HO</td>
<td>Home Office</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon Gamma</td>
</tr>
<tr>
<td>Inv</td>
<td>Investigator</td>
</tr>
<tr>
<td>ISO</td>
<td>Injection Site Observations</td>
</tr>
<tr>
<td>IVP</td>
<td>Investigational Veterinary Product (BCG Vaccine)</td>
</tr>
<tr>
<td>LCA</td>
<td>Latent Class Analysis</td>
</tr>
<tr>
<td>LL</td>
<td>Lung Lesions</td>
</tr>
<tr>
<td>MA</td>
<td>Marketing Authorisation</td>
</tr>
<tr>
<td>MoU</td>
<td>Memorandum of Understanding</td>
</tr>
<tr>
<td>mth</td>
<td>Month</td>
</tr>
<tr>
<td>MS</td>
<td>Milk Sample</td>
</tr>
<tr>
<td>NC</td>
<td>Natural Challenge</td>
</tr>
<tr>
<td>NS</td>
<td>Nasal Swab</td>
</tr>
<tr>
<td>OIE</td>
<td>&quot;Office International des Epizooties&quot; World Organisation for Animal Health</td>
</tr>
<tr>
<td>PASS</td>
<td>Procurement and Social Sciences (Defra advisory group)</td>
</tr>
<tr>
<td>PM</td>
<td>Post Mortem</td>
</tr>
<tr>
<td>PMA</td>
<td>Provisional Marketing Authorisation</td>
</tr>
<tr>
<td>Post-vacc</td>
<td>Post Vaccination</td>
</tr>
<tr>
<td>Pre-vacc</td>
<td>Pre Vaccination</td>
</tr>
<tr>
<td>RCVS</td>
<td>Royal College of Veterinary Surgeons</td>
</tr>
<tr>
<td>RT</td>
<td>Rectal Temperature</td>
</tr>
<tr>
<td>SAE/SAEs</td>
<td>Serious Adverse Event(s)</td>
</tr>
<tr>
<td>SD</td>
<td>Study Day</td>
</tr>
<tr>
<td>SICCT</td>
<td>Single Intradermal Comparative [test] Cervical Tuberculin [test]</td>
</tr>
<tr>
<td>SIT [test]</td>
<td>Single Intradermal Tuberculin [test]</td>
</tr>
<tr>
<td>SOG</td>
<td>Scientific Oversight Group***</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>TA</td>
<td>Treatment Administrator</td>
</tr>
<tr>
<td>TBC</td>
<td>To Be Confirmed</td>
</tr>
<tr>
<td>UC</td>
<td>Untreated Controls</td>
</tr>
<tr>
<td>Vacc</td>
<td>Vaccination</td>
</tr>
<tr>
<td>VICH</td>
<td>International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products</td>
</tr>
<tr>
<td>VMD</td>
<td>Veterinary Medicines Directorate</td>
</tr>
<tr>
<td>WG</td>
<td>Welsh Government</td>
</tr>
<tr>
<td>WP</td>
<td>Work Package</td>
</tr>
<tr>
<td>WPPn</td>
<td>Work Package Project number ‘n’</td>
</tr>
<tr>
<td>y</td>
<td>Year</td>
</tr>
</tbody>
</table>

Note: Standard Système Internationale (SI) units and other generally accepted text abbreviations have been excluded from this list.

* Farms which receive cattle from herds under bTB control restrictions.

** Consortium members and core expertise are given on Page 6.

*** Group of independent scientific advisors to Defra on cattle vaccine field trial issues.
Consortium Members and Core Expertise

- Triveritas Ltd - design and conduct of field studies, regulatory affairs expertise and project management
- School of Management and Business (SMB), Aberystwyth University - socio-economics and the dairy industry
- The Disease Dynamics Unit (DDU), Department of Veterinary Medicine, University of Cambridge - modelling and trial design expertise
- BioSS - statistical input to trial design and results analysis
- XLVets Ltd – sourcing and co-ordination of participating veterinarians, AFUs and farms
- APHA - technical input on DIVA test and BCG vaccine
- Two independent veterinarians – providing independent expertise on bTB.

The structure of the Consortium is a simple, collaboration relationship with Triveritas as Lead Consortium Partner.

Disclaimer

The information presented in this report is based on current thinking and understanding within the Consortium at the time of writing. Study designs and other proposed actions may require revision as new information becomes available. Information from other studies subsequently completed as a part of the same project or external studies with relevance to the objectives of this project and any regulatory development should be taken into account when finalising study specifications.
Executive Summary

BCG vaccine sensitises cattle to the tuberculin test used for TB surveillance. As a result its use is currently banned in the EU. Defra and the Welsh Government commissioned a Consortium led by Triveritas to produce proposals for the design of cattle field trials of a BCG based vaccine and for a test to differentiate infected from vaccinated animals (DIVA) that can be undertaken in England and Wales.

The Consortium comprises: Triveritas Ltd, Cumbria; Disease Dynamics Unit, Department of Veterinary Medicine, University of Cambridge; School of Management and Business (SMB), Aberystwyth University; XLVets Ltd UK Ltd, Carlisle; the James Hutton Institute, Biomathematics & Statistics Scotland (BioSS), Edinburgh; the Animal and Plant Health Agency (APHA) Weybridge; and two veterinary surgeons with specific bTB experience.

The proposals put forward for the design of trials should provide sufficient evidence: to support a Marketing Authorisation application for the vaccine in the United Kingdom; and to provide evidence to secure international support for cattle vaccination and validation of the DIVA test. Our findings, which we think indicate this study could not be performed as part of these initial field trials, should be reviewed with international regulators.

Field Safety trials (a) are to confirm safety to consumers and unvaccinated cattle during field use. These trials which are to validate existing laboratory safety studies could be started relatively promptly. They are the lowest price, and the socio-economic evidence suggests that they should be a priority because farmers see this as a business critical issue. The results could be a go or stop decision point for the progress of further trials.

Field Efficacy trials (c) is to confirm the suitability of the test to international standards. This report suggests that before starting these trials additional regulatory guidance should be sought to ensure that some technical issues can be feasibly and cost-effectively addressed such that the subsequent trial results should be acceptable to the regulators.

International scientific opinion suggests the trials to investigate efficacy of the BCG vaccine and its safety to vaccinated cattle during field use (c), demonstrate a statistically significant effect (which means that some trial cattle would be unvaccinated). A validated DIVA is required for evaluation of the

<table>
<thead>
<tr>
<th>Field trials</th>
<th>Trial designs / options</th>
<th>Suggested locations and size</th>
<th>Approx. costs</th>
<th>Approx. duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Field Safety</td>
<td>WPP 8.1, 8.2, 8.4</td>
<td>Low risk TB areas. Approx. 7 farms. Approx. 340 cattle</td>
<td>£1.3 million</td>
<td>2-3 years</td>
</tr>
<tr>
<td>b) DIVA test validation</td>
<td>WPP 2.1, 2.4, 2.5, 2.6</td>
<td>Mainly high risk TB areas. Approx. 100+ sites *(AFUs +/- farms). Approx. 32,400 cattle</td>
<td>£20.2 million</td>
<td>3-4 years</td>
</tr>
<tr>
<td>c) Field Efficacy</td>
<td>WPP 6.1, 6.2</td>
<td>Mainly high risk TB areas. Approx. 100 farms. Approx. 12,000 cattle</td>
<td>£35.0 million</td>
<td>4-8 years</td>
</tr>
</tbody>
</table>

*Requires sites with a prolonged high incidence of TB, such as AFUs (Approved Finishing Units)

This summary does not include a very large TB transmission cattle vaccine field trial suggested by an international scientific opinion. Our findings, which we think indicate this study could not be performed as part of these initial field trials, should be reviewed with international regulators.

No attempt has been made to prioritise the other studies or consider other break points since this will depend on further regulatory guidance and policy discussions between Defra and the Welsh Government. The study designs described could be considered to be building blocks which might be assembled in a number of ways into a suitable programme of work.

International scientific opinion suggests the trials to investigate efficacy of the BCG vaccine and its safety to vaccinated cattle during field use (c), demonstrate a statistically significant effect (which means that some trial cattle would be unvaccinated). A validated DIVA is required for evaluation of the
efficacy trials and generally this would be completed first, but this would cause a delay of several years to the start of these efficacy trials. Consequently the report discusses how vaccine efficacy trials (c) and DIVA validation (b) could be performed at the same time.

A Scientific Opinion produced by the European Food Safety Authority (EFSA) indicates that the efficacy trials should ideally use a double-blind randomised test design (or for there to be justification of an alternative approach). This implies that a placebo is used. Development of a suitable placebo to use, on unvaccinated cattle in efficacy trials could be challenging and time-consuming. If the Safety trials (a) were to initiate any field trial programme this would provide vital early safety information for farmers and other stakeholders in the food chain, be a potential early break point for any trials programme, and could also allow more time to:

i. clarify whether a true placebo is required for unvaccinated cattle or whether other approaches (e.g. a blinding mechanism) could be justified. For example a small low cost trial could be conducted to investigate if “masking” (to see if sham vaccination +/- an inert injection) was a potential and economic alternative to a placebo.

ii. consider if there should be investigation into production and/or testing for a suitable placebo (a work plan or budget has not been devised but a notional amount of up to £250,000 is estimated). This could be run concurrent with any “masking” trial to avoid a potential additional time delay, or after it.

iii. progress suggested regulatory guidance on the DIVA.

iv. progress development of a new, potentially lower cost, skin DIVA test.

In summary it is estimated that it could cost approximately £57 million to deliver the trials, but both the price and length of time to complete the trials discussed in the report are only indicative and will be affected by a number of factors. These include policy decisions affecting when trials start and which designs are used, future TB disease incidence in cattle and recruitment rate of cattle into trials, UK regulatory permissions to allow the trials, and resolving some regulatory requirements (including regarding DIVA validation and the potential need for a placebo).

The report also looks at the socio-economic issues surrounding the deployment of this vaccine and DIVA test in the field. The social science work informed the need for a strong communications policy to inform stakeholders, and suggests that trials involving not vaccinating some cattle could be very challenging. This is important when considering the EFSA Scientific Opinion seeking a TB transmission cattle vaccine field trial that we think would potentially need 500-750 entire herds to remain unvaccinated during trials lasting several years (and compared to 500-750 ideally identical but partially vaccinated herds). Our opinion of the research suggests it could be challenging to persuade enough farmers to participate in a field trial of this design, and consequently further discussions with EFSA should be considered. Additional detailed socio-economic work is needed although the headline messages are that this work has confirmed support for cattle vaccination but farmers are cautious about using the vaccine and DIVA because of the unknown impact on their business.

A cost-benefit analysis suggested that the estimated price of field trials is likely to be relatively small (less than 5%) compared to the subsequent estimated costs of both vaccine use and TB testing over a 20 year period. Our analysis indicates that the bulk of the costs and benefits will be realised more than a decade after the start of any trials. It includes a very substantial level of uncertainty due to forecasting over a long period. Based on various assumptions, the estimated Net Present Value* balance of costs over benefits of using the cattle BCG vaccine and DIVA over a 20 year time horizon was £768 million to £986 million. However significant improvements in the cost-benefit analysis were observed after considering several factors including: “intangible” benefits (e.g. animal welfare benefits, psycho-social costs to farmers), efficient and effective targeting of vaccination, exploiting economies of scale in cattle TB testing and ensuring benefits are obtained in larger herds. Further economic assessment would be required if cattle vaccination were to become a component of TB control strategies.

*Net Present Value (or NPV) is a standard method in such work regarding the value of money over periods of time (to appraise long-term projects such as the TB trials and TB vaccine use). NPV compares the present value of money today to the present value of money in the future, taking into account society’s preference for money today over the future and expectations of future economic growth.
1 INTRODUCTION GENERAL CONSIDERATIONS

The call for tender for the design of field trials of a cattle BCG vaccine and associated diagnostic test in England and Wales, was published on 27th December 2013. A Consortium of public and private organisations headed by Triveritas Ltd ("Triveritas Consortium" or "the Consortium") was established and commissioned to undertake this research under Defra project SE3287. This report summarises the research outputs and conclusions for Defra and WG consideration for execution of a programme of trials both in response to the requirements laid-out in the initial call for tender and subsequent discussions with various stakeholder organisations.

Tuberculosis in cattle is an infectious disease presenting a serious and ongoing challenge to the cattle industry in affected regions (notably south-west England and Wales). A final vaccine product must meet the quality, safety and efficacy requirements of EU veterinary medicines legislation in order to be awarded a Marketing Authorisation (MA) for the BCG cattle vaccine by the Secretary of State through the VMD and subsequently for the European Commission to permit its use with associated free trade of vaccinated cattle and their products.

Previously published results from limited field trials outside Europe suggest that BCG will provide protection to approximately 63% of vaccinated cattle but its safety and efficacy in UK field use has not been established. The current SICCT test to diagnose bTB in cattle cannot differentiate between vaccinated cattle and infected cattle and therefore a new test is required to accurately make this differentiation.

The overall aim of project SE3287 is to provide design options for undertaking cattle vaccine field trials in England & Wales and in collecting safety/efficacy study as part of the dossier of evidence towards establishing the component parts of a bTB vaccination policy. The key components are as follows:

1. To consider options for field trials providing validation samples for a novel DIVA diagnostic test.
2. To devise options for efficacy/safety field trial designs for a BCG cattle vaccine.
3. Model likely impacts of such a policy on the UK bTB disease situation and control programme.
4. Establish the acceptability, to relevant stakeholders, of a bTB vaccination and associated testing policy.
5. Establish the work required and analyse the cost/benefit of vaccine deployment in the UK.

This document reports on achievement of the project objectives and makes suggestions for consideration by Defra and WG for viable courses of action to be taken by the organisations eventually charged with execution of the studies identified.

The report is structured to be consistent with both the initial tender request and the research proposal compiled by the Consortium. Four overall Work Packages (WP A, B, C and D) were identified in the tender request and these definitions have been retained. Sections 2 to 5 of this report address these WPs in order. Within the tender itself each WP was further divided into identifiable Work Package Proposals (WPP1 to WPP13), each identifying a study/studies or a parcel of work required to meet the demands of the project. This report presents the results of the Consortium’s deliberations and research under each WPP heading.

In April 2013 APHA received a list of data gaps which would need to be addressed in order to receive an MA following an assessment of a dossier submitted to the Veterinary Medicines Directorate (VMD). This informed the basis of this project alongside the recommendations and conclusions of an EFSA Scientific Opinion document (2013). These are given under the following two sub-headings alongside a short reference to the part(s) of this report which respond to the point(s) made. Other general considerations are discussed under subsequent sub-headings.

1.1 Summary of data gaps to be addressed for a Marketing Authorisation

The tables below list the main areas which could be addressed by the provision of field trial data. In this report, the response to each of these requirements may be covered by single or multiple WPPs.

It should be noted that additional options for the WPP2 study designs are also provided in Annex 1.
Safety

V1 Injection Site Reactions – heat and pain data in calves. Heat and pain in adults from field. Size and duration in all categories of cattle from field, i.e. data from field needed.

V2 Spread – “shed and spread” data required, i.e. monitoring of potential shedding of the BCG vaccine, non-target exposure, environmental survival, etc.

V3 Vaccination of the milking cow – field data required: verification of lab milk culture, and milk yield figures, or dairy cows to be excluded.

V4 Pregnant Cows – field data required: fertility data, abortion data, body temperature data, heat and pain at injection site data.

V5 Breeding males – field data required.

V6 Pre-exposure and Breeds – field data required (injection site, temperature, fertility) for all categories of pre-exposure, and range of breeds.

Efficacy

V7 Reduction in lung lesions.

Associated report section/WPP

Injection site reactions are to be recorded as an integral part of the clinical trials
- WPA, WPP2.1-2.3, Section 2.4, Page 21
- WPB, WPP6.1-6.2, Section 3.3 Page 27
- WPC, WPP8.1-8.3, Section 4.3, Page 33

Protocols for “Shed and spread” studies in milk and nasal secretions are described in the vaccine safety section of the report. Following discussion with Defra and WG other studies (non-target exposure, environmental survival etc.) were not considered to be critical
- WPC, WPP8.1-8.4, Section 4.3, Page 33

Protocols for clinical studies involving pregnant and lactating dairy cattle are described in the vaccine efficacy and vaccine safety sections of the report.
- WPB, WPP6.1-6.3, Section 3.3 Page 27
- WPC, WPP8.1-8.3, Section 4.3, Page 33

Protocols for vaccine safety in breeding males, including dissemination of BCG in semen are described in the vaccine safety section of the report.
- WPC, WPP8.4, Section 4.3, Page 33

The described clinical trials for DIVA evaluation, efficacy and vaccine safety all have requirements to widen the range of breeds and ages recruited as far as feasible.
- WPB, WPP6.1-6.3, Section 3.3 Page 27
- WPC, WPP8.1-8.4, Section 4.3, Page 33

Efficacy

V7 Reduction in lung lesions.

Associated report section

The PM requirements in the clinical trials for DIVA validation and efficacy all require assessment of lung lesions and comparisons between treated and vaccinated animals.
- WPA, WPP2.1-2.3, Section 2.4, Page 21
- WPB, WPP6.2, Section 3.3 Page 27
V8 Field efficacy data – to confirm the dose required. Dose of vaccine used in field trials preferably to include at least some close to the minimum titre.

The clinical trials described in the efficacy section all require a proportion of animals to be tested at the minimum recommended titre.

WPB, WPP6.1-6.2, Section 3.3 Page 27

DIVA

V9 Age restrictions – data on the performance of the DIVA test must be relevant to animals from minimum recommended age of vaccination, or restrictions will apply.

Associated report section
EFSA (2013) stated that a key difference between neonatal calves and older animals that could be important for vaccination and immune response induction is the relatively high circulating numbers of innate T cells in young calves. The adaptive immune system is relatively immature in neonates and increased numbers of innate effector T cells present in young animals may enable effective immune responses to vaccination (Siddiqui et al., 2012).

DIVA validation will be performed in animals >6 months of age. Field data will be collected to demonstrate DIVA performance in animals <6 months.

WPB, WPP6.1-6.2, Section 3.3 Page 27

V10 Full confirmation of performance of the DIVA test under field conditions.

A range of sample sets for DIVA validation will be collected under true field conditions for WPP6.1-6.3. WPP2.1-2.3 provides supportive data.

WPB, WPP6.1-6.3, Section 3.3 Page 27

V11 Impact of repeat vaccination on performance of DIVA test – field data on consecutive annual vaccinations required to confirm absence of impact.

The study designs presented in the efficacy section address this issue.

WPB, WPP6.1-6.3, Section 3.3 Page 27

1.2 EFSA Conclusions and Recommendations

The tables below lists EFSA’s conclusions and recommendations.

Conclusions

E1 The strategy of establishing and attempting to simultaneously evaluate DIVA and vaccine performance in the same field trial poses considerable risks, because only limited conclusions about vaccine performance can be made if test performance is later found to be poor.

Associated report section
Efficacy clinical trials are separated from those designed to have the production of DIVA Validation sample sets as their primary objective.

WPA, WPP2.1-2.6, Section 2.4, Page 21
WPB, WPP6.1-6.3, Section 3.3 Page 27

E2 Evaluation of DIVA test performance (sensitivity and specificity in vaccinated animals) under field conditions should be

A range of sample sets for DIVA validation will be collected under true field
based on a representative sample of the target population. Any proposed deviations from such a design should be justified and the bias that may subsequently be introduced should be accounted for.

E3 At slaughter, all trial animals should be examined in greater detail than is the case with established routine post-mortem inspection, in order to increase the diagnostic sensitivity of the post mortem procedure and enable better estimation of the performance of the DIVA test. Furthermore, in a select number of animals, the determination of the true disease status should be improved by slicing through and inspecting all pre-defined sets of lymph nodes, and by applying supplementary tests.

E4 The DIVA test(s) should be standardised using an SOP and quality assurance that includes, but is not limited to, sampling, test set-up, test data analysis and test interpretations. Training in the correct procedures for sampling should be provided to all relevant persons involved in the field trials. Variations in test parameters can be introduced during the trials, but any change in test format, including the use of new antigens, should be carried out in addition to the original DIVA test set-up.

E5 The double-blind randomised controlled trial design is recommended as it guarantees the lowest possible level of bias. Any proposed deviations from the preferred trial design should be justified and any biases that may subsequently be introduced should be accounted for.

E6 Estimates of the reproductive number should be sufficiently precise to enable conclusions to be drawn about the performance of bTB vaccination, in conjunction with test and cull using the DIVA test, for bTB eradication.

E7 The risk posed by transmission of bTB infection from wildlife to cattle enrolled in the field trials should be considered and accounted for in any trial design.

Enhanced post-mortem procedures will be used in the clinical trials described in:
WPA, WPP2.1-2.3, Section 2.4, Page 21
These procedures will also be used in WPP6.1-6.3
WPB, WPP6.1-6.3, Section 3.3 Page 27

All of the described clinical trials will be conducted to GCPv which requires that analytical procedures should be fully and precisely detailed in SOPs, that adequate training is document for all study personnel and that Quality Control and Quality Audits are an integral feature of the protocols. GCPv also requires that any changes to test parameters are justified, accepted by the trial sponsor and documented.

Blinding issues are discussed in Section 1.3, Page 14, and in the individual trial protocols given under the DIVA validation and efficacy headings.
WPA, WPP2.1-2.6, Section 2.4, Page 21
WPB, WPP6.1-6.3, Section 3.3 Page 27
WPC, WPP8.1-8.4, Section 4.3, Page 33

From research undertaken as part of this report, it seems unlikely that farmers would agree to participate in a trial which would involve 100% of animals in the herd potentially remaining unvaccinated. Particularly if previous field studies (WPB, WPP6.3) performed to generate MA data have shown the vaccine to be effective.
WPB, WPP6.3, Section 3.3 Page 27

All clinical trials require that multiple farm sites are used and that each farm site has a group of animals on site which is a negative control group. This will eliminate bias from variable environmental Mycobacterium challenge
WPA, WPP2.1-2.4, 2.6, Section 2.4, Page 21
E8 Data generated in the trials for DIVA and vaccine performance should include detailed quantitative test signals at the level of the individual animal and data normally recorded in identification and registration systems. At the herd level, annual official bTB status for the previous five years should be provided, as well as possible risk factors associated with both the risk of bTB introduction and within-herd bTB transmission.

E9 Data collected should enable estimation of vaccine-induced reduction of bTB transmission, and not just reduction of bTB incidence, in order to reach a conclusion regarding the contribution of vaccination to eradication.

E10 The field trials should provide safety data to confirm the results obtained in laboratory conditions according to the requirements of the Commission Directive 2009/9/EC. Specifically, data regarding the potential shedding of the BCG vaccine Danish strain via the respiratory route and milk should be provided in order to allow risk assessment of trade in vaccinated animals.

**Recommendations**

E11 A simulation analysis of potential trial results should be performed prior to the start of the trials to ensure that sufficient data are collected during the trials, appropriate to the analytical methods to be used. There is also a need to ensure that the data required for test validation and vaccine performance can indeed be obtained, and that the DIVA test and BCG vaccine performs according to expectations based on the laboratory experiments, while taking account of the many factors that may generate additional variation in the field.

E12 DIVA test performance should be analysed as early as possible in the trials, while continuing to generate more data for analysis of vaccine performance in greater detail. Once the results from test validation are known, the analysis for the power of the field trials for vaccine performance can be repeated, using the updated results.

E13 The use of the tuberculin skin test should be avoided if possible as its use would limit the

**Associated report section**

An *a priori* statistical modelling study for the DIVA validation is presented and discussed in Appendix 2 (Page A5) and modelling of DIVA requirements in Appendix 3 (Page A15). These studies provided a rational calculation of sample numbers required to obtain a reliable DIVA validation and statistically significant results to the pre-defined regulatory requirements and statistically significant data on vaccine performance.

WPP2 studies for generating sample sets for DIVA validation are programmed as the initial activity. It is noted in the protocols for these studies that vaccine performance during these studies will be used to inform the design (by statistical power analysis) of the efficacy studies.

The use of the tuberculin skin test is kept to a minimum although there are potential...
1.3 Blinding and the use of a Control Product

The “gold standard” in regulatory, scientific trial designs is for double blinding to be in place thus eliminating chances of bias. EFSA suggest this should always be the default goal but accept that a pragmatic approach accepting less than perfect procedures may still be justified. Blinding and use of placebo control products (CP) are appropriate to consider under WPA and WPB of this study.

It should be noted that if serial SICCT/DIVA testing is adopted it is probable that vaccinated cattle would be identified through a positive response to the SICCT test. This would particularly affect the ability to undertake blinding in the field trials.

Studies supporting claims for MA applications should be ‘blinded’ according to the VICH GCP guidelines, which EFSA also recommends. These require that farmers and veterinarians, or other technicians assessing vaccine performance, are not aware of the animals’ vaccination status during a study and thereby unable to bias results. It is known that subcutaneous BCG vaccination gives rise to injection site reactions (skin thickening/lump) and therefore control animals should ideally be injected with a CP (placebo) capable of inducing similar reactions while itself being non-immunologically-active and similar in appearance to the BCG vaccine.

The nature and manufacture of this CP and the feasibility of its use should be carefully considered. Factors influencing the eventual decision on any use of a CP include the practical and scientific challenges of its manufacture and the need to obtain a licence under A(S)P/A for its use. Ideally the CP would have the same presentation as the BCG vaccine, i.e. that of a vaccine requiring on-site reconstitution.

Alternative blinding methods (‘masking’) could also be considered such as blinding individuals by study ‘function’. For example nominating one person as a “treatment dispenser” for each site.
This person prepares and is aware of the identity of the treatments at administration, but then takes no further part in any assessments or samplings at that site. Likewise personnel who are responsible for the assessment of the efficacy and safety of the vaccine will not be present during administration and will not have access to the associated documentation. This is a typical industry procedure for masking veterinary clinical trials with the disadvantage that it increases the number of personnel involved in the work.

It should be noted however that masking would not necessarily prevent farmers or farm staff (particularly during milking of dairy cows) from potential identification of vaccinates and controls.

Other, alternative methods could be considered:

- Use of physical methods (e.g. plasters) to make injection sites less visible to study personnel performing day to day husbandry. Whilst simple in concept this is not currently considered practical for a number of reasons. The plasters have to remain fixed in place yet be removable so that an assessor can measure reaction sizes and irritation may lead animals to rub them off against surfaces, possibly aggravating any local reactions.
- Use of a “treatment dispenser” to physically prepare the vaccine in either opaque or otherwise masked syringes prior to handing to the Investigator for administration. This would not address the issue of differences in injection site reactions.
- A comparator CP (not necessarily a true placebo), without immunologically active components, may be produced to closely mimic the reactions cattle raise to the full vaccine formulation.

If blinding options are preferred, Defra and WG would need to discuss these with EFSA before setting on a final approach. It is suggested for consideration by Defra and WG that studies performed to generate DIVA validation samples are performed first within a suite of initial studies, including safety, it is within these studies that there is a proposal to generate data to assist in the assessment of masking/blinding and the practicalities of its role out into the pivotal field efficacy studies.

1.4 Derogations

Field trials will need to be undertaken under conditions agreed with the EU to ensure that they meet current EC Directives and regulations for TB testing.

In order to conduct trials involving BCG-vaccinated cattle various derogations from existing legislation are desirable for the efficient and optimal conduct of the proposed work. During the course of this study the Consortium has discussed several derogation options with Defra and WG. It is for Defra and WG to make separate applications for appropriate derogations enabling the conduct of work within a legal framework.

1.5 Study Animal Traceability

EFSA recommendation E16 indicates that measures and controls should be in place to ensure traceability of all BCG vaccinated cattle participating in the studies such that they can be prevented from moving out of the trial area and the country. Fate of study cattle and stipulations qualifying the terms of their release from study and ATC/A(SP)A control is a requirement under 6.3.15 of VICH GCP GL9 and will ensure compliance with this point.

1.6 Matching Herds and Confounding Factors

EFSA conclusion E7 and recommendations E14 and E15 mention the recording of potential influences of confounding factors e.g. the level of the wildlife reservoir challenge during the course of a study. Suggested study protocols address these issues in general (e.g. recording farm details including biosecurity measures) and the final study protocols should document how these confounding factors should be managed.

1.7 Animal Inclusion and Removal

Records of the fate of all study animals at the end of the any clinical study should be made.

It should be ensured that details of the appropriate withdrawal period (for any veterinary medicinal product) are observed for all animals destined for the food chain.
2 WORK PACKAGE A – DIVA TEST PERFORMANCE

Provide data on the performance of the DIVA test (sensitivity and specificity) to fulfil OIE validation requirements

A summary of proposed study options is given in Table 1 and the associated advantages and constraints of each is shown in Table 2.

2.1 Introduction

The current SICCT or SIT tests used in EU ‘test and cull’ bTB control programmes are incapable of distinguishing between vaccinated and infected cattle. Presently anti-tuberculosis vaccination of cattle (e.g. BCG) is prohibited in the EU (directive 78/52/EEC). Under current legislation all cattle testing positive to skin test are culled and restrictions are placed upon the source farm with consequences also for neighbouring farms.

Vaccination with BCG will elicit positive results in response to SICCT and SIT. A reliable and validated DIVA test which distinguishes between cattle testing positive due to vaccination from those infected with bTB is essential.

The development of the blood IFN-γ DIVA test is currently the most advanced and is the one to be initially promoted in this project, referred to hereafter as the “DIVA”. Defra and WG are investigating a skin test DIVA. In practice the skin DIVA test will be subjected to essentially the same validation tests protocol as the blood test DIVA.

EFSA opinion on behalf of the European Commission has stipulated that the performance of all the proposed tests must be evaluated under UK field conditions as part of the UK case for trade in bovine products from vaccinated herds.

2.2 Work Package Project Requirements

2.2.1 Aim

Provide data on the performance of the DIVA test (sensitivity and specificity) to fulfil OIE validation requirements (Chapter 1.1.5. Principles and Method of Validation of Diagnostic Assay for Infectious Diseases. OIE May 2013).

The EU veterinary medicines legislation and EFSA requirements addressed are V1, V7, V9, V10, V11 and E1, E2, E3, E4, E5, E8, E12 and E15 respectively (see Sections 1.1 and 1.2).

2.2.2 Requirements/Objectives

- WPP1: Review of pre-clinical derived data package in support of blood IFN-γ DIVA test validation.
- WPP2: Study designs and options for generation of field-derived blood samples to validate the blood IFN-γ DIVA test to OIE standards.
## Table 1: WPP2 - Summary of Proposed Study Options for the Collection of Blood Samples for DIVA Validation

<table>
<thead>
<tr>
<th>Primary objective</th>
<th>Secondary Objectives</th>
<th>WPP reference (Appendix)</th>
<th>Design Features</th>
<th>Targets</th>
<th>Estimated Duration</th>
<th>Estimated Cost /M£</th>
</tr>
</thead>
</table>
| To obtain blood samples from vaccinated cattle which subsequently are DIVA bTB-ve (Critical)* | – ISO and lung lesions  
– Supportive safety and efficacy data  
– Vaccine influence on SICCT  
– Generate samples for LCA | Option 1  
WPP2.1 (Appendix 4) | Challenge phase to be performed on AFUs  
Cattle -ve pre-vacc., exposed to unvacc. challenge cattle  
– 50% cattle sourced from bTB free farms (BCG vacc.)  
– 50% cattle sourced from bTB breakdown farms (unvacc.)  
– Annual review of results | 300 bTB +ve blood samples  
30,000 cattle* | 3-4 y | 15.25 |

| | | Option 2  
WPP2.2 (Appendix 5) | Challenge phase to be performed on AFUs  
Cattle –ve pre-vacc., exposed to unvacc. challenge cattle  
– 33% cattle sourced from bTB free farms (BCG vacc.)  
– 33% cattle sourced from bTB breakdown farms (BCG vacc.)  
– 33% cattle sourced from bTB breakdown farms (unvacc.)  
– Annual review of results | 300 bTB +ve blood samples  
30,000 cattle* | 3-4 y | 15.16 |

| | | Option 3  
WPP2.3 (Appendix 6) | Challenge phase to be performed on AFUs  
Cattle –ve pre-vacc., exposed to unvacc. challenge cattle  
– 50% vacc. and 50% unvacc. cattle sourced from AFU normal intake  
– Annual review of results | 300 bTB +ve blood samples  
30,000 cattle* | 3-4 y | 15.26 |

| Blood samples from vaccinated cattle which remain bTB DIVA -ve. (Critical) | – ISO  
– Supportive safety data  
– Vaccine influence on SICCT  
– Generate samples for LCA | WPP2.4 (Appendix 7) | Commercial farms in low risk area  
Cattle –ve pre-vacc. and which remain –ve  
– 100% vacc.  
– Annual review of results | 1000 bTB –ve blood samples  
1100 cattle (ca. 10 herds) | 2-3 y | 0.69 |

| Blood samples from unvaccinated cattle (DIVA bTB +ve or -ve). (If required by OIE) | – | WPP2.5 (Appendix 8) | Conducted at farms/AFUs in high risk, low risk or ‘edge’ areas  
– Suggested 1000 -ve samples, 300 +ve samples  
– Sampling at routine national surveillance SICCT | 1000 bTB-ve samples  
300 bTB +ve samples  
1300 cattle | 1-2 y | 3.9 |

| Collection of blood samples to assess the impact of SICCT on subsequent DIVA results (Optional) | – DIVA and SICCT results at repeated individual animal level | WPP2.6 (Appendix 9) | Farm site in low risk area.  
Cattle –ve pre-vacc. and which remain –ve  
– 50% vacc. and 50% unvacc.  
– Possible PM at end of the study phase | 500 blood samples  
100 cattle | 1-2 y | 0.33 |

---

*This primary objective has 3 optional designs and these can be performed in isolation or as a combination.b. Based on modelling projections 1000 AFUs to obtain 300 +ve sample. Numbers given in the table illustrate if the study was performed for a 2 year period only, involving 100 AFUs. (see Appendix 3).

Key: vacc. = vaccinated, unvacc. = unvaccinated, -ve = bTB negative, +ve = bTB positive.
### Table 2: WPP2 - Summary of the advantages and constraints of each proposed study design

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Advantages</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPP2.1</td>
<td>- Scientifically robust study design</td>
<td>- Significant management changes at AFU, impacts on supply chain and costs</td>
</tr>
<tr>
<td></td>
<td>- Generates DIVA validation samples (300) from vaccinated bTB positive animals</td>
<td>- Impact on challenge rate of introducing 50% of bTB free animals is unclear</td>
</tr>
<tr>
<td></td>
<td>- PM endpoint will confirm true bTB status (within limitations) of each animal</td>
<td>- Impossible to model impact of known bTB status on transmission rates at AFU</td>
</tr>
<tr>
<td></td>
<td>- Generates supporting safety/efficacy data and specific data on lung lesions</td>
<td>- CP administration and/or blood sampling may necessitate an ATC and/or A(SP)A</td>
</tr>
<tr>
<td></td>
<td>- Generates data to confirm animal numbers in WPP6 trial design</td>
<td>permissions in advance</td>
</tr>
<tr>
<td></td>
<td>- Use of AFUs to enhance challenge relative to farm sites – reduction in timeline</td>
<td>- Large numbers of animals required to generate 300 samples</td>
</tr>
<tr>
<td></td>
<td>- Use of a terminal SICCT enables assessment of SICCT use at ca. 12 mth post</td>
<td>- Disruptive to AFU business</td>
</tr>
<tr>
<td></td>
<td>vaccination – possibly helpful for animal removal from field studies(WPP6)</td>
<td></td>
</tr>
<tr>
<td>WPP2.2</td>
<td>- As for WPP2.1.</td>
<td>- Similar to WPP2.1, but less disruptive to AFU business as a proportion of animals will be obtained from the AFUs standard supply chain.</td>
</tr>
<tr>
<td></td>
<td>- Vaccination of cattle of uncertain bTB status (EU veterinary medicines legislation, V6)</td>
<td>- 2/3 of cattle vaccinated therefore this may decrease challenge rate at AFU.</td>
</tr>
<tr>
<td></td>
<td>- More vaccinated cattle a proportion of which will not be protected and may generate bTB positive samples.</td>
<td></td>
</tr>
<tr>
<td>WPP2.3</td>
<td>- As for WPP2.1.</td>
<td>- Similar to WPP2.1. Least disruptive to AFU business</td>
</tr>
<tr>
<td></td>
<td>- No change to animal source, therefore AFU bTB challenge rate should be unaffected</td>
<td>- Pre-vaccination bTB free status unknown - impossible to determine if vaccine protection was insufficient or if animals had latent pre-vaccination infections</td>
</tr>
<tr>
<td></td>
<td>- Easy sell to farmers as limited disruption to business</td>
<td>- Data unusable to support efficacy as does not reflect actual usage, normally only DIVA or SICCT –ve animals would be vaccinated</td>
</tr>
<tr>
<td>WPP2.4</td>
<td>- Generates bTB - ve samples required for validation</td>
<td>- No positive confirmation via PM.</td>
</tr>
<tr>
<td></td>
<td>- Contributes towards safety data</td>
<td>- Derogation needed for animal follow-up during study period and longer if SICCT cannot confirm that animals are unreactive 12 mth post vaccination</td>
</tr>
<tr>
<td></td>
<td>- Using animals from bTB free source minimises animals required (1000 samples)</td>
<td>- Does not contribute to the efficacy data</td>
</tr>
<tr>
<td></td>
<td>- Occasional bTB +ve’ animals despatched for slaughter for status confirmation if -ve, farm bTB status unaffected</td>
<td></td>
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<tr>
<td></td>
<td>- Could generate ISO data to address ‘blinding’ concerns prior to WPP6 field trials</td>
<td></td>
</tr>
<tr>
<td>WPP2.5</td>
<td>- Uses standard SICCT testing to obtain positive and negative blood samples</td>
<td>- Uncertain if required for DIVA validation - confirmation needed from OIE</td>
</tr>
<tr>
<td></td>
<td>- PM endpoint confirms true bTB status of positively testing animals (SICCT or DIVA)</td>
<td>- Reimbursement for animals which are positive to either the SICCT or DIVA test</td>
</tr>
<tr>
<td></td>
<td>- Relatively few animals to obtain data set</td>
<td>- Does not contribute to the efficacy or safety data</td>
</tr>
<tr>
<td></td>
<td>- Provides DIVA data for animals bought onto farm and left unvaccinated</td>
<td></td>
</tr>
<tr>
<td>WPP2.6</td>
<td>- Demonstrates the likelihood of SICCT testing impacting on DIVA results, combination of tests likely to be EC preferred test associated with use of BCG vaccine. (Further study designs describing validation of DIVA in series with SICCT are given in Annex 1). Experimental study which would create the opportunity to look at repeat DIVA results and SICCT results at an individual animal level.</td>
<td>- No positive confirmation of bTB negative status via PM.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Derogation needed for vaccinated animal follow-up during study period and longer if SICCT cannot confirm that animals are unreactive 12 mth post vaccination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Does not contribute to the efficacy data as animals required to be from a bTB free source.</td>
</tr>
</tbody>
</table>
2.2.3 General Considerations

For its evaluation the DIVA test will be validated to OIE standards demonstrating its ability to discriminate between infected and non-infected cattle, irrespective of vaccination status. Initial epidemiological modelling, carried out as part of Defra Project SE3127, explored how the diagnostic performance of prospective DIVA tests could affect the probability of restrictions being applied to herds, duration of breakdowns, or the numbers of animals condemned as reactors. This modelling work suggested that a DIVA test specificity (true negatives per total reported negatives) of >99.85% will be necessary for DIVA testing to break-even against SICCT testing in terms of these three key metrics of the economic impact of breakdown testing on farmers (Conlan et al. PLoS Comp Bio 2014 (In press, Appendix 14)).

At a minimum blood samples have to be collected from cattle held under standard UK commercial conditions that fall into two categories:

1. Vaccinated, infected with bTB
2. Vaccinated, not infected with bTB

It is debatable whether or not it is necessary to present an equivalent data set from unvaccinated animals. WPP2.5 is included to cover this eventuality.

Cattle will be confirmed as bTB-infected from PM investigations in approved abattoirs conducted by trained operatives to a standardised procedure. This will involve identification of visible lesions at carcass inspection in predilection sites plus confirmatory microbiological culture recovering M. bovis.

The specification of PM procedure is to be determined but must be sustained throughout and across all studies contributing data gathered as a result. Personnel conducting the procedures should have acknowledged and documented training in the process.

Based on current understanding of OIE requirements it is proposed to obtain blood samples from 300 known bTB positive and 1000 known bTB negative cattle, all previously vaccinated and all tested with the DIVA test prior to any PM inspection, with microbiological culture for M. bovis confirmation. This would enable the true sensitivity and specificity to be defined with reasonable precision (Altman and Bland, 1994).

These numbers correspond to those required using a gold-standard test, with the further assumption that the true sensitivity and specificity are known in advance (Altman and Bland, 1994) details a more realistic method which allows for uncertainty in the values assumed for the sensitivity and specificity. The results from the more realistic analysis indicate that somewhat larger numbers are required.

However, there is no “gold standard” test for bovine TB. In particular post mortem examination and culture may miss some positive animals. In the absence of a “gold standard” test alternative methods are available. These methods are referred to as latent class analyses, since the true status of an animal: infected or not, is not known. The true status is then imputed from the data, along with the parameters of interest, in this case the sensitivity and specificity of the DIVA test.

However, to apply latent class methods, data must be available from more than one population, where the proportion of animals that are truly positive is different in each population. In addition two conditionally independent tests have to have been applied to each animal. In layperson’s terms, this is a requirement that should we know both the true status of an animal and the result from one test, our best estimate of the result from the other test is the same as what we would have if we knew only the true animal status.

The key advantage of latent class methods is that they do not need a “gold standard” test. The main disadvantage is that, subject to the availability of population and test data as specified above, they also require much larger numbers of animals to establish the test sensitivity and specificity to any required precision. We therefore propose to use the indicated number of positive and negative samples to compare to post mortem investigation as a pseudo “gold standard”. This will produce estimates of test sensitivity and specificity;
strictly speaking the relative sensitivity and specificity. This will then enable us to demonstrate that the DIVA test has sufficient sensitivity and specificity in the field to proceed with the larger vaccine trial. We will then apply a latent class analysis to all the animals involved in the vaccine trial. We have demonstrated by statistical simulation that this will give us unbiased estimates of the true sensitivity and specificity of the DIVA test. We have also demonstrated the level of precision that we can expect given the number of test results expected from the vaccine trial (Altman and Bland, 1994).

As a part of WPP2.4 a sub-population of cattle may be assessed for discernible differences in injection site reactions between IVP and control animals.

Blood samples will be collected for DIVA validation, if blood sample volumes allow, surplus plasma/serum could form a reference sample set for the possible development of other tests for bTB based on alternative biological markers (to contribute towards existing Defra studies). If appropriate the opportunity will be taken to acquire such material.

The APHA report on finishing herds (Defra report, DA4500, 22.10.2013) suggested that AFUs licensed to accept cattle from high-risk areas for finishing prior to slaughter would be the preferential test site for generating positive samples from BCG vaccinated cattle. In order to obtain samples from BCG vaccinated cattle which contract bTB, it is necessary to expose them to challenge from unvaccinated animals. The designs proposed in Table 1 included options for 50:50 and 67:33 vaccinated to unvaccinated ratios of cattle but it is noted that there are features of any design which will be in conflict. i.e. a higher proportion of vaccinates decrease the number of cattle within a herd likely to succumb to bTB thus generating the sought after vaccinate bTB positive blood sample for DIVA validation. However it simultaneously reduces the number of non-vaccinates within the herd which are the principal source of challenge within AFU facilities.

AFUs have a quick turnover of animals and are known to present higher levels of disease challenge compared to conventional farms thus they represent the best field opportunity to generate blood samples from vaccinated, bTB positive animals. On the negative side the typical residence time of an animal on an AFU prior to slaughter is less than six months thus leaving a relatively short period in which animals may contract and express bTB such that they show DIVA test positive results. In addition to this the number of AFUs at any given time point is variable.

The regular commercial slaughter of animals from AFUs would facilitate PM assessment of DIVA negative animals, essential for identifying false negative animals and confirming true negatives. For a sub-population of animals a standardised, enhanced PM procedure is proposed including bacterial culture from collected tissue swab samples. All lesioned animals will have microbiological culture of material taken from swabs of affected organs/tissues for follow up identification of M. bovis as confirmation of bTB positive status. An approximately equal number of lesion-free animals would receive the same processing to show representative confirmation of bTB negative status.

2.2.4 Efficacy Data Derived From DIVA Validation Sample Generation

Generating blood samples from vaccinated and infected animals will entail vaccinating animals from bTB free areas and mixing them with CP animals from high risk areas. The relative infection rates of vaccinated vs. unvaccinated animals in these trials can be used as an initial indicator of vaccine efficacy. This will inform the design of later large-scale efficacy studies (in particular the number of subjects required to obtain statistically significant results), but will make a limited contribution to the data generated in wider field efficacy trials.

It has been estimated by mathematical modelling (Appendix 3) that at least 120 herds with a recent past history of bTB must be recruited for the simplest designs with 50:50 vaccinates:controls within-herd coverage or herd-by-herd controls (100% vaccinated herd:0% vaccinated herd) in order to provide useable efficacy data. The study designs have been powered (estimates of recommended sample numbers) to estimate direct efficacy.

In all cases the clinical end-points for estimating vaccine efficacy are reduction in visible bTB lesions and the results of validated DIVA tests. The conclusions of the mathematical modelling study Appendix 3 are as follows:
Indirect reduction of bTB transmission may have a non-linear and unpredictable impact on observed efficacy due to change in age-structure of positive testing animals.

Predicted power (probability of a statistically significant result) is based on assumed relationship between visible lesions and animal age.

It is unknown to what extent vaccination may change this relationship. It is indeed an important limitation of the modelling, but we would like to emphasise that the design of this field trial is as much, if not more so, influenced by the practical, political and legal requirements surrounding the use of vaccination. The role of the modelling in this design is to predict the likely effect of vaccination and sample sizes required based on the information and biology as we understand it now. Should a field trial progress, statistical significance could be achieved with far less, or far greater, numbers of samples.

Within-herd transmission models suggest that the magnitude of the indirect efficacy will be close to zero. Given the low within-herd prevalence of bTB, this leads to a high probability that an underpowered trial could estimate a negative efficacy of vaccination just by random chance, even if a positive protective effect exists.

2.3 WPP1: Completion of pre-clinically derived data package in support of blood IFN-γ DIVA test validation

WPP1 was informed by the current data pack of validation sample held by APHA in support of an application to OIE for a validation of the DIVA blood test. An overview of results is presented in Appendix 1.

The DIVA test will comprise of two reagents (i) a peptide cocktail of ESAT-6/CFP-10 and (ii) a peptide Rv3615c. A DIVA test positive result is recorded if a response is detected to either of these reagent sets.

APHA have separately calculated that samples from 1000 vaccinated animals which remain bTB-negative and samples from 300 vaccinated animals which subsequently test bTB positive will deliver satisfactory validation data. This was separately confirmed by BioSS (Appendix 2). The same target number of samples (1000 unvaccinated animals which remain bTB negative, 300 unvaccinated animals which subsequently test bTB positive) may be required in respect of unvaccinated animals.

APHA currently holds data from blood samples (of variable provenance) as follows:-

1. Vaccinated, infected with bTB
   75 samples
2. Vaccinated, not infected with bTB
   214 samples

The research carried out in this study suggests that additional samples should be collected from:

- Vaccinated and bTB positive animals using options WPP2.1, WPP2.2 or/and WPP2.3.
- Vaccinated and bTB negative animals using option WPP2.4.

The need to obtain the target numbers of unvaccinated animals should be discussed with the OIE as it is not the primary category of interest.

2.4 WPP2: Study designs and options for generation of field-derived blood samples to allow the validation of the IFN-γ DIVA test to OIE standards

2.4.1 Study Design Options

Six potential study designs (WPP2.1 to WPP2.6) have been identified and are outlined in Appendix 4 to Appendix 9. In addition to this, further design options are listed in Annex 1 describing the designs wherein DIVA is validated in the context of association with a preceding SICCT. The primary and secondary objectives, as well as estimated timelines and costs, are summarised in Table 1. A listing of the perceived advantages and constraints for each design is given in Table 2.

All the proposed studies are blinded (as applicable), controlled (placebo or negative controls), multicentre clinical field efficacy and safety studies to be carried out in different
geographical regions within the UK. All will be conducted to the principles of GCPv (overview in Appendix 10).

Blood samples from vaccinated animals subsequently determined bTB positive (study designs WPP2.1, WPP2.2, and WPP2.3) could be generated on high risk area AFUs. Only animals greater than six months of age would be blood sampled for DIVA analysis and subsequently slaughtered as normal. All study animals would be consigned directly to slaughter to allow confirmation of bTB status by PM; a sub-population of these animals being sampled for *M. bovis* culture. Carcasses would be duly processed and enter the food chain. Applicable guidelines for processing of any carcasses from bTB positive animals would be followed.

Blood samples from vaccinated animals subsequently determined bTB negative and samples from vaccinated animals subsequently exposed to repeat SICCT testing (study designs WPP2.4 and WPP2.6 respectively) will be generated in animals sourced from bTB free farm sites (previous 5-10 years). Documented herd history is considered sufficient to obviate the need for confirmatory PM (see Appendix 2).

The standard SICCT testing regimen under current field conditions would allow blood sample generation from unvaccinated animals (study design WPP2.5). Any animal testing positive to either the SICCT or DIVA test will be despatched to slaughter with PM to confirm bTB status.

Where possible, diverse animal populations should be used (breed, sex, husbandry conditions etc.). AFUs receive cattle from pure dairy and beef breeds as well as crossbreeds.

### 2.4.2 Considerations on Study Design Options

A combination of one or more of studies WPP2.1, WPP2.2 or WPP2.3 plus WPP2.4 is critical for generating the types and quantities of samples required for DIVA test validation. Designs have been/are suggested based on the predicted number of blood samples needed for validation to an acceptable sensitivity/specificity level.

Generating samples from animals with unknown bTB status at study start (WPP2.3) adds an additional test sample set to the validation – to demonstrate the DIVA test's efficiency at identifying animals that were potentially bTB positive prior to transfer to AFUs, including animals infected prior to vaccination. Generating these samples, however, is not currently a primary objective for DIVA validation identified by either EFSA or EU veterinary medicines legislation. These samples may be required, or alternatively generated as part of field studies (WPP6), once there is confidence that the DIVA test is fundamentally robust.

WPP2.5 is included as an option but would only be conducted under one of the following circumstances:

- Additional samples from unvaccinated animals are considered necessary to give statistical significance to the DIVA test validation.
- If it is considered that new samples need to be generated from animals covering a wider age range prior to performing the field trials in WPP6: Samples could be generated as part of WPP6 or alternatively a sub-population will be included in WPP2. However, for WPP2 the samples required to generate the validation data set will be collected from animals greater than six months of age.

Pre-vaccination SICCT testing would confirm animals' bTB status at study start. Note that experimental challenge studies have observed a positive interaction of recently applied skin tests on the DIVA response, equating to more animals testing positive.

### 2.5 Consortium Proposals

Final expert advice and opinion from regulatory authorities will be required to confirm the exact DIVA validation requirements and EFSA’s agreement on the study designs should be sought (plus OIE’s view on animal numbers). The presented options include those that the Consortium consider to be the most likely scenarios. In default of advice to the contrary, undertaking studies WPP2.2 or WPP2.3 (both more likely to be practical than WPP2.1) plus WPP2.4 should suffice to generate the required samples.
3 WORK PACKAGE B – BCG VACCINE PERFORMANCE

Assess BCG vaccine performance (direct and indirect efficacy) in support of gaining an MA and its capacity to effectively assist in the eradication of bTB alongside a test and cull strategy.

3.1 Introduction

The primary aim of this WP is to provide data that will help to assess vaccine efficacy/safety and provide data to support MA application. Study designs investigating direct efficacy of the BCG vaccine in the field have been informed by mathematical modelling conducted by Cambridge University DDU.

The within-herd modelling carried out by the University of Cambridge team, based on our current understanding of the efficacy and duration of protection of BCG, predicts that the effect of vaccination on rates of within-herd transmission will be marginal with an indirect efficacy of vaccination of close to 0%. These models are not appropriate to inform the potential impact on rates of herd-to-herd transmission or the burden of infection in wildlife populations. Such questions were out of the scope of the tendered project, but could be addressed using a validated national level model of transmission, such as the recently published model by Brooks-Pollock et al. Nature 2014. Field trial designs which inform the impact of bTB transmission have also been considered within the mathematical modelling to satisfy EFSA requirements (E9).

In addition, for vaccine use on UK farms, the primary aim of generating an MA, data on direct efficacy would be required.

3.2 Work Package Project Requirements

3.2.1 Aim

Assess BCG vaccine performance (direct and indirect efficacy) in support of gaining an MA and its capacity to effectively assist in the eradication of bTB alongside a test and cull strategy.

The requirements addressed are V7, V8 and E5, E6, E7, E8, E9, E11, E12, E15 respectively (see Sections 1.1 and 1.2).

3.2.2 Requirements/Objectives

- WPP6: Consideration of the design of a VICH GCP protocol for the field trial of the BCG vaccine for determination of efficacy and field safety to satisfy the requirements of an application for granting of an MA.
- WPP7: Proposal, subsequent to modelling, to determine the appropriate measure of vaccine efficacy in terms of individual animal, herd or regional measures.

3.2.3 General Considerations

The proposed protocol designs for WPP6 have been based on the results of the modelling work conducted under WPP7.

The proposed designs all assess efficacy at the level of the individual animal (i.e. the animal is the experimental unit) with the exception of WPP6.3 in which the proposed experimental unit is the individual farm.

Vaccine efficacy is assessed as direct efficacy in WPP6.1 and WPP6.2, and as indirect efficacy in WPP6.3.

Numbers are based on power calculations given in the statistical report presented in Appendix 2. Animal numbers may be modified in the light of efficacy results from WPP2 studies that could, potentially, be completed earlier.

3.2.4 Results of the Mathematical Modelling Study

Modelling predicts that the benefit of vaccination will be modest, with at most a 15% reduction in the herd level incidence, duration of breakdowns and probability of recurrence compared to unvaccinated but DIVA tested herds. These models demonstrate the considerable challenge of achieving a benefit of vaccination above and beyond the impact of
an ongoing test-and-slaughter program. Indeed in these model simulations, the benefit of vaccination relative to SICCT testing is provided almost entirely by the relative test performance of DIVA testing (assumed) compared to the estimated performance of tuberculin testing.

Consideration should be given to a staged design with:

- An initial trial to assess vaccine efficacy (50% target vaccination coverage to assess direct efficacy using within-herd controls, WPP6.1)
## Table 3: WPP6 - Summary of Proposed Study Options

<table>
<thead>
<tr>
<th>WP Primary Objectives</th>
<th>Secondary Objectives</th>
<th>WPP reference (Appendix)</th>
<th>Design Features</th>
<th>Recruitment Targets:</th>
<th>Estimated Duration</th>
<th>Estimated Cost /M£</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field safety and efficacy</strong></td>
<td>Generate data:  - Intra-farm transmission  - ISO  - LL +ve animals  - Milk production (including pregnant cows)  - Fertility  Blood samples:  - Range of animal ages (incl. minimum of 15 days)  - DIVA validation LCA</td>
<td>WPP6.1 (Appendix 11)</td>
<td>- Range of breeds, ages, weights and genders  - High risk areas to demonstrate efficacy  - Treatments assigned by animal at 1:1 IVP:CP  - Five annual vaccinations (based on modelling advice) with pre-vacc. BS  - 6-12 monthly DIVA testing to confirm bTB status  - ISO sub-population of pregnant/lactating animals  - 1 y BS used for DIVA validation by LCA  - Consider sub-pop. BS at 6 month intervals from last vacc  - PM on all animals which die/are slaughtered (incl. DIVA +ve test)  - Possibility of interim analysis at Day 720 and option of study termination if results are definitive</td>
<td>100 farms (12 000+ animals)  Mix of beef and dairy animals</td>
<td>8 y</td>
<td>33.5</td>
</tr>
<tr>
<td><strong>Field safety and efficacy data in neonates</strong></td>
<td>Generate data  - LL (all animals)  - ISO (minimum age animals)  Blood samples:  - Range of animal ages (incl. minimum of 15 days)</td>
<td>WPP6.2 (Appendix 12)</td>
<td>- Animals sourced from bTB -ve farm sites (previous 5 y)  - Range of breeds, ages, weights and genders from 15 days old  - Treatments assigned by animal at 1:1 IVP:CP  - 2 annual vaccinations with pre-vacc. BS  - 6-12 monthly DIVA testing to confirm bTB status. Transfer to high risk area AFU 30 days after initial vacc  - ISO for 30 days post vacc  - Possible termination at 1 y if bTB challenge rate high  - Consider PM on all animals which die/are slaughtered (incl. DIVA +ve test)  - PM on surviving animals slaughtered at 2 y mark</td>
<td>10 AFUs</td>
<td>4 y</td>
<td>1.53</td>
</tr>
<tr>
<td><strong>Field efficacy data</strong></td>
<td>Generate data on Intra-farm transmission rates of bTB</td>
<td>WPP6.3 (Appendix 13)</td>
<td>- 240 farms (30% power) proposal to pilot the project  - Treatments assigned by farm at 1:1 IVP:CP  - Pairing of farms Farm 1 IVP, Farm 2 CP  - Allocation of farms to treatment group is sustained for entire study  - Consider up to 10 y for a full study powered to 80% i.e. extend after pilot phase (1000 to 1500 farms)  - Assessment per farm of overall bTB incidence  - 6-12 monthly DIVA testing to confirm bTB status  - Mix of beef and dairy animals</td>
<td>240 farms (30,240 animals)</td>
<td>Initial 2 y</td>
<td>20.3m (consider further 10 y, 80m to 120m)</td>
</tr>
<tr>
<td>Study Design</td>
<td>Advantages</td>
<td>Constraints</td>
<td></td>
<td></td>
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</tbody>
</table>
| WPP6.1       | - Generates the minimum data required for MA  
- 50:50 IVP:CP minimises impact of environmental challenge - all animals exposed to same risk, with least variability.  
- Generates additional safety data  
- Provides optimal experimental power | - Use of DIVA which has not be fully validated  
- Large numbers of animals  
- Logistical difficulties in handling farm site movements  
- Needs further exploration of supply chains to confirm that farms can still sell product  
- Education of farms/veterinarians needed. This will add substantially to time and cost.  
- Some farmers may not be prepared for the use of a CP  
- Some farmers may not be accept a 60% efficacy estimate  
- CP administration and/or BS may necessitate an a priori ATC and/or A(SP)A project and personal licence | |
| WPP6.2       | - Generates data to compare incidences of lung lesions in IVP and CP animals  
- End point is slaughter therefore bTB status can be assessed by PM  
- Similar to WPP2 study designs – efficiency gains in time/cost and identification of knowledge gaps  
- Generates additional safety data (e.g. lung lesions data) | - Significant management changes at AFU - impacts on supply chain and costs  
- Impact on challenge rate of introducing 50% of bTB free animals is unclear  
- Model does not inform on impact of known bTB status on transmission rates at AFU  
- CP administration and/or BS may necessitate an a priori ATC and/or A(SP)A project and personal licence | |
| WPP6.3       | - Follows vaccine use once registered - all animals on a given farm vaccinated  
- Allows post vaccination farm site follow-up (number and duration of breakdowns once vaccination regimen is in place)  
- Addresses EFSA concern E9  
- Generates additional safety data  
- Can adapt to introduction of evolving diagnostic tests e.g. Skin DIVA | - Farmers may not want to remain as a CP farm for a period of up to 8 y  
- Possibility of farmers discontinuing vaccination if positive effect is observed if costs are high (i.e. complacency)  
- CP administration and/or BS may necessitate an a priori ATC and/or A(SP)A project and personal licence  
- Extended study duration, challenge to sustain commitment to study disciplines |
A second Review Point based on achieving a given efficacy (in WPP6.1, WPP6.2) before field trials to assess the effectiveness (WPP6.3) of vaccination under field conditions using whole herd vaccination and herd-by-herd controls.

Combined results of vaccine efficacy and vaccine effectiveness trials can be used to assess direct, total and indirect efficacy of vaccination and potentially inform impact on transmission (which no single trial design will do).

3.3 WPP6: Consideration of the design of a VICH GL9 GCP protocol for the field trial of the BCG vaccine

3.3.1 Study Design Options

A total of three possible study designs (WPP6.1 to WPP6.3) have been identified and these are detailed in Appendix 11 to Appendix 13 respectively. The primary and secondary objectives of these studies, including estimated timelines and costs, are summarised in Table 3 and a listing of the perceived advantages and constraints for each design is given in Table 4.

Prior to first vaccination, all animals would be subject to SICCT testing; only negative animals will be recruited. All animals bought-in or born onto site between vaccinations would remain as 'in-contact negative controls' until the next vaccination. This would, however, impact the number of negative control animals (and therefore study powering). Consideration should be given to the vaccination of introduced cattle at six monthly intervals (midway between annual vaccinations). Annual vaccination has been selected in trial designs as this mirrors the currently drafted Summary of Product Characteristics. The alternative to vaccination at six monthly intervals would be to leave the animals as in-contact negative controls, which may risk upsetting the target ratio of vaccinates to controls.

All deaths for any cause, routine or not, will be subject to PM analysis to assess bTB status using standardised systems. Other assessments may also be considered.

Collection of milk yield and composition data will be undertaken in dairy animals 30 days pre- and 30 days post-vaccination for each of the first two vaccinations.

All three options take the form of randomised, blinded, placebo/negatively-controlled, multi-centred clinical field efficacy studies. They would involve multiple beef and dairy farms in high risk areas covering different geographical regions within the UK. Farms in high risk areas would be recruited because they offer the potential to generate a greater bTB challenge plus based on initial social feedback these farms may be more willing to participate in trials. Farms would be selected on the basis of their previous five years' bTB history and if possible, with consideration to the environmental Mycobacterium status (incidence of different M. bovis and M. avium strains).

All studies will be conducted to the principles of VICH GCPv (overview in Appendix 10).

3.3.2 Considerations on Study Design Options

Studies WPP6.1 and WPP6.2 have been designed to optimise the quantity and quality of data available from clinical field trials to inform the MA application. As such there is relatively little margin for flexibility in the designs and both will be necessary to achieve the two primary objectives of this WP. The final proposed cattle numbers (the primary determinant of study costs) will be based on standard statistical approaches using initial data generated from WPP2.

WPP6.3 will generate data on efficacy at the farm level and on the indirect efficacy of vaccination on the rate of transmission within a single farm. EFSA have specifically emphasised the importance of quantifying the potential impact of vaccination on transmission (E9). The most complete solution to address this question would be a trial design where the proportion of vaccination is varied across the study population. A design structured in this way offers the potential to estimate the impact of vaccination on both susceptibility and infectiousness (transmissibility) (Longini et al. Statist. Med. 17, 1121-1136). However, this extra information comes at a cost to the power to estimate the direct efficacy of vaccination which, it is straightforward to show, is optimised for a 50:50 split between vaccinates and
controls. Given the marginal impact of vaccination on transmission predicted from the University of Cambridge within-herd models we do not believe that estimates of transmission are a proper basis on which to hinge the design of a field trial. Therefore, in order to maximise the power to estimate the direct efficacy of vaccination, and better control for the potential of intra-herd variation we exclusively consider designs with a 50:50 configuration. (Appendix 3).

The direct vaccine efficacy is defined by the ratio of the Attack Rate Vaccinate and Attack Rate Unvaccinate within a partially vaccinated population. For a design using only within-herd controls changing the target vaccination coverage level directly changes the number of animals that can be recruited in the two categories. Given the relatively small indirect impact of vaccination, the power to estimate a protective effect of vaccination is driven almost completely by the number of animals that can be recruited in each category.

Increasing vaccination coverage to greater than 50% reduces the variability in estimate of ARV but conversely increases the variability of estimates of the ARU. As efficacy depends on the estimates of both the ARV and ARU the least variability (and greatest power) is achieved for a 50:50 coverage.

Simply, if there is only single vaccine coverage with only within-herd controls then maximum power is achieved at 50% coverage. If a mixture of different vaccine coverages are introduced, power is reduced and more herds are needed to get the basic estimate of direct efficacy.

With regards to WPP6.2 different numbers of AFUs were considered but were not thought to offer any significant benefit in terms of cost/time, i.e. using fewer AFUs would merely increase the time to completion.

With regards to WPP6.3 using statistical modelling (Appendix 3), increasing the power of the study design from the current ‘pilot’ configuration powered at 30% up to 80% results in a requirement for an approximate 4 to 6 times increase in the number of herds required (1000 to 1500 herds).

Also regarding WPP6.3 designs providing shorter periods for farms to remain unvaccinated were carefully considered. A rotational design type, although potentially more desirable to farmers, was unfortunately found not to be statistically viable as it did not deliver continuity of control sites.

As discussed in Appendix 3, the combined results of efficacy (WPP6.1) and efficiency (WPP6.3) trials can be jointly analysed to estimate direct, total and indirect efficacy of vaccination and potentially inform impact on transmission (which no single WPP will do, as it is not considered practical to perform both studies under one protocol).

3.4 WPP7: Proposal, subsequent to modelling, to determine the appropriate measure of vaccine efficacy in terms of individual animal, herd or regional measures.

WPP7 has been completed and is reported in Appendix 3.

The conclusions are presented below.

3.4.1 To achieve the primary trial aim of demonstrating direct efficacy of BCG vaccination:

- Consider validation of a DIVA specificity of at least 99.85% before the commencement of efficacy trials.
- Visible lesions (supported by confirmatory culture) should be used as the end-point for estimating efficacy.
- Another clinical endpoint is considered to be results of a validated DIVA test (if validation is performed prior to the initiation of the field studies).
- Trials should be powered to estimate Direct Efficacy.
- The indirect protection of cattle vaccination predicted by our within-herd models is small due to the assumed constant environmental risk of infection.
• Power is defined as the probability of estimating a protective benefit of vaccination when it exists (net improvement relative to unvaccinated animals/herds).

• Statistical powering of the proposed trial designs is dependent on targeting herds with a history of bTB such that recruited herds have a bTB incident rate of ~ 90% for vaccinated herds and 80% for unvaccinated herds over the trial period.

• Power calculations are based on a DIVA test sensitivity of at least 73.3% and specificity of 99.85% and should be recalculated if this is not achieved.

3.4.2 To achieve the primary trial aim of demonstrating significant direct efficacy of BCG vaccination to achieve MA (WPP6.1), and to demonstrate an individual animal benefit (WPP6.1)

• At least 100 herds with a recent past history of bTB must be recruited for a period of three years (total trial time will be longer, due to staggered recruitment of farms over 2 years and a study phase of each farm being 5 vaccination cycles) for designs with either within-herd or between-herd controls to achieve a power of > 90%.

To demonstrate herd level benefit of vaccination (WPP6.1 and 6.3):

• At least 100 herds with a recent past history of bTB must be recruited for a period of three years (total trial time will be longer) for designs with either within-herd or between-herd controls to achieve a power of > 90%
  o Herd level incidence.
  o Duration of restrictions (probability of prolonged breakdown).
  o Probability of recurrence (within trial period).

3.4.3 Final Proposals:

• Combined results of efficacy (WPP6.1) and efficiency (WPP6.3) trials can be used to assess direct, total and indirect efficacy of vaccination and potentially inform impact on transmission (which no single WPP will do, it is not considered practical to perform both studies under one protocol).

• Alternatively, demonstration of significant protective efficacy of vaccination in WPP6.1 should be used as a Review Point for the commencement of efficiency trials (WPP6.3).

3.5 Consortium Proposals

It is currently proposed that initially WPP6.1 and WPP6.2 be conducted but consideration should be given to WPP6.3 in order to address transmission rate and satisfy EFSA conclusion E9.
4 WORK PACKAGE C – BCG VACCINE SAFETY

Assess the safety of the vaccine, both in relation to vaccinated animals as well as humans through contact or animal products (sufficiently to support the grant of an MA).

4.1 Introduction

Safety assessments for veterinary medicinal products used in production animals have to be considered at four levels: safety for the animal, safety for the administrator and/or owner, safety for the consumer, and safety for the environment. It is a formal requirement for an MA application that safety is demonstrated at these four levels. With a project that potentially attracts public concern there is also a potential additional interest to produce clear and unequivocal evidence that can be used to reassure stakeholders and the general public.

The work discussed in this WP is designed to meet these requirements.

4.2 Work Package Project Requirements

4.2.1 Aim

Assess the safety of the vaccine, both in relation to vaccinated animals as well as humans through contact or animal products (sufficiently to support the grant of an MA)

The requirements addressed are V1, V2, V3, V4, V5, V6 and E10 (see Sections 1.1 and 1.2).

4.2.2 Objectives

Injection site reactions are assessed as an element of the field studies presented in WPP2 and WPP6. In addition fertility and abortion data in pregnant cows, and milk yield and composition in lactating dairy cows will be collected. This data will cover a range of breeds and pre-exposure conditions.

- WPP8: Consideration of components within the VICH GCP protocol for the field trial making provision for the generation of samples for analysis and separate protocols in respect of post-vaccination bacterial shedding in milk and the respiratory tract.
- WPP9: Proposal for a GLP protocol for analysis of milk samples gathered in the field from vaccinated and unvaccinated animals.
- WPP10: Proposal for a GLP protocol for analysis of e.g. nasal swabs, gathered from vaccinated and unvaccinated animals under field conditions.

4.2.3 General Considerations

These studies have a requirement to be conducted under minimal chance of exposure of study animals to natural bTB challenge. This is in order to avoid confounding the observation of bacterial shedding in samples from vaccinated animals with bacterial shedding from infected animals. These studies would therefore be undertaken in regions with a low challenge risk and at sites with no prior history of bTB (minimum five years bTB free). Ideally cattle will be selected from closed-herds, and if possible neighbouring farms will have similar bTB status/closed herds.

Analysis of milk and respiratory tract swab samples from WPP8 will be undertaken according to the protocols proposed in WPP9 and WPP10. Semen samples from WPP8 studies in breeding bulls will require additional work to develop and validate a bacteriological analysis adapted to this matrix.

NB Good Laboratory Practice embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. GLP is intended to assure regulatory authorities that the safety data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments.
### Table 5: WPP8 - Summary of Proposed Study Options

<table>
<thead>
<tr>
<th>WPA Primary Objectives</th>
<th>Secondary Objectives</th>
<th>WPP reference (Appendix)</th>
<th>Design Features</th>
<th>Considerations</th>
<th>Targets:</th>
<th>Estimated Duration/ Cost /K£</th>
</tr>
</thead>
<tbody>
<tr>
<td>EITHER Milk samples for bacterial shedding</td>
<td>Generate data: - ISO &amp; rectal temperature - Milk yield and composition</td>
<td>WPP8.1 (Appendix 14)</td>
<td>- Farm sites low risk area - 20 cows&lt;sup&gt;a&lt;/sup&gt; of each parity 0-5&lt;sup&gt;b&lt;/sup&gt; (120 total per farm, 2 farms). - Animals at least 21 days post calving - Two vaccinations, one year separation - Pre- and post-vaccination data (milk parameters, milk bacterial dissemination). - Post-vaccination ISO and rectal temperature.</td>
<td>- Lactating dairy cows of any weight - SICCT used to confirm negative status</td>
<td>240 cattle</td>
<td>3 y 400 – 650</td>
</tr>
<tr>
<td>AND Nasal samples for bacterial shedding</td>
<td>Generate data: - ISO &amp; rectal temperature</td>
<td>WPP8.2 (Appendix 15)</td>
<td>- Farm sites low risk area - 80 cows (20 per farm site) - Two vaccinations, one year separation. - Pre- and post-vaccination data (bacterial dissemination) - Post-vaccination ISO and rectal temperature.</td>
<td>- Dairy or beef cattle of any age and weight - SICCT used to confirm negative status</td>
<td>80 cattle</td>
<td>3 y 300 – 450</td>
</tr>
<tr>
<td>OR Milk and nasal samples for bacterial shedding</td>
<td>Generate data: - ISO &amp; rectal temperature - Milk yield and composition</td>
<td>WPP8.3 (Appendix 16)</td>
<td>- Farm sites low risk area. - 20 cows&lt;sup&gt;a&lt;/sup&gt; of each parity 0-5&lt;sup&gt;b&lt;/sup&gt; (120 total per farm, 2 farms) - Animals at least 21 days post calving - Two vaccinations, one year separation - Pre- and post-vaccination data (milk parameters, milk and nasal bacterial dissemination) - Post-vaccination ISO and rectal temperature</td>
<td>- Lactating dairy cows of any age and weight - SICCT used to confirm negative status - Combination of WPP8.1 and WPP8.2</td>
<td>240 cattle</td>
<td>3 y 450 – 700</td>
</tr>
<tr>
<td>Semen samples for bacterial shedding and semen quality</td>
<td>None</td>
<td>WPP8.4 (Appendix 17)</td>
<td>- CRO - 20 bulls - Two vaccinations, one year separation - Pre- and post-vaccination data (semen bacterial dissemination) - Cull and post mortem to determine bTB status</td>
<td>- Mature dairy or beef bulls of known reproductive history - SICCT used to confirm negative status</td>
<td>20 bulls</td>
<td>2 y 300 – 450</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> Minimum number, reserves will be recruited to allow for drop-out during the study

<sup>b</sup> Parity is counted as the number of established pregnancies – parity ‘0’ is a heifer
Table 6: WPP8 - Summary of the Advantages and Constraints of Each Proposed Study Design

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Advantages</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPP8.1 and</td>
<td>– Standard designs for shed and spread study</td>
<td>– Possibility of higher execution costs compared with combined design of WPP8.3</td>
</tr>
<tr>
<td>WPP8.2</td>
<td>– Robust design which should produce good quality and unequivocal data in a range of farming situations.</td>
<td>– High cost associated with validation of analytical method (nasal only)</td>
</tr>
<tr>
<td>WPP8.3</td>
<td>– As an alternative to WPP8.1 and WPP8.2 will require overall fewer animals than running two independent studies and is therefore cheaper than running two separate studies</td>
<td>– Range of animal breeds/types for collecting nasal samples is restricted to dairy breeds.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– More complex study management</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Risk of interference between two different primary objectives resulting in poorer data quality.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Any repeat study could result in a higher expenditure than 8.1 plus 8.2</td>
</tr>
<tr>
<td>WPP8.4</td>
<td>– Would generate unequivocal data on shed and spread via semen, and semen quality</td>
<td>– Likely to have a relatively high cost for demonstrating aspects of safety not widely considered to be significant cross infection risk. May, however be deemed significant to stakeholders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– High cost associated with validation of analytical method</td>
</tr>
</tbody>
</table>
If a placebo were to be used in BCG efficacy studies, consideration should be given to the studies in WPP8. Alternatively animals allocated to CP groups may be left unvaccinated as ‘in-contact controls’. The benefits of a placebo would be the collection of injection site observation data in lactating cattle within this WPP rather than as a part of WPP6. It is, however, currently understood that using a placebo may require a project licence under A(SP)A. It may therefore not be practical to use a placebo. Use of a placebo can be considered as the “gold standard” to generate additional safety data prior to conducting the pivotal field studies and its use has been considered in the presented designs.

4.3 WPP8: Field trial making provision for the generation of samples for analysis of post-vaccination bacterial shedding in milk and the respiratory tract

4.3.1 Study Design Options WPP8.1 to WPP8.3

A total of three possible study designs (WPP8.1 to WPP8.4) have been identified and these are detailed in Appendix 14 to Appendix 17 respectively. Their primary and secondary objectives as well as estimated costs and timeframes are summarised in Table 5.

WPP8.1 and WPP8.2 are designed to collect milk and nasal swab samples respectively from vaccinated animals in separate trials whilst WPP8.3 is an alternative design to collect both sample types from the same animals. All options are randomised, blinded, placebo-controlled studies (as per EMEA/CVMP/VICH/359665/2005 and EMEA/CVMP/852/99). All will test equal numbers of animals tested with IVP and CP and would be vaccinated using the highest recommended vaccine titre.

4.3.2 Study Design Options WPP8.4 (breeding males)

WPP8.4 is a design for a study in breeding bulls to satisfy EU veterinary medicines legislation requirement (V5) and would take the form of being a randomised, blinded, controlled study. The study would test equal numbers of vaccinated and unvaccinated bulls and would use the highest recommended vaccine titre for vaccination.

4.3.3 Considerations on Study Design Options

Animal numbers for studies WPP8.1 to WPP8.4 are expected to satisfy the Commission Directive 2009/9/EC that govern such studies. “Shed and Spread” studies are routinely undertaken for vaccine development and are a standard requirement for a MA application.

As indicated in Table 5 there is a choice to be made of either WPP8.1 and WPP8.2 in combination, or WPP8.3 on its own. As a general rule it is best practice to have just one primary objective for each study so that the achievement of each individual objective cannot interfere with the other. An argument in favour of WPP8.3 is economic and the risk is that an extension or repeat of this study to counter any difficulty would render this option a false economy.

An alternative design option for WPP8.4 is to vaccinate and then euthanise/PM bulls recruited to this study. This design has the flaw that any pre-existing semen quality anomalies could be wrongly ascribed to the BCG vaccine.

WPP8.4 is the study that could potentially be the most rapidly executed. This study could potentially be undertaken under GLP standards as opposed to GCP. Its design and scale are essentially dictated by the requirements of the relevant legislation.

4.4 WPP9 and WPP10: Proposal for a GLP protocol of analysis of milk samples and/or nasal swabs gathered in the field from vaccinated and unvaccinated animals

4.4.1 Study Outlines

A protocol outline for WPP9 is presented in Appendix 18; a protocol outline for WWP10 is presented in Appendix 19.

4.4.2 Considerations on Study Designs

It may be advantageous, for both financial and time line considerations, for WPP9 and WPP10 to be conducted at the same facility, possibly under a single protocol.
Selection of the facility should take into account the following: GLP status; capacity for receiving large numbers of field samples; capacity for dealing with potentially pathogenic samples.

Transfer validation of the test method should be performed where applicable.

4.5 Consortium Proposals

The Consortium suggests that the studies WPP8.1, WPP8.2 and WPP8.4 are conducted in the first instance. There is no scientific constraint on running these studies in parallel with those of WPP2 or WPP6 however the social assessment identifies these studies as critical to realising the benefit of sharing all safety data as early as possible with interested stakeholders.

It is recommended that regulatory scientific advice is sought prior to execution to confirm the data requirement, and in case there is adequate literature data available to address the EFSA/EU veterinary medicines legislation requirements.
5 WORK PACKAGE D – FEASIBILITY OF WIDER USE OF BCG VACCINE

Contribute to assessment of the feasibility of wider use of vaccine beyond the trials.

5.1 Introduction

The preceding WPs are concerned primarily with scientific and technical requirements for assessing efficacy and safety of BCG vaccine use in cattle. This WP, however, takes a broader view of the project’s requirements, impacts and utility and essentially covers three, diverse aspects;

1. The socio-economic impacts of undertaking large scale field studies on commercial farms and of wide scale vaccination campaigns for bTB control.
2. The possibilities of prediction (using modelling) of the effectiveness of vaccination as a tool for controlling bTB spread in the UK.
3. Efficacy must also be considered not only in terms of disease reduction but also of its cost-benefit analysis.

The final cost-benefit analysis will be based on all available information, both the results of this project (WPP2 and WPP6) as well as any other pertinent data that is, or becomes, available.

Finally, this WP also includes a brief implementation plan for the whole project.

The socio-economic effects and effectiveness modelling are clearly linked since effectiveness will include cost-benefit or cost-utility analyses of potential scenarios for vaccine deployment.

By necessity the implementation plan has to be provisional and flexible and it will be subject to change as the project progresses and develops. The project is predicted to last for at least seven years in which time strategies and approaches will change and develop as new information on bTB, that is to say data, understanding and insight, becomes available.

5.2 Work Package Project Requirements

5.2.1 Aim

Contribute to assessment of the feasibility of wider use of vaccine beyond the trials.

There are no specific regulatory requirements applicable to this WP, rather this deals with addressing the practicalities and economics of implementing cattle vaccine field trials in the UK.

- WPP11: Design of group discussion and individual in depth interviews to define the fundamental structure of the investigative process.
- WPP12: Cost-benefit analysis and modelling of use of BCG vaccine in bTB control.
- WPP13: Design of the coordination plan to bring all facets of the investigation together both during the in life phase and as the project concludes.

5.3 WPP11: Design of group discussion and individual in depth interviews to define the fundamental structure of the investigative process

5.3.1 Objectives

The key informant study had the objectives of exploring practical issues of farmer participation in the implementing of the BCG vaccine trials and discussing other issues which might need to be taken into account in optimising the designs. The methodology used was semi-structured interviews with farming, veterinary, processing and retailing sectors stakeholder representatives.

5.3.2 Summary and Conclusions

A network recruitment process was adopted. Two waves of recruitment were undertaken with the second wave being recruited from suggestions by interviewees in the first wave. After briefing on trial design and the issues raised, participants were questioned on their
attitudes to vaccination, willingness to participate, incentives, market and supply chain issues.

Many respondents emphasised the need for a proactive communications campaign so that all stakeholders (including retailers) are engaged and fully informed. Most importantly the farmers should be informed by their veterinarians and/or farming organisations. Farmers could be engaged through public meetings but then smaller events would be needed for potential participants so that the details could be worked out. The ideal facilitators for the meetings would be farmer groups or unions – it would be important for Defra or APHA to take a back seat to suppress any cynicism felt by some farmers towards these bodies.

Key informant interviews indicated that it would be important for the entire farming family including stock-men/women to be involved because complete commitment would be needed. Participants indicated there would be an advantage in issuing contracts or having MoUs so that all engaged are very clear what commitments are required.

Financial incentives were not seen by the interviewees as vital, but full compensation would be, particularly in areas of crisis (i.e. areas where breakdowns had occurred).

Interviewees indicated that if there is a risk that any breakdowns suffered whilst participating in the trials, this would be seen as caused by participation, therefore direct or indirect losses related to any adverse reactions would need to be covered. The potential cost implications for this situation have not been considered in this report.

5.3.3 Consortium Proposals

Preliminary social research has informed some general conclusions important to successful field trial execution;

1. Need to engage and involve all farm staff and family.
2. Full information needs to be provided to stakeholders at the start of various scenarios and related compensation issues need to have been thought through to achieve credibility.
3. Messages (to farming stakeholders) would have more impact coming from trusted sources (farm veterinarians and/or farming organisations) rather than from APHA or Defra.
4. Engage with processors and retailers so farmers are assured that their supply markets will not be disrupted.
5. A contract or MoU with farmers participating in field trials would be important.
6. Facilities and human resources for gathering stock may be limiting, particularly in extensive holdings.

Information and education;

1. It would be beneficial to clarify that a vaccine is only part of a wider strategy, not a solution in itself.
2. Biosecurity has an important role to play; just as important to maintain during trial.
3. Expectations have to be managed; in particular participants need to understand the time-scale for a vaccine being available.

Consideration should be given to a comprehensive communication plan to keep all stakeholders and the general public informed on this project.

5.4 WPP12: Cost benefit analysis and modelling of the use of BCG vaccine in bTB control

Implementation costs for the studies described in this report include professional fees for implementing, conducting, analysing and reporting the field trial designs. These take account of costs of recruiting and providing information and training for participating farmers, veterinary practitioners’ involvement in administering vaccines and placebos, incentives to participate and compensation for any consequential losses which may arise. In net present value terms, these costs will vary according to the length of trial period and the numbers of animals and farms which are required. Once the trials are concluded, there will be additional costs associated with the
DIVA test (which is likely to be more expensive than the current SICCT test) which will need to be used where animals are known to be vaccinated. While this could result in more false positive results requiring additional compensation, it would be compensated for by lower rates of transmission.

Benefits will arise from the adoption of the BCG vaccine which, despite current existing evidence of its partial protective effect, is likely to have two major effects:

- To reduce within-herd transmission in the case of a breakdown, and consequently the average costs of compensation involved in each breakdown (Appendix 21).
- To reduce between-herd transmission because of direct and indirect protective effects (Appendix 21).

These benefits will also be realised during the trials themselves in participating farms, although these will be minor compared with anticipated usage once the BCG vaccine can be used without restriction. Costs and benefits are assessed, in net present value constant price terms, over a 20 year time horizon.

As with all cost-benefit analyses, there are some very substantial uncertainties to consider. In terms of costs, the level of incentives to participate and more particularly compensation required for any market risk, should be subject to sensitivity analysis. Likewise, predicted reductions in within-herd transmission are derived from modelling studies and the rate of new breakdowns from time trend projections. The total cost of breakdowns, both public and commercial, depends on overall market conditions which may affect the value of stock and of the opportunity cost of labour. Finally, there is also interdependence between costs and benefits, to the extent that earlier utilisation of vaccines following a shorter but possibly more expensive trial will bring forward anticipated benefits.

There are also intangible factors which can be estimated, but not with any certainty. These include psycho-social costs of breakdowns on farmers and their businesses which, if reduced, would increase the value of benefits. Also on the positive side, reduction in the overall incidence of bTB would provide a more stable primary production base for the dairy and beef supply chains, which would improve their overall efficiency and profitability. Vaccination may be voluntary or compulsory (either across all of England and Wales or in high risk areas); if voluntary, some farmers may choose not to use it. Intra-EU trade in vaccinated animals would probably not be permitted until substantial practical experience of vaccination and the DIVA test had been acquired; farmers may decide to opt not to vaccinate for export markets, or to vaccinate and forego export opportunities. In 2012, just fewer than 39,000 cattle were exported from the UK, mainly to Spain and Ireland. These issues should be noted when reviewing and interpreting the overall net present value measures.

As a part of the sensitivity analysis in Appendix 21 it is concluded that, except in substantially changed circumstances, monetary costs of the trials and additional post-trial vaccination and testing outweigh the benefits substantially. If vaccination reduces the incidence of the disease or the value of savings from reduced herd breakdowns is greater than assumed in the central estimates on which this conclusion was based, the extent to which costs exceed benefits in net present value terms only diminished by between 10-20%. In view of this analysis, the case for public support of vaccine field trials rests on the importance of intangible but nevertheless significant non-monetary benefits which can be obtained from reduction in the incidence of bTB. Three such benefits were identified:

- The welfare and psychological costs to farms and farm families from the experience of a bTB breakdown.
- The improved efficiency of the beef and dairy supply chains which would result from reduced disruption of production volume.
- The reduction in the compulsory slaughter of bTB suspect positive cattle, which is an animal welfare issue of significant public and political concern.

Notwithstanding the need to avoid what has been termed ‘confirmation bias’ in policy choice, the conclusion of this analysis is that substantial weight would need to be given to these intangible elements for the field trials to achieve greater benefits than costs.
6 DISCUSSION

6.1 Introduction

Development and regulatory approval of a cattle BCG vaccine and associated DIVA test(s) by the use of field trials would allow their use alongside other bTB control measures. This study has taken an interdisciplinary approach to informing field trial designs, incorporating scientific information produced by APHA during BCG vaccine and DIVA diagnostic test development, predictive modelling research at the University of Cambridge Department of Veterinary Medicine, statistical analyses by BioSS, regulatory compliant study design outlines by Triveritas, and socio-economic information and cost benefit analyses produced by Aberystwyth University. Help from XLVets and two veterinary surgeons with specific TB expertise, feedback from stakeholder representatives and written regulatory guidance was also used. The field trial designs could be used to assess the field performance of the vaccine and validate the associated DIVA test(s) in England and Wales.

Computer modelling and statistical analyses have been used to optimise the field trial designs especially to estimate the minimum numbers of cattle and herds required for the DIVA validation and vaccine field efficacy trials. Information is provided on estimated costs and benefits, including significant non-monetary (“intangible”) impacts, for farmers, veterinarians and other stakeholders of deploying the vaccine and the DIVA test(s).

The field trial designs include application of the appropriate quality standards (e.g. GCPv), and consideration of specific written regulatory requirements and advice (supplied via Defra). Performance of any field trials would need continuing expert regulatory awareness during their execution to try to ensure regulatory compliance. Some of the field trial designs provide data collection both for their core and other regulatory requirements such that executing these designs within an interlinked programme should be cost-effective.

The time to approvals and use of a BCG vaccine and associated DIVA test(s) would depend on several factors including: the precise trial designs selected, appropriate clarifications with regulatory agencies, the design of the overall trials programme, the speed of the trials, the trial results and their assessment time by regulatory agencies.

6.2 Feedback from key informant interviews

The key informant feedback from 21 interviews indicated that a well-planned communications campaign aimed at farmers, their veterinarians and other key stakeholders would be important to ensure good compliance with any field trials. The role of Defra and APHA in such a campaign should be carefully considered with the use of “trusted” sources of information such as veterinarians and farmers unions being reported as beneficial. Interviewees indicated that the key information and education issues include: vaccination being only part of a bTB strategy, biosecurity remaining important, and expectations of the vaccine and the timescale for its availability.

Key informants considered that engagement with bTB trials was more likely to be highest in high risk areas. The field trial designs are concentrated on these areas, and estimated payments for farm labour for trial work were included in the costs of field trials. Full field safety information at the outset for the food supply chain was considered very important (which is also reflected in the suggested sequence of field trials), with early and full engagement with processors and retailers. Publicity using for example farmer meetings and involvement of all farm staff was suggested together with contracts (which could be field trial agreements) for those involved in trials. Comments regarding potential interactions of the BCG vaccine with other vaccines or medications were raised but the potential for such interactions would normally be addressed by laboratory studies, not field trials. Recording concomitant use of other vaccines and veterinary medicines is included in the field trial designs. Participants also considered it important to address the wildlife reservoir during field trials.

6.3 Cost-benefit analysis

The cost-benefit analysis indicates that the cost of the field trials is small compared to subsequent use of the vaccine and testing over a 20 year period.
Using Net Present Values (NPVs)* the estimates for post-trial vaccination and testing costs were approximately £1 to 1.4 billion compared to £32 to £45 million for the trials (i.e. trial costs were 2.4% to 4.4% of the overall cost estimate, Appendix 21 Table A1.3.5). The trial costs used for this calculation excluded the costs of any field efficacy "transmission" study (WPP6.3) in the initial trials, and as discussed in Section 6.5 below we think that subject to discussion with regulators that this is an appropriate approach.

The conclusion of the analysis is that substantial weight would need to be given to the significant “intangible” elements for the field trials to achieve greater financial benefits than costs over a 20 year time horizon.

The NPV balance of costs over benefits of using the cattle BCG vaccine and DIVA over a 20 year time horizon was £768 million to £986 million without sensitivity testing the data or consideration of intangible factors. Significant improvements in the cost-benefit analysis were observed when post trial costs and “intangible” benefits were considered.

Three “intangible” elements were considered:

- psycho-social costs of bTB breakdowns on farmers and their businesses which, if reduced, would increase the value of benefits.
- Reduction in the overall incidence of bTB would provide a more stable primary production base for the dairy and beef supply chains, which would improve their overall efficiency and profitability.
- The compulsory slaughter of cattle suspected of having bTB is an animal welfare issue of public and political concern.

The effects of “intangible” factors, and sensitivity testing using different scenarios are reported in Appendix 21, and as with all such cost-benefit analyses, there are some very large uncertainties to consider. One important conclusion to note is that post-trial costs would be reduced by the use of a validated skin DIVA test rather than a blood-based gamma DIVA test. This highlights the potential financial benefit of having a skin DIVA test ideally replacing the current SICCT.

### 6.4 Safety

Confirming the absence of bacterial shedding into milk or from the respiratory tract of BCG vaccinated cattle in the field is a regulatory request and demonstration of BCG vaccine safety in the field would also help alleviate concerns of processors, retailers and farmers prior to their participation in large-scale trials. The shedding trials would be simpler and shorter than any of the other trials required. The likely cost of this key field safety data is estimated at approximately 2% of the total trial costs (£1.3 million of approximately £56.4 million). The field safety trial designs proposed for consideration (WPP8.4 plus either WPP8.1 + WPP8.2, or WPP 8.3) would also generate data on injection site reactions, milk yield and composition, and vaccine use in breeding bulls.

Additionally small scale studies to assist regulatory justification of blinding or masking procedures to be used for untreated cattle in the later larger field trials could be performed alongside these safety studies. The important regulatory issue of blinding or masking is further discussed in 6.7 below.

Consequently these safety trials should be considered as an important, early, relatively rapid (estimated completion approx. 3 years from a decision to start) and economical part of any development programme.

* In this section of the Discussion only (6.3 Cost-benefit analysis) the monetary values used are Net Present Values (or NPV), which is a standard method in such work regarding the value of money over periods of time (to appraise long-term projects such as the TB trials and TB vaccine use). NPV compares the present value of money today to the present value of money in the future, taking inflation and returns into account (and in our case provides lower values than those estimated as current 2014 levels).
The completion of the field safety trials would provide a clear go/no go decision point and provide an opportunity to demonstrate safety to the wider public, and stakeholders, before commencement of full trials.

6.5 Field efficacy and in use safety

Trial designs have been considered taking account of key informant responses, modelling, statistical and regulatory considerations. Study designs such as WPP6.1 and WPP6.2 are to facilitate assessment of the direct efficacy of the vaccine (e.g. the reduced risk of vaccinated animals having confirmed bTB lung lesions).

The suggested main study design to investigate the direct efficacy of the vaccine (WPP6.1) uses 12,000 or more cattle on approximately 100 commercial farms (not AFUs) in high risk areas in England and Wales. The design WPP6.1 also provides data required for DIVA validation (using latent class analysis). In addition to the Safety studies above this study should provide in-use vaccine safety data (which is a regulatory requirement) including: injection site observations, milk production, use in pregnancy, and effects on fertility. Such a trial would provide data regarding vaccine use in a range of ages, breeds, weights, dairy and beef cattle, on farms with different environmental challenge and farms with different husbandry and management conditions.

Investigation of the incidence of confirmed bTB lung lesions in vaccinated versus unvaccinated cattle, and the prevention of these lesions (based on previous laboratory research results which were statistically significant) is considered a key claim to permit authorisation of a UK MA. This prevention of lung lesions would be investigated further in the field by using the suggested design.

Modelling has indicated the optimal design within each farm on a randomised basis would be 50% vaccinated cattle and 50% unvaccinated. This design would minimise any bias affecting vaccinated or unvaccinated cattle due to wildlife or environmental factors. Also this design using only within-herd controls ensures maximum power for 50% coverage. If a mixture of different vaccine coverage was used (for example 75% vaccinated compared to 25% unvaccinated) it would reduce the study power such that more herds would need to be recruited to provide the basic estimate of direct efficacy.

The estimated cost indication to execute the efficacy trial design WPP6.1 is £33.5 million (59% of the estimated cost indication for all field trials excluding WPP6.3). Such a study would take an estimated 4 to 8 years, with a suggested review period, (e.g. after two years of live phase), and with the option of early termination should results be statistically definitive. Precise timing would depend on a variety of currently undefined factors including but not limited to: success of a communications policy and farmer participation, resource allocation, and disease incidence.

A second relatively small field trial design in young cattle (WPP 6.2) should be considered. WPP 6.2 (which has an estimated cost indication of £1.6 million with an estimated duration of approximately 4 years) is designed to address several issues:

1. to provide efficacy data to support vaccine use in young cattle for the MA application;
2. by vaccinating young cattle on bTB negative farms but subsequently rearing them on an estimated 10 AFUs it could also provide further data to support the MA application especially regarding the key claim of prevention of lung lesions;
3. a specific regulatory request that a DIVA test should be relevant to animals from the minimum recommended age of vaccination (the current target minimum age for vaccination is 15 days) such that this "efficacy" study is therefore also part of a DIVA test validation.

Successful execution and results from study design WPP6.1 should address most of the EFSA Scientific Opinion efficacy requests and WPP6.2 similarly should address potential data gaps that are likely to be required for regulatory purposes. However, the EFSA Scientific Opinion makes an additional comment regarding efficacy: "Data collected should enable estimation of vaccine-induced reduction of bTB transmission, and not just reduction of bTB incidence, in order to reach a conclusion regarding the contribution of vaccination to eradication." (E9).

We have considered this issue carefully and feel that based on the key informant study, and modelling to optimise study designs, that it would be extremely challenging (indeed we believe
not possible) to address this request using an initial field trial in England and Wales. There are other potential ways this request might be addressed including subsequent field trials after the BCG vaccine is in use.

The investigation of the contribution of vaccination to eradication (E9) is not addressed by study designs such as WPP6.1 and WPP6.2 (these designs consider direct efficacy of the vaccine for example the reduced risk of vaccinated animals having confirmed bTB lung lesions). E9 is considering what is known as “indirect efficacy” of the vaccine which is not a requirement for a vaccine to be authorised (i.e. to obtain a UK Marketing Authorisation). It is currently thought that vaccinated animals that become infected might have a slower rate of progression or reduced infectiousness and there might be an additional reduction in the rate of bTB transmission (which is termed indirect efficacy).

To assess this indirect efficacy request E9 one could compare the incidence rate in unvaccinated controls within a partially vaccinated herd and completely unvaccinated control herds. Furthermore this comparison should be between paired farms (one partially vaccinated farm versus one unvaccinated) which are considered similar throughout a trial that would need to last several years. This would mean trying to identify a large number of similar paired farms which would be very difficult. Additionally for the study to be conducted on a blinded basis it would mean that none of the farmers would know if their herd was either partially vaccinated or completely unvaccinated.

This forms the basis for study design WPP6.3 and the unvaccinated control herds are a critical requirement to estimate the indirect efficacy (and each unvaccinated herd must have a bTB challenge which is similar to that for the paired partially vaccinated herds). An initial attempt to minimise the size of this trial, due to its inherent difficulties, resulted in the WPP6.3 design provided which uses a total of 240 herds (including 120 herds completely unvaccinated for approximately 5 years) and has a cost indication of £20.3 million.

Unfortunately our subsequent findings indicated that a study of this size would only provide an approximately 30% power of estimating any statistically significant effect – and this is not recommended due to the low statistical powering. Based on the effect size predicted by the model (Figures A3.7-A3.12 of Appendix 3), for 80 to 90% power levels (which are normally used) there would need to be between 1,000 and 1,500 herds to estimate a statistically significant (protective) effect in a study design like WPP6.3. Apart from the major increase in cost indication (estimated in the region of perhaps £80 to £120 million for this single larger trial using WPP6.3) the key informant study suggests that few or no farmers would be willing to be involved (Appendix 20, Section 3. Specific Questions, responses regarding placebo or blinding).

The responses indicate that a sufficient number of farmers are very unlikely to agree to a blinded or masked study in which 500-750 whole herds would be totally unvaccinated but involved in repetitive GCPv trial procedures for approximately 5 years.

Based on the detailed assessment within this report the Consortium suggest that EFSA is consulted regarding options for assessing the indirect effect of BCG vaccine upon transmission, as this is not required for a UK Marketing Authorisation.

6.6 DIVA test(s) validation

The BCG cattle vaccine could not be used as part of any control programme, and efficacy field trials could not be assessed, until one or more suitable DIVA test(s) has been validated. A blood DIVA test is currently available for validation, and a skin DIVA test is in research. The latter is likely to be significantly more economical for use and the key informants have indicated that farmers are wary of the cost implications of any new test. Therefore a cheaper skin DIVA test is likely to be preferred to the blood DIVA test. Validation of either the blood or more cost-effective skin DIVA test using field trials is estimated to cost approx. £20 million (36% of total field trial costs excluding WPP6.3).

There are several options for DIVA validation as shown in Table 7.
**Table 7: Cost and Time Indication of some DIVA validation Options**

<table>
<thead>
<tr>
<th>Option</th>
<th>Est. approx. cost indication, £m</th>
<th>Est. delay to use of BCG vaccination in England &amp; Wales vs. option (a). Years</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Blood DIVA test only (started at similar time to Safety trials WPP8.1, 8.2)</td>
<td>20.2</td>
<td>N/A¹</td>
<td>Relatively expensive blood test is the only DIVA available resulting in major increased costs in bTB testing during vaccine use. Not recommended for consideration based on cost benefit analysis in Appendix 21.</td>
</tr>
<tr>
<td>(b) Skin DIVA test only</td>
<td>20.2</td>
<td>&gt;1.5²</td>
<td>Main efficacy field trials &amp; BCG vaccine use could not start until the skin DIVA test became available (at least 1.5 years). Any delay to the development of the skin DIVA test would cause further additional delays to the main trials and vaccine use.</td>
</tr>
<tr>
<td>(c) Blood DIVA test (started at similar time to Safety trials); then Skin DIVA test (with “300 positives” generated after vaccine is in use, without much or any use of AFUs).</td>
<td>30.2</td>
<td>0</td>
<td>Avoids unforeseen development delays to the skin DIVA test causing delays to vaccine use.</td>
</tr>
<tr>
<td>(d) Blood DIVA test followed by Skin DIVA test (attempt to generate “300 positives” for each DIVA before BCG vaccine is approved and in use)</td>
<td>Up to 40.4</td>
<td>0</td>
<td>Higher cost than option (c) but might be a shorter period of blood DIVA test use in England &amp; Wales than in option (c). More challenging and less feasible than (c) to generate “300 positives” for each DIVA, and this might result in similar timing to (c) – which would mean higher cost but no additional benefit.</td>
</tr>
</tbody>
</table>

Notes: 1 N/A: Not Applicable, 2 Best estimate from APHA given what is currently known about the skin DIVA test. Performing validations of both the blood DIVA test and a skin DIVA test would increase overall DIVA test validation costs and this has not been used in the trial costs or cost benefit forecasts.

The current understanding of OIE requirements for field trial data to assist validation includes advice from APHA of a need for 300 cattle that are vaccinated but subsequently become infected with bTB (“300 positives”) plus 1,000 cattle that are vaccinated but do not become infected with bTB (“1,000 negatives”).

It will be challenging to generate “300 positives” and consideration of this should be discussed further with the OIE. One option is to use AFUs which have a higher level of bTB infection. However, our modelling suggests that the current number of AFUs in England and Wales would be insufficient to practically generate these “300 positives” rapidly. We estimate the validation trials would last 4 years at 100 AFUs (or, for example, 8 years using 50 AFUs) plus additional time during efficacy trials (for latent class analysis to complete the validation).

An alternative option would be to consider use of some farm sites in addition to AFUs to generate “300 positives”. Such farms would need to have a high level of bTB challenge but against this the farmer normally would be working to decrease the presence of bTB, and bTB reactors would be
removed from the farm. Trying to retain bTB reactors on farms could have legal and Health and Safety implications. Any possibility of retaining reactors on a farm would create an experimental study (not a field trial due to the change in husbandry), and if a farmer agreed to a study then we assume financial compensation could be required (e.g. due to increased risks to herd health). Therefore, other farms might be used however this would require more animals, more time and cost more.

Due to the challenges envisaged regular reviews (e.g. annual or biennial) should be considered to monitor DIVA validation progress. This report indicates that the trial design options (WPP2.1 or WPP2.2 or WPP2.3) for “300 positives” account for 76% of an estimated total blood or skin DIVA validation cost of £15.3 million.

Three other potentially more cost-effective approaches to generate some or all of the “300 positives” were considered: use of bTB infection models in cattle at research facilities, the use of field trials outside UK, and the vaccination of bTB reactor cattle. However, during the development of feasible options these alternative approaches were not favoured by Defra, the Welsh Government and their advisors, but the option remains of discussing these approaches with the appropriate regulators (e.g. OIE and possibly EFSA or EC).

As noted by EFSA the strategy of validating a DIVA test and vaccine performance at the same time (by latent class analysis in the efficacy study) poses risks because only limited conclusions about vaccine performance could be made if test performance was later found to be poor.

The trial design (WPP2.4) presented here for the “1,000 negatives” should be relatively simple, economical (c. £0.69 million), and quick (estimated 2 to 3 years) to execute using suitably selected cattle and commercial farms in low risk areas. However, key informants have advised that willingness for trials in these areas might be more problematic and incentives might be required for these relatively small trials.

There are further trial designs included in the DIVA validation field trial programme which relate to investigating any potential interference between a SICCT and a DIVA. Firstly a trial (WPP2.6) for the blood DIVA test to investigate any potential impact of using the current SICCT on subsequent use of a DIVA (this would not be required for a skin DIVA test if it was not used with the SICCT).

Depending on regulatory advice, the results might be required to address potential validation questions, and could be beneficial in decreasing future bTB testing expenditure in a control programme by avoiding continuing use of SICCT in addition to a DIVA. The cost indication and duration of WPP2.6 are relatively limited at approx. £0.33 million and 1 to 2 years, but the design is for it to be performed on farms in low risk areas (where incentives might be required to encourage trial participation).

An alternative option which could avoid WPP2.6 is to perform DIVA validation(s) which incorporates the use of the SICCT test (e.g. Annex 1 Variants on WPP2 studies) which would mean the DIVA test would be applicable in such a situation (i.e. following use of SICCT).

Written guidance from EFSA regarding the approach to DIVA validation and the use of SICCT has not been seen, and in the Consortiums’ opinion it is currently uncertain which of these above approaches should be used. Consequently we strongly suggest that there is interaction with regulators and if applicable the European Commission to obtain clarification of their precise views.

Using SICCT plus an additional DIVA test after approval of the BCG vaccine would increase the costs of bTB testing and would adversely affect the cost benefit and acceptability over a 20 year horizon. A novel option suggested for consideration (dependent on regulatory discussions) is to validate the blood DIVA with the SICCT but any subsequent skin DIVA test validation to be without the SICCT (and possibly perform a design similar to WPP2.6 for the skin DIVA test only). If this approach were to be acceptable to regulators it could have significant benefits:

i. no delays to the overall programme or trials due to DIVA validation following a WPP2.6 study,

ii. reduction in costs for two DIVA validations,

iii. the cost of DIVA validation could be reduced due to concurrent evidence of a vaccine being safe and effective,
iii. later skin DIVA test validation trials should be easier and more feasible than current challenging validation trial designs (if vaccination were approved and in use it should minimise any need for further AFU based trials),

iv. unforeseen delays in R&D of the skin DIVA test should not affect trial timings or BCG vaccine approval,

v. permitting a period of post-authorisation vaccine use when the SICCT and blood DIVA could be used together (e.g. if required by the European Commission),

vi. and subsequent introduction of a skin DIVA test which is used without the SICCT (e.g. to replace the blood DIVA test), hence maximising cost benefits for bTB testing and presumably having the acceptability of a single skin test.

Another validation trial design WPP2.5 concerns a potential OIE requirement using unvaccinated cattle to assist blood DIVA validation and has a cost indication of £3.9 million and estimated duration of 1 to 2 years.

Consideration should be given to further discussions with OIE (before and during DIVA validation trials) regarding the difficulties in meeting the requirements discussed above.

6.7 Other

The cost indications are approximate estimates based on available information and from our experience of conducting field trials of this type. The following non-exhaustive list of field trial costs were not included: a communications policy, further socio-economic work, compensation if data to support a zero milk and/or meat withdrawal is not achieved, regulatory fees or work (e.g. to obtain ATCs, for MA approval, discussions with OIE or EFSA, etc.), any compensation to farms which have cases of bTB during trials, any compensation for slaughter of cattle that are reactors, any incentives to encourage farmer participation (e.g. which might be required for the relatively limited trials in low risk areas), and any items associated with blinding (see below).

EFSA have advised “The double-blind randomised controlled trial design is recommended as it guarantees the lowest possible level of bias. Any proposed deviations from the preferred trial design should be justified.” (E5). This design implies that an indistinguishable placebo should be administered to unvaccinated cattle such that everyone directly involved in the trial would be unaware which cattle were vaccinated (alpha-numerical codes to identify treatments used would be stored confidentially at a secure location). Our understanding is that any justification to explain why a placebo was not to be used should be based on scientific data that would be acceptable to EFSA. Performing relevant trials without a placebo and then finding EFSA did not accept that design could result in the trial(s) being unacceptable to EFSA and hence a significant loss of investment, work and time.

We are currently unaware of any suitable placebo for the BCG vaccine and production of a placebo might prove challenging and take considerable time. If it is thought that there is already sufficient information (e.g. laboratory investigations, scientific literature and arguments) to scientifically justify not using a placebo then it should be discussed with EFSA as soon as possible.

Other approaches to attempt to make treatment groups anonymous that are often used include “masking” which involve those administering the vaccine (or a negative control substance or a sham administration) not being involved in any other part of the trial. This is relatively labour intensive and more logistically complex at farm level and in large multi-site trials might prove to be less cost-effective. However, it is strongly suggested that an acceptable scientific justification is required if a placebo were not to be used before the commencement of any field trial for which a double-blind design could be considered. The written opinion of EFSA and others on this topic is vital and this might differ from that of the trial designer, sponsor, or those performing the field trials.

Investigating alternatives to a placebo could be relatively inexpensive and could be started early, for example alongside the Safety field trials (e.g. WPP8.1 and 8.2). This is because these Safety trials do not require double-blinding and the current designs would allow data generation to investigate the feasibility of “masking” which might prove helpful in any subsequent justifications to EFSA. However, if this approach is adopted, the Consortium suggests that manufacturing of a
placebo is also simultaneously considered because if any “masking” investigation should prove unsuccessful then without detailed justification on placebo production it might lead to the entire cattle BCG vaccine project being delayed. Active investigation of placebo production should be seen as a time critical issue for the field trials. If it was progressed one approach would be an initial feasibility investigation (a work plan or budget has not been devised but a notional amount of up to £250,000 is estimated as possibly appropriate). Any potential costs for placebo development or masking trials have not been included in the current costings.

There are several further regulatory issues which could have significant time and/or cost implications to field trials, including whether or not there would need to be a withdrawal period for milk and/or meat following BCG vaccination of cattle, and whether or not personal protective equipment (e.g. face mask) would need to be worn during BCG vaccine administration. Additionally the Consortium suggests that the selected field trial designs are described to the RCVS in detail to establish whether the procedures involved are considered Recognised Veterinary Practice (RVP) or whether A(SP)A licences from the HO would be required to cover elements of the trials.
7 CONCLUSION

This study has led to the proposal of potential field trial designs to evaluate the performance of a cattle BCG vaccine against bovine tuberculosis (bTB) and validate associated DIVA diagnostic test(s) for future use in England and Wales. A number of designs, informed by key informant opinions, cost-benefit analysis, regulatory requirements, computer models and statistical analyses have been described.

Key informant interviews highlighted several important issues, including that a well-planned communications campaign to inform and educate stakeholders is vital for the field trials. Also field confirmation that bacteria are not shed into milk, or from the respiratory tract, of BCG vaccinated cattle will be required by processors, retailers and farmers prior to their participation in large-scale trials. This confirmation is also a regulatory requirement and the field safety trial designs proposed to address this issue are relatively economical (2% of the estimated total field trial costs). These trials should be considered as an early, cost effective, and relatively rapid start to a field trial programme. The results from safety trials would provide a clear, timely, go/no go decision point.

Cost-benefit forecasts indicate that the total cost of field trials would be relatively small (2.4% to 4.4%) compared to costs associated with twenty year use of the BCG cattle vaccine. Approximate estimated cost indications for the conduct of the field trials are £56 million spread over several years, but the final cost depends on several variables including which of the trial designs are selected, the results and better defined regulatory requirements. As with all such cost benefit analyses there are large uncertainties, but over a twenty year time horizon of BCG vaccine use it is concluded that substantial weight would need to be given to significant "intangible" elements in order for the field trials to achieve greater financial benefits than costs. "Intangible" elements include: psycho-social costs of bTB breakdowns on farmers and their businesses, a reduction in the overall incidence of the disease which would provide a more stable primary production base for the dairy and beef supply chains (improving their overall efficiency and profitability) and that the compulsory slaughter of cattle suspected of having bTB is an animal welfare issue of public and political concern.

Significant improvement in the cost-benefit balance occurs where assumptions concerning post trial costs are modified, and following financial consideration of "intangible" benefits. For example post-trial costs would be reduced by the use of a successfully developed, validated lower cost DIVA test (such as a DIVA skin test).

A design for the main efficacy and in use safety field trial suggests 12,000 cattle on approximately 100 commercial farms in high risk areas of England and Wales should be used. This trial would also generate data to assist DIVA test validation.

The Consortium suggests that there should be consultation with EFSA regarding their request for field trial data to investigate vaccine efficacy on bTB transmission (which is not a requirement for a UK MA). This is because the results of our work from a disease model and statistical calculations, plus the views of key informants indicate that unfortunately it appears impossible to address this issue in an initial UK field trial. Our findings for such a study would mean having 500-750 unvaccinated herds in a field trial lasting several years (with the lack of vaccination being unknown to the farmers involved), and key informant feedback suggest that this would not be possible. The scientific information within this report should provide a suitable basis for a discussion with EFSA regarding this request.

Field trials are needed to validate a DIVA test(s) for use with the BCG vaccine. The report findings indicate that validating only the blood DIVA test would adversely affect cost benefits over a twenty year horizon of vaccine and DIVA test use. Whereas waiting for the completion of development of the cheaper skin DIVA test to be available prior to starting any DIVA validation field trials could cause a delay to both efficacy trials and general BCG vaccine use by at least 1.5 years. Such delays could be avoided by starting the blood DIVA test validation field trials as soon as possible and subsequently the skin DIVA test validation trials. This approach would result in a post approval use period of the BCG vaccine where the SICCT and blood DIVA test were used together, prior to potentially their replacement with just the skin DIVA test. Clarification of various details is required from OIE and possibly the European Commission to allow the selection of the most suitable trial designs for DIVA validation.

Using the information provided in this report, further opinions from regulatory agencies are required to finalise the selection of some field trial designs. The topics for clarification include: EFSA regarding the blinding or masking of efficacy trials; and the VMD regarding withdrawal periods, protective clothing, communications campaign to inform and educate stakeholders is vital for the field trials. Also field confirmation that bacteria are not shed into milk, or from the respiratory tract, of BCG vaccinated cattle will be required by processors, retailers and farmers prior to their participation in large-scale trials. This confirmation is also a regulatory requirement and the field safety trial designs proposed to address this issue are relatively economical (2% of the estimated total field trial costs). These trials should be considered as an early, cost effective, and relatively rapid start to a field trial programme. The results from safety trials would provide a clear, timely, go/no go decision point.

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Significant improvement in the cost-benefit balance occurs where assumptions concerning post trial costs are modified, and following financial consideration of "intangible" benefits. For example post-trial costs would be reduced by the use of a successfully developed, validated lower cost DIVA test (such as a DIVA skin test).

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Using the information provided in this report, further opinions from regulatory agencies are required to finalise the selection of some field trial designs. The topics for clarification include: EFSA regarding the blinding or masking of efficacy trials; and the VMD regarding withdrawal periods, protective clothing.
pharmacovigilance during trials, and ATCs. Derogations regarding EU bTB policy are also needed to perform the field trials.

The start of the execution phase of field trials would indicate an end to the current field trial design phase. The execution and management of field trials would require a focussed development approach using the selected trial designs with prior guidance on key issues from regulators. Some of the field trial designs provide data collection both for their core and other regulatory requirements such that executing these designs within an interlinked programme would maximise cost-effectiveness. The Consortium suggests the use of an interdisciplinary approach to delivery of the field trials using experts with experience of socio-economics (where further work is needed), regulatory requirements and advice, trial clearances, derogations, vaccine supply and blinding. The launch of a BCG cattle vaccine addressing the current EU regulatory requirements might subsequently lead to commercial development and launch of new bTB vaccines and associated diagnostic tests.
APPENDICES
APPENDIX 1 WPP1 - PRE-CLINICAL DATA PACKAGE IN SUPPORT OF BLOOD IFN-γ DIVA TEST VALIDATION

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1 INTRODUCTION
WPP1 has been completed by the APHA and has been independently reported to Defra.

The blood IFN-γ DIVA test will comprise two reagents (i) a peptide cocktail for ESAT-6/CFP-10 and (ii) a peptide cocktail for Rv3615c. A DIVA test positive result is declared if a response is detected to either of these reagents.

It is currently understood that APHA have a draft OIE ‘Application Form for the Certification of Diagnostic Test as validated fit for specific purposes’ for both the blood DIVA and skin DIVA tests. This is a working document and is under revision as new data becomes available. The document contains data generated to date describing the sensitivity and specificity, and reproducibility of the blood IFN-γ DIVA test. As this work by APHA forms part of a separately funded Defra project it is not presented within this report. However a brief outline of the current status of blood IFN-γ DIVA test validation process is summarised below.

1.1 Unvaccinated

Before APHA began evaluating the performance of the DIVA test performance in relation to use of the BCG vaccine they were generating data on the DIVA antigens in non-vaccinated, non-infected animals and in skin test positive, bTB reactor animals with confirmed infections. The data, upon which APHA’s standard ELISA cut off of >0.1 for the DIVA test reagents has been applied are summarised below.

<table>
<thead>
<tr>
<th>Animal status</th>
<th>DIVA characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bTB reactors</strong></td>
<td></td>
</tr>
<tr>
<td>Number positive / total</td>
<td>107 / 118</td>
</tr>
<tr>
<td>Sensitivity (95% Confidence Level)</td>
<td>90.68%</td>
</tr>
<tr>
<td>Range</td>
<td>(83.93% to 95.25%)</td>
</tr>
</tbody>
</table>

| Controls |                        |
| Number positive / total | 9 / 691 |
| Sensitivity (95% Confidence Level) | 98.70% |
| Range | (97.55% to 99.40%) |
A total of 118 unvaccinated bTB naturally-infected animals were tested. For these animals, 107 of the 118 tested positive to either ESAT-6/CFP-10 or Rv3615c reagents giving an estimated sensitivity of 90.68% (83.93% – 95.25%). Based on these results, APHA conclude that there is a need for more data (samples) from unvaccinated/naturally infected animals to reach the target of less than 5% error in the estimate of the sensitivity at the 95% confidence level.

A total of 691 samples have been collected from unvaccinated control animals (bTB negative). Out of the 691 samples, nine tested positive to either ESAT-6/CFP-10 or Rv3615c reagents giving an estimated specificity of 98.70% (97.55% – 99.40%), which meets the OIE requirement of less than 5% error in estimate of the specificity at the 95% confidence level.

It is unclear whether it is a requirement to include unvaccinated reference samples (not the intended target population). It is suggested that further clarification on this point is sought from OIE.

1.2 Vaccinated

1.2.1 Serial testing (to confirm or negate the results of an intra-dermal skin test)

APHA define the intended purpose of the blood DIVA test as being to detect the IFN-γ response against bTB, (M. bovis) specific antigens, with at least equal certainty, in individual BCG-vaccinated cattle, which have tested positive to the current tuberculin skin test (specificity approximately 99.9% and sensitivity range of 65-80%, nb test characteristic depends on source reference); such a test will allow differentiation of infected and vaccinated animals for the purpose of resolving positive tuberculin skin test results in vaccinated animals.

APHA assessed the diagnostic sensitivity and specificity of the DIVA peptide cocktails using negative reference samples obtained from BCG-vaccinated, non-infected animals and positive reference samples obtained from BCG-vaccinated animals subsequently experimentally infected with M. bovis. The results of the blood IFN-γ DIVA are given below.

<table>
<thead>
<tr>
<th>Animal status</th>
<th>DIVA characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG vaccinated/experimental infected</td>
<td></td>
</tr>
<tr>
<td>Number positive / total</td>
<td>72 / 75</td>
</tr>
<tr>
<td>Sensitivity (95% Confidence Level)</td>
<td>96%</td>
</tr>
<tr>
<td>Range</td>
<td>(89% to 99%)</td>
</tr>
<tr>
<td>BCG vaccinated non-infected</td>
<td></td>
</tr>
<tr>
<td>Number positive / total</td>
<td>11 / 214</td>
</tr>
<tr>
<td>Sensitivity (95% Confidence Level)</td>
<td>95%</td>
</tr>
<tr>
<td>Range</td>
<td>(91% to 97%)</td>
</tr>
</tbody>
</table>

The performance of the DIVA test was evaluated in 75 BCG-vaccinated, M. bovis-infected animals and 214 BCG-vaccinated, non-infected animals.

It was noted that three of the BCG-vaccinated animals that were experimentally infected with M. bovis escaped detection with the DIVA test. By reducing the cut-off value used to classify a positive response to ESAT-6/CEP-10 peptide (from 0.1 to 0.0484) the test sensitivity for the DIVA test was increased to 99% (93% - 100%). However, this decreased the test specificity to 86% (81% - 90%).
1.2.2 Stand-alone testing (DIVA test used to screen BCG-vaccinated cattle for M. bovis infection as an alternative to the skin test)

A disadvantage of using the blood DIVA test as a serial test post skin testing is that, unless the skin test is 100% sensitive, it is possible that truly infected animals may be missed on the initial skin test and not be subjected to the DIVA test. Thus, APHA evaluated the DIVA test as a stand-alone test. More specifically, APHA compared the performance of the DIVA test with the SICCT and SIT skin tests, tested on the same animals at the same time. In total, 73 animals were studied and the results are shown below:

<table>
<thead>
<tr>
<th>Animal status</th>
<th>Test characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DIVA</td>
</tr>
<tr>
<td>BCG vaccinated/experimental infected</td>
<td></td>
</tr>
<tr>
<td>Number positive / total number</td>
<td>58 / 73</td>
</tr>
<tr>
<td>Sensitivity (95% Confidence Level)</td>
<td>79%</td>
</tr>
<tr>
<td>Range</td>
<td>(68% to 88%)</td>
</tr>
<tr>
<td></td>
<td>SICCT</td>
</tr>
<tr>
<td>Number positive / total number</td>
<td>65 / 73</td>
</tr>
<tr>
<td>Sensitivity (95% Confidence Level)</td>
<td>89%</td>
</tr>
<tr>
<td>Range</td>
<td>(80% to 95%)</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
</tr>
<tr>
<td>Number positive / total number</td>
<td>69 / 73</td>
</tr>
<tr>
<td>Sensitivity (95% Confidence Level)</td>
<td>95%</td>
</tr>
<tr>
<td>Range</td>
<td>(87% to 98%)</td>
</tr>
</tbody>
</table>

The results demonstrate lower test sensitivity for the DIVA reagent when compared to the skin tests.

2 CONCLUSIONS FROM WPP1

It has been concluded based on the above data that 1000 samples from vaccinated animals sourced from bTB free herds (negative reference samples) will be required to confirm the validity of the blood IFN-γ DIVA test. The test does not have 100% specificity and therefore some animals may test positive even though truly they are negative. Likewise 300 samples from vaccinated animals with confirmed bTB (positive reference samples) are estimated to be required.

2.1 Identified objectives for WPP2

2.1.1 Vaccinated – no challenge with M. bovis

One thousand (1000) samples are required from vaccinated animals (no challenge with M. bovis).

This population of samples will inform on how many of the 1000 vaccinated animals test negative or positive in the blood IFN-γ DIVA test thus giving an estimate of specificity. This number (as confirmed by statistical prediction) is thought sufficient to satisfy the OIE requirements.

For these samples APHA and Defra consider that history of the animals, herd and farm is sufficient provenance of bTB negative status i.e. animals sourced from herds located in low risk regions of England/Scotland with no history of bTB is sufficient and therefore it would not be necessary to PM these animals to confirm absence of bTB.

Currently there are a total of 214 samples generated from the clinical studies performed at APHA which may contribute towards the blood IFN-γ DIVA test validation data set. In order to determine the suitability of these samples, the data set will need to be fully reviewed by the contractor responsible for performing WPP2.

2.1.2 Vaccinated – M. bovis challenge

Three hundred (300) samples are required from vaccinated animals/M. bovis exposed. This population of samples will inform on how sensitive the blood IFN-γ DIVA test is. Again, this number (as supported by statistics) is thought sufficient to satisfy the OIE requirements.

For these samples there is a need to confirm if the animals had bTB at PM, so that APHA can make estimates of the sensitivity of the blood IFN-γ DIVA test in identifying these animals.
Currently there are a total of 75 samples generated from the animal studies performed at APHA which may contribute towards the blood IFN-γ DIVA test validation data set. In order to determine the suitability of the data from these there should be a review by the contractor responsible for performing WPP2. In addition to this, scientific advice should be sought from OIE to determine whether these samples will be acceptable as the samples were generated as part of challenge studies (i.e. not natural challenge).

3 REFERENCES


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Gareth Jones (APHA). Document (working): Application Form for the Certification of Diagnostic Tests as validated fit for specific purposes. Supplied in communication e-mail dated 04APR14, 09:30.

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Gareth Jones (APHA). E-mail title: PN1567 DIVA in unvaccinated animals. Supplied in communication e-mail dated 15MAY14, 10:04.
APPENDIX 2  REPORT ON THE SAMPLE SIZE OF A STUDY EVALUATING THE DIVA TEST FOR BOVINE TB IN GREAT BRITAIN.

Defra SE3287

Biomathematics & Statistics, Scotland

G.T. Innocent, I.J. McKendrick, G. Rydevik

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1  METHODS

1.1  Determining the test sensitivity and specificity using a "gold standard" test

If there exists a test that has a one-to-one relationship with reality, i.e., if the test results indicate that an individual is positive, then that animal is truly positive, and conversely if the results indicate that the individual is negative then it truly is negative, then that test is referred to as a "gold standard" test.

Determining the test sensitivity and specificity in the presence of a "gold standard" test is relatively simple. Using the "gold standard" test identify a group of positive animals, and a group of negative animals. The proportion of the positive animals that test positive is then our estimate of the sensitivity, the proportion of negative animals that test negative is the estimate of specificity. If we can assume that the event of an animal being tested is a random sample from the population of all possible animals being tested (either negative or positive), and across each population all animals have a common probability of being tested as positive, then the number of test positives from a sample of animals come from a binomial distribution. Provided our sample size is sufficiently large, we can then use the normal approximation to the binomial to estimate the errors associated with these estimates. Alternative methods exist to estimate errors for smaller samples, but the assumption that there is a "gold standard" is the least defendable element in this set of assumptions.

For bovine TB (bTB) there is no "gold standard" test. It is generally accepted that post-mortem identification of lesions typical of infection, and subsequent identification of the organism, is proof that an animal is infected. However, the converse cannot be said to be true: early in infection lesions may be small or non-existent, and even late in infection lesions may be small and few in number in some individuals. Hence, genuinely infected animals may be misclassified.
In the absence of a “gold standard”, we propose to use two groups of animals, one group being composed of animals selected as being very likely to be uninfected with bTB, the other group being composed of animals that are very likely to have bTB. The selection process for these animals can be thought of as implicitly defining a specificity and sensitivity:

1. A group of putative negative animals. These will be sampled from an area recognised as bTB free. They will have been declared single intradermal comparative tuberculin test (SICCT) negative within the previous 12 months, and they will come from a farm with no animals bought in since that previous test. This selection process for negative animals itself has a specificity of well over 99%. For example, if we consider Scotland, between 2008 and 2013, the percentage of tested farms that have had movement restrictions put in place is consistently at or below 2% [1,2]. We can use this as an estimate of the probability that we might inadvertently select animals from a farm undergoing a breakdown. Note that this probability is likely to be an overestimate, since the 2% relates to herds that have not undergone testing during the previous 4 years, whereas we would select animals from farms tested in the previous 12 months. Pooling over farms that experienced a breakdown in bTB status between 2002 and 2008, 1.2% of animals were infected with bTB (59 out of 4958 animals) [2]; it follows that buying animals randomly from a number of previously-bTB-free herds might be expected to result approximately 0.02% of animals being bTB-positive i.e. classing them as negative on the basis of the herd history has an implicit specificity of 99.98%. This is based on sampling a large number of farms, each contributing a small number of animals, i.e. we do not need to account for the overdispersion expected, and the expected association of disease status with herd size, and management. Further post mortem examination will be used to demonstrate a lack of lesions in these animals, increasing this notional specificity further. Further constraints could be implemented to increase the notional specificity if it was thought appropriate (e.g. use of closed herds).

2. A group of definitively positive animals. These will be SICCT positive, and subsequent to testing with the DIVA test will be subjected to post mortem examination. Only the results for those animals with typical lesions identified on post mortem and subsequent identification of the causal organism will be retained for calculating the sensitivity. This will ensure that the sensitivity is only calculated using animals that are very likely to have had bTB, and the implicit “gold standard” is assumed therefore to be close to 100%.

Unfortunately, using post mortem and organism isolation as a “gold standard” for the identification of bTB cases and non-cases is fallacious. Although positive animals are truly positive, they do not represent the whole population of positive animals; there will be a sub-population of positive animals with either lesions too small to find, or no lesions at all. Furthermore, as already discussed, negative animals may not be truly negative. Where no “gold standard” tests exist we can employ a latent class analysis, described in the next section.

1.2 Determining the test sensitivity and specificity in the absence of a “gold standard” test

If we have two diagnostic tests where the probability of a positive result for one test in a positive animal is independent of the probability of being positive on the second test, and both probabilities only depend on the sensitivity of the relevant tests, then by using the laws of independent probabilities the probability that a positive animal is positive to both, test 1 only, test 2 only and neither test is:-

\[ Se_1 \times Se_2 \], \( Se_1 \times (1 - Se_2) \), \( (1 - Se_1) \times Se_2 \) and \( (1 - Se_1) \times (1 - Se_2) \) respectively.
If we have two independent populations, with different prevalences and two conditionally independent tests we can produce results for the number in each population that are positive to neither test, to the first only, to the second only and to both. Using these results we can apply the following to determine an estimate of the posterior distribution of sensitivities, specificities and prevalences:

\[
\begin{align*}
Pr(++) & = Se_1 \times Se_2 \times p_1 + (1 - Sp_1) \times (1 - Sp_2) \times (1 - p_1) \\
Pr(+-) & = Se_1 \times (1 - Se_2) \times p_1 + (1 - Sp_1) \times Sp_2 \times (1 - p_1) \\
Pr(-+) & = Se_1 \times Se_2 \times p_1 + Sp_1 \times (1 - Sp_2) \times (1 - p_1) \\
Pr(--) & = Se_1 \times Se_2 \times p_1 + Sp_1 \times Sp_2 \times (1 - p_1).
\end{align*}
\]

Where \( Pr(++) \) is the probability that both tests are positive for an animal in population 1, \( Se_1 \) is the sensitivity of test 1, \( Sp_1 \) is the specificity of test 1, \( p_1 \) is the proportion of animals in population 1 that are truly positive, and other parameters are defined by analogy.

Hence, in population 1 the numbers positive to both tests, positive to the first only, to the second test only and to neither test will follow a multinomial distribution with the above probabilities. We can construct similar probabilities for population 2. With these values we can make estimates for sensitivities, specificities and prevalences.

This is the approach proposed by Hui and Walter [3]. We have extended this approach to account for vaccination. Each test now has two sensitivities and two specificities: those for vaccinated animals and those for unvaccinated. In order to solve the new equations, and be able to make estimates for the entire set of four sensitivities and four specificities we require four populations. This is simplified since two of the populations are now vaccinated, and two unvaccinated, and this status is known. If the prevalences in the different populations are sufficiently different, then we can calculate estimates of all four sensitivities, four specificities, and four prevalences. By a slight re-parameterisation and making an additional assumption of equal vaccine efficacy, we can replace the need for four prevalence estimates with two prevalence estimates and a single value for the vaccine efficacy.

If two populations with different prevalences are not available, then it is possible to use the standard Hui Walter model, and consider vaccinated animals as a separate population to unvaccinated animals, with a lower prevalence. However in such an analysis it is necessary to assume that the sensitivity and specificity are the same for vaccinated and unvaccinated animals.

In the following sections we provide the results from using these statistical estimation methods to estimate sensitivities and specificities and the associated 95% confidence intervals under different scenarios. We will vary the assumed ‘true’ value of the sensitivity and specificity, using values relevant to the decisions being made in the design of the DIVA trial, and we will vary the number of animal tests being analysed, using values consistent with the resources available to the proposed study and the likely properties of the bovine TB infected animal population, as indicated by the work of Cambridge University.

“Gold standard” assessments are calculated analytically and by numerical solution of equations. A precision associated with 95% confidence is an estimate of how wide the 95% confidence interval around the point estimate will be. We report two sets of figures: those arising from the standard method of equation inversion as described in Thrusfield [4] (the ‘Approximate’ method), and an alternative method based on exact confidence intervals and binomial distribution probabilities (the ‘Exact’ method). The latter estimates are larger than the former because the former inappropriately assumes a normal approximation to the confidence intervals and because the latter also allows for sampling variability. In both approaches we neglect the fact that where specificities are close to 100%, the confidence interval will be non-symmetric, and make calculations relative to an average precision over the upper and lower elements of the confidence interval. The numbers reported in the Exact method section of the
“gold standard” tables will be the minimum sample sizes associated with at least an 80% chance of achieving the stated level of precision. The approximate method has been provided since, although we would not encourage its use in this context, it is the method most widely used in practice, and we believe that it is likely to have been the method used to provide the EFSA advice.

Latent model assessments are calculated via simulation: pseudo datasets with the predicted characteristics are simulated and then analysed using latent class methods, and the results across multiple runs are summarized.

2 RESULTS

2.1 Determining the test sensitivity and specificity using a “gold standard” test

If we assume that we have a “gold standard” test then Tables 1 and 2 give the numbers of samples needed to determine the sensitivity or specificity of a test to a given precision at 80% power.

<table>
<thead>
<tr>
<th>Assumed Sensitivity</th>
<th>Precision</th>
<th>Number Required (Approx. Method)</th>
<th>Number Required (Exact Method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>±5%</td>
<td>323</td>
<td>353</td>
</tr>
<tr>
<td>70%</td>
<td>±1%</td>
<td>8003</td>
<td>8230</td>
</tr>
<tr>
<td>75%</td>
<td>±5%</td>
<td>289</td>
<td>320</td>
</tr>
<tr>
<td>75%</td>
<td>±1%</td>
<td>7152</td>
<td>7382</td>
</tr>
</tbody>
</table>

**Table 1**: Table of the numbers of samples needed to determine sensitivity to certain precisions with 95% confidence and 80% power in the presence of a “gold standard” test.

<table>
<thead>
<tr>
<th>Assumed Specificity</th>
<th>Precision</th>
<th>Number Required (Approx. Method)</th>
<th>Number Required (Exact Method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.5%</td>
<td>±0.5%</td>
<td>764</td>
<td>1226</td>
</tr>
<tr>
<td>99.5%</td>
<td>±0.2%</td>
<td>4778</td>
<td>5974</td>
</tr>
<tr>
<td>99.85%</td>
<td>±0.5%</td>
<td>231</td>
<td>696</td>
</tr>
<tr>
<td>99.85%</td>
<td>±0.2%</td>
<td>1439</td>
<td>2508</td>
</tr>
</tbody>
</table>

**Table 2**: Table of the numbers of samples needed to determine specificity to certain precisions with 95% confidence and 80% power in the presence of a “gold standard” test.

Given our uncertainty about the true sensitivity of the test, there is actually little or no scientifically or logistically relevant difference in the two sets of figures for estimating sensitivity. However, because the assumed values of the specificity are very close to 100%, there are appreciable differences in the estimates arising from the two methods. In this situation, we would recommend use of the exact method.

Evidence from AHVLA (now APHA) suggest that the DIVA test can have a cutoff set that results in a specificity of 0.999 and a sensitivity of 0.733. From Table 1 we can see that if the sensitivity of the proposed DIVA test is about 75% then 300 positive animals will probably allow us to say that the true sensitivity lies between 70% and 80%. From Table 2, if the true specificity is 99.9% then we would require about 1,000 samples to determine that the specificity lies between 99.4% and 100% with a 95% confidence. If we are required to demonstrate cost effectiveness then the work of our Cambridge collaborators elsewhere in this project suggests that the specificity needs...
To be as high as 99.85%. To demonstrate this we would need around over 20,000 samples. Note that these samples must be independent, i.e. it is not appropriate to test multiple samples from a single animal, as these are possibly highly correlated, unless separated widely in time. Here we assume false positives are produced by concurrent, short-term infections with cross-reacting organisms and false negatives by non-specific effects such as immuno-suppression following metabolic disease.

2.2 Determining the test sensitivity and specificity in the absence of a “gold standard” test

In the absence of a “gold standard” test then the latent class analysis is essential. If we have two populations, of equal size, but dissimilar prevalences, then the precision to which we can determine the test sensitivity for varying total population size is detailed in Figure 1 and for specificity in Figure 2. As can be seen, with 50,000 samples we expect to be able to determine a test with sensitivity of 70% to a precision of about ± 5% and a test with specificity of 99.9% with precision of about ±0.4% (Figures 1 and 2).

![Figure 1: The effect of total sample size on the estimates of the upper and lower 95% confidence limit for a latent class analysis of sensitivity, true value 0.70. The box and whisker plots denote the smallest, first quartile, median, third quartile, and maximum values obtained by analysing 1000 simulated data sets. Clear box and whisker plots represent the upper confidence limit, blue the lower.](image-url)
Figure 2: The effect of total sample size on the estimates of the upper and lower 95% confidence limit for a latent class analysis of specificity, true value 0.999. The box and whisker plots denote the smallest, first quartile, median, third quartile, and maximum values obtained by analysing 1000 simulated data sets. Clear box and whisker plots represent the upper confidence limit, blue the lower. The red line represents a specificity of 0.9985: the limit for economic equivalence to the present situation, according to the modelling from the Cambridge University group.

2.3 Combined approach

Although not a true “gold standard”, post mortem identification of lesions with subsequent identification of the causal agent has 100% specificity. We can use the results from the post mortem examination to identify assuredly positive animals. Specifically we can use the results to inform our priors for the latent class analysis. These results, assuming a test with sensitivity of 70% and specificity of 99.9% are detailed in Figure 3 for the sensitivity and Figure 4 for the specificity.

Figure 3: The effect of total sample size on the estimates of the upper and lower 95% confidence limit for a combined analysis of sensitivity, true value 0.70. The box and whisker plots denote the smallest, first quartile, median, third quartile, and maximum values obtained by analysing 1000 simulated data sets. Clear box and whisker plots represent the upper confidence limit, blue the lower.
Figure 4: The effect of total sample size on the estimates of the upper and lower 95% confidence limit for a combined analysis of specificity, true value 0.999. The box and whisker plots denote the smallest, first quartile, median, third quartile, and maximum values obtained by analysing 1000 simulated data sets. Clear box and whisker plots represent the upper confidence limit, blue the lower. The red line represents a specificity of 0.9985: the limit for economic equivalence to the present situation, according to the modelling from the Cambridge University group.

Briefly, the method involves simulating 1000 data sets for each sample size with known prevalence, vaccine efficacy, test sensitivity and specificity. For this analysis vaccine efficacy is defined as the relative decrease in prevalence in the vaccinated group compared to the unvaccinated group. Each data set is then analysed using a Bayesian MCMC approach with prior distributions defined using simulated data representing the results of the "gold standard" approach. We then record the median and 95% credible interval for the posterior distribution, and present the distribution of the median or upper and lower limits of the 95% CI across the 1000 data sample simulations/analyses.

The plots produced from these simulated analyses summarise two important aspects of the effect of increasing sample size. Looking at the distance between appropriate measures of the upper and lower limits of the confidence interval (e.g. median to median, first quartile to third quartile), these decrease with increased sample size. This result demonstrates that the larger sample size does tend to reduce the size of the resulting confidence interval for the sensitivity. Also, looking at the width of the box plots for all the upper limits and all the lower limits for different sample sizes, it is notable that the box plot width tends to shrink with larger samples. This result demonstrates that the uncertainty in the values taken by the confidence intervals will tend to reduce also (i.e., results are likely to be more consistent). Hence, the extra information in the combined analysis both decreases the range of the confidence interval somewhat, and decreases the uncertainty in the value of the confidence limits. However for 300 positive animals and 1000 negative animals the benefit of a combined analysis would be small. It is likely that more information will be available. There are likely to be a number of animals that are challenged as part of the protocol to produce the known positive animals for the "gold standard" approach, but remain negative at post mortem inspection. These can be included in the ultimate analysis. However, since the exact number is unknown this has not been included in the present hypothetical analyses.

From Figures 2 and 4 it is clear that even with 50,000 samples we would not be able to say with 95% certainty that the specificity is above the 0.9985 limit set by the work of Cambridge University. This is while assuming a true specificity of 0.999 - the figure
suggested as the true value by APHA. If we assume the true value to be 0.9995 then the results are as presented in Figure 5. Even in this situation we have only around a small chance of saying with 95% certainty that the real value is indeed above 0.9985.

![Figure 5: The effect of total sample size on the estimates of the upper and lower 95% confidence limit for a combined analysis of specificity, true value 0.9995. The box and whisker plots denote the smallest, first quartile, median, third quartile, and maximum values obtained by analysing 1000 simulated data sets. Clear box and whisker plots represent the upper confidence limit, blue the lower. The red line represents a specificity of 0.9985: the limit for economic equivalence to the present situation, according to the modelling from the Cambridge University group.

However, is this a reasonable approach? The original simulation model of bTB in the UK states that the true value for the DIVA specificity has to be at or above 0.9985 for there to be no associated increase in the number of culled animals. If the true value were in fact 0.9985 then, in practice, this test would be sufficient. However, statistical analysis of the data produced by such a test may not be able to support a claim: since the randomness in the results and the subsequent uncertainty in the estimates derived from statistical analysis of the results may result in a 95% CI that includes the target value. This results in us being unable to say with reasonable certainty that the true value is not less than 99.85%. An alternative approach is to demonstrate non-inferiority, information about which, in the current context, can be thought of as being summarized by the distribution of estimates of the lower credibility interval point. Hence, in Figure 6, we present the distribution of the median estimates for the specificity where the true value is 0.999. With 50,000 samples we can say that over 50% of the time we would expect a median in excess of 0.998, and from Figure 5 a lower 95% CI limit of over 0.9965.
Figure 6: The effect of total sample size on the estimates of the median for a combined analysis of sensitivity, true value 0.999. The box and whisker plots denote the smallest, first quartile, median, third quartile, and maximum values obtained by analysing 1000 simulated data sets.

3 DISCUSSION AND RECOMMENDATION

The sample size required to estimate sensitivity and specificity to a specific precision is smaller when a “gold standard” test is available than when a “gold standard” is unavailable. In the absence of a “gold standard”, the true status of an individual is unknown and so a latent class analysis is required to obtain estimates of sensitivity and specificity. A combined approach improves the precision of the latent class analysis by using information derived from the “gold standard” model, even if we do not consider the “gold standard” to be perfect.

We recommend making an initial assessment of sensitivity and specificity using 300 known positive animals and 1000 known negative animals, all vaccinated and all tested with the DIVA test prior to post mortem inspection and causal agent identification. This would enable us initially to define the true sensitivity and specificity with reasonable precision, as specified in section 2.1, confirming that the properties of the diagnostic test are such that it is not inappropriate for the vaccine trial to proceed. Thereafter we recommend employing vaccine trial animals to further increase precision. This will enable us to not only make more precise estimates of the sensitivity and specificity (reducing the uncertainty in trial results relating to cost effectiveness, as well as providing more precise estimates of the properties of the DIVA test) but will also allow us to make an estimate of the efficacy of the vaccine, as defined by the relative decrease in the prevalence of infection in vaccinated animals as opposed to unvaccinated. Since Bayesian analysis does not require awaiting all the results being obtained prior to performing the analysis, this approach could be used to update the estimates of DIVA test sensitivity and specificity at regular intervals, perhaps annually.

If the vaccine trial population can be divided into two groups with appreciably different disease prevalences, then the extended Hui Walter method can be used, providing valuable information about the properties of the DIVA test in the vaccinated and unvaccinated populations. If this is not the case, we will be forced to assume that the sensitivity and specificity are the same for both vaccinated and non-vaccinated animals and the standard latent class analysis employed. If this is necessary, and the sensitivities and specificities of the test in the two populations are indeed similar, then with around 40,000 samples we would expect similar results to the values presented here for 80,000 samples, since there would be only half the number of parameters to estimate in the model. If the sensitivities and specificities are not similar, however, the
gain in power is irrelevant since the resulting single set of estimates will be biased. It is preferable to design the study on the assumption that we can and will estimate the sensitivities and specificities separately, unless we are sufficiently confident that they are identical across the vaccinated and unvaccinated populations that we do not wish to provide evidence to regulatory agencies about the properties of the test in the two populations.

4 REFERENCES


APPENDIX 3  MODELLING TO SUPPORT CATTLE BCG FIELD TRIALS

The Disease Dynamics Unit (DDU), Department of Veterinary Medicine, University of Cambridge

Dr Andrew Conlan, MPhys, PhD

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EXECUTIVE SUMMARY

General requirements for efficacy trials:

- Recommend validation of a DIVA specificity of at least 99.85% before the commencement of
  efficacy trials (Section 3.5)
- Visible lesions should be used as the end-point for estimating efficacy (Section 3.5)
- Trials should be powered to estimate Direct Efficacy (Section 2.3.3.3)
- The indirect protection of cattle vaccination predicted by our within-herd models is small due to
  the assumed constant environmental risk of infection (Section 3.3)
- However, trials should include a mixture of herds with 0%, 50%, 100% target coverage of
  vaccination to provide the potential to assess these predictions (Section 3.3)
- Power is defined as the probability of estimating a protective benefit of vaccination when it
  exists (net improvement relative to unvaccinated animals/herds)

Powering is dependent on targeting herds with a history of bTB such that at least 90% of recruited
unvaccinated herds and 80% of vaccinated herds disclose at least one positive DIVA test or confirmed
slaughterhouse submission over a three-year trial period (Tables A3.1, A3.2).
Power calculations are based on a DIVA sensitivity of at least 73.3% and specificity of 99.85% and should be recalculated if this is not achieved.

Number of herds required to recruit 300 lesioned vaccinates for DIVA validation (WPP2.1, WPP2.2, WPP2.3):

DDU within herd models estimate that 1,000 randomly selected AFUs would be required to recruit 300 lesioned vaccinates under the proposed designs with a full one year residence time.

To achieve the primary trial aim of demonstrating significant direct efficacy of BCG vaccination to achieve marketing authorization (WPP6.1):

Designs are powered to estimate Direct Efficacy (Section 3.3).

At least 100 herds with a recent past history of bTB must be recruited for a period of three years for designs with either within-herd or between-herd controls to achieve a power of > 90% (Section 3.1).

To demonstrate herd level benefit of vaccination (WPP6.3):

- For a reduction in susceptibility of ~ 60% models suggest that at least 150 herds over three years will be required to achieve an approximately 80% power and at least 300 herds to achieve 90% power of estimating a protective benefit of vaccination in terms of:
  - Herd level incidence
  - Duration of restrictions (probability of prolonged breakdown)
  - Probability of recurrence (within trial period)

Final Recommendations:

- Combined results of efficacy (WPP6.1) and effectiveness (WPP6.3) trials can be used to assess direct, total and indirect efficacy of vaccination and potentially inform impact on transmission (which no single design will do).

- Consideration should be given to running WPP6.1 and WPP6.3 in parallel to control for potential changes in extrinsic (environmental) risk of infection to trial herds. Alternatively, demonstration of significant protective efficacy of vaccination in WPP 6.1 should be used as a stop/go point for the commencement of effectiveness trials (WPP6.3)
INTRODUCTION

The EFSA Scientific Opinion on the design of field trials for cattle vaccination for bovine Tuberculosis (EFSA, 2013) emphasized the importance of using mathematical modelling to inform the design, scope and timescale of any proposed trials (see EFSA Conclusion C5 & Recommendation R1). We address these questions using within-herd transmission models developed by the Disease Dynamics Unit (DDU), University of Cambridge during Defra projects SE 3230 and SE 3127. In this report we outline the result of our simulation studies and cross-reference our conclusions and recommendations to both the EFSA scientific opinion and the proposed field trial designs.

Statistical inference of dynamic transmission models for bovine tuberculosis is a considerable technical challenge. There are fundamental knowledge gaps with respect to the relationship between diagnostic and epidemiological statuses and the timescales between infection and infectiousness. Furthermore the surveillance system is complex and dynamic. Scheduling and interpretation of tests is linked to the (apparent) burden of infection within herds. Exact likelihood based methods of inference, such as data-augmented MCMC (Jewell et al. 2009), have so far proven to be computationally intractable. Only four fully estimated models of bovine Tuberculosis transmission in the United Kingdom have been published to date, all of which depend on approximate methods (Conlan et al. 2012, O’Hare et al. 2014, Conlan et al. 2014, Brooks-Pollock et al. 2014). Parameter values for our within-herd models are estimated using Approximate Bayesian Computation (ABC). ABC is a systematic procedure to estimate distributions of model parameters that are consistent with metrics derived from the epidemiological data. We used this framework to provide the first published estimates of cattle-to-cattle transmission rates in Great Britain and the hidden burden of infection missed by testing (Conlan et al., 2012). These models implicitly include estimates of the background (extrinsic) force of infection acting on herds including from wildlife reservoirs (addressing EFSA conclusion C8). We have since extended our original models to incorporate more realistic herd demographics and applied these new models to inform strategies for the use of cattle vaccination within an ongoing test and slaughter regime (Conlan et al., 2014).

In this report we apply our within-herd transmission models to predict effect sizes for the efficacy of BCG vaccination in cattle and the corresponding statistical power of specific randomised control trial designs. We will explore and compare alternative measures of vaccine efficacy at the herd and individual level. In addition to considering the basic requirements of demonstrating a protective effect of BCG, we consider the potential for field trials to address all the wider requirements raised by EFSA with respect to the population level effectiveness of vaccination in combination with DIVA testing and the impact of cattle vaccination on transmission rates of bTB (EFSA C7 and C10).

Demonstrating an impact of vaccination on transmission rates is by far the most challenging objective of the proposed field trials. As previously discussed, published estimates of transmission rates for bovine tuberculosis in Great Britain have all used approximate methods of inference, dependent on aggregating data at the population level from large numbers of herds (Brooks-Pollock et al., 2014; Conlan et al., 2012; O’Hare et al., 2014). Given the current lack of a robust theoretical framework to directly estimate rates of transmission for bTB, we do not consider that transmission rates are an appropriate measure upon which to power a field trial (EFSA C7). Rather we propose that the impact of BCG on transmission should be estimated indirectly through measures of relative risk and we explore designs that provide the potential to do so (EFSA C10). Specifically, we explore the utility of classical measures of vaccine efficacy (Direct, Indirect and Total Efficacy) to capture systematic reductions in both the susceptibility and infectiousness of vaccinates.

Finally, we note that data on the prevalence of bTB within environmental reservoirs is not routinely collected, or intended to be collected as part of trials of cattle vaccination. As a consequence, trials can only be designed to estimate the impact of vaccination on rates of cattle-to-cattle transmission. Fundamental data gaps on the prevalence of bovine tuberculosis in environmental reservoirs preclude designing a trial to quantify the impact of cattle vaccination on the total burden of infection within the whole disease ecosystem (cattle, environment and wildlife).

In Section 2 we briefly describe the basic assumptions, simulation protocols and measures of vaccine efficacy and effectiveness used for this report.

In Section 3.1 we explore the recruitment rate of visibly lesioned animals within Approved Finishing Units as a basis to estimate the number of such herds required for DIVA validation studies (WPP2.1, WPP2.2, WPP2.3).
Section 3.2 explores the number of herds necessary to estimate the direct efficacy of vaccination using different levels of within-herd vaccination coverage using either herd level or individual level control. (WPP6.1, WPP6.2)

In Section 3.3 we consider designs with both within- and between-herd controls that allow for alternative measures of vaccine efficacy to be defined and estimated. (WPP6.1, WPP6.2, WPP6.3)

In Section 3.4 we consider herd level measures of vaccine effectiveness, predicting the impact of vaccination on the herd level incidence of breakdowns, duration of breakdowns and probability of recurrence. (WPP6.1, WPP6.2, WPP6.3)

Finally, in Section 3.5 we highlight the central importance of test specificity, rather than sensitivity, to the success of field trials to assess the efficacy (and effectiveness) of vaccination.

2 METHODS

2.1 Within-herd transmission models for bTB

Details of the DDU within-herd transmission models can be found in the published literature (Conlan et al., 2012, 2014) and project reports to Defra (SE 3230 and SE 3127). The specific models used in this report are described in full in the forthcoming paper in PLoS Computational Biology (Conlan et al. 2014) which is included with this report as Appendix A14. Briefly, these herd level models were designed to estimate the effectiveness of testing at clearing infection from herds set against the rates of within-herd transmission and extrinsic introduction of disease from cattle-movements and environmental reservoirs. Herds are treated as independent, coupled to an extrinsic constant reservoir of infection. This is an unrealistic, but pragmatic assumption, given the complete lack of information routinely collected on the burden of disease within the environmental and wildlife reservoirs. More complex dynamic models of the reservoir could be easily constructed, but with no prior information to inform the parameters describing transmission to and from cattle these parameters would be statistically unidentifiable. The appropriateness of this assumption will depend on the extent to which the rate of extrinsic infection into herds varies over the course of simulation. For this reason, our within-herd models are not appropriate for long-term predictions of the impact of control measures, rather we apply these herd level models to only consider the events before, during and immediately after a so-called bTB "breakdown". Triggered by the disclosure of at test positive animal through routine or slaughterhouse surveillance, breakdowns result in movement restrictions being applied to herds and a sequence of repeated short-interval (60 day) tuberculin testing of herds until clear.

The study population used to estimate these models includes a representative sample of herd sizes and management models of GB herds. The herd size for model simulations is fixed, with the rate of on and off movements of animals sampled from the cattle tracing system (CTS) to generate a realistic age-structure and distribution of residence times for individual animals as described in Conlan et al. 2014. Parameter distributions for the DDU within-herd models are estimated using Approximate Bayesian Computation (ABC) based on statistical measures of herd-level incidence and persistence of bTB. These approximate, posterior distributions capture both the uncertainty in estimates of model parameters and the sensitivity of model predictions to the value of these parameters.

2.2 Testing and vaccination schedules for efficacy trials

To accommodate the practical requirements of efficacy trials, herds are subjected to a simplified schedule of testing and surveillance based upon the current regulatory regime.

To allow trials to be blinded, tuberculin testing is replaced by DIVA testing for all vaccinated and unvaccinated animals on trial herds (EFSA R3). Herds are re-vaccinated on an annual schedule, with the entire herd vaccinated on day 0. Imports and births into the herd are batched and vaccinated at 180-day intervals and then re-vaccinated according to the herd schedule. Breakdowns are triggered on trial herds by failure of routinely scheduled 6 monthly tests or by detection of a lesioned animals at slaughter. Once infection is disclosed in a trial herd it is subject to 60-day short interval DIVA testing. After a single clear DIVA test short interval testing is suspended and follow-up 6 month and 12 month tests are scheduled. In contrast to the status-
DIVA specificity was the greatest. VA specificity of at least 99.85% is included as a requirement for the success and appropriate powering of field trials (Section 3.5). We therefore recommend that validation of a DIVA specificity of at least 99.85% is included as a stop/go point for efficacy trials.

The potential misclassification of infected individuals by the imperfect “gold standard” measurement of visible lesions complicates the translation of empirical estimates of test characteristics to model parameters. In the absence of a true gold-standard test, the relationship between absolute test characteristics and relative measures cannot be modelled a priori. Care must therefore be taken in comparing between the absolute test sensitivity and specificities used as model parameters and estimates relative to the presence of visible lesions (which can also be considered as an imperfect diagnostic test). The proposed latent class analysis will address this critically important question. However, in the absence of further information for the purposes of powering these trials we assume a (true) DIVA sensitivity of 73.3% and specificity of 99.85% for both vaccinates and unvaccinated controls. While a sensitivity analysis around these assumed figures would have been informative, the priority given to assessing sensitivity to the impact of vaccination on infectiousness meant this was not possible within the time-constraints of the project. Sensitivity analyses of the model from Conlan et al. 2014, and preliminary explorations suggest that the impact of test specificity is likely to be far more detrimental to the successful outcome of a trial than sensitivity. We explore this issue in more detail, highlighting the risks of proceeding to a field trial based on a DIVA test with poor specificity in section A3.5.

2.3 Simulation protocols

For each scenario we simulate 5,000 herds from a sample of herds representative of the range of herd sizes and demography seen in Great Britain. We only include herds from high incidence (historic annual testing) areas. Simulations are initiated with no infection within herds. However, as the model is estimated from breakdown herds only, the background rate of infection within the model should be considered as representative of herds with a past history of bTB. Herds with no previous history of bTB may be expected to experience a lower rate of challenge from the outside of the herd. The total number of herds that must be recruited to achieve a given power will also depend on the proportion of study herds that experience a breakdown. The predicted herd level incidence rates under the trial testing schedule are tabulated in Tables A3.1 and A3.2.

Animals are assigned as vaccinates or controls on entry to a herd. Therefore, even for a 100% target vaccination coverage the instantaneous level of vaccination coverage will change...
dynamically over a simulation. The variability in coverage over time will depend on the demography of the herd and in particular the rate of demographic turnover (i.e. moves on/moves off). This turnover of animals will limit the effectiveness of vaccination at the herd level compared to that which could be achieved under more controlled trial conditions. However as the EFSA opinion requires that trials are carried out under field conditions, these demographic effects must be accounted for.

Sensitivity to model parameters is implicit in our analysis as we sample the parameters for each simulation from the approximate posterior distributions of the relevant model. As described below (Section 2.2) we estimate the expected effect sizes and statistical power based on a range of specified “true” efficacies in terms of the assumed proportionate reduction in both susceptibility and infectiousness for vaccines.

We explore the sensitivity of our results to model structure by comparing two alternative within-herd transmission (Susceptible-Occult-Reactive-Infectious: SORI, Susceptible-Occult-Reactive: SOR) models that differ in terms of the assumed timing of the onset of infectiousness (Conlan et al., 2012, 2014). The SORI model is the more traditional view of bTB progression in cattle where susceptible animals (S) must progress through a series of latent classes where they are first undetectable (or occult O), detectable (or reactive R) before finally becoming infectious (I). The SOR model accounts for evidence for the potential ‘early’ transmission of bTB and assumes all infected animals are potentially infectious, but still differ in their detectability.

### 2.4 Individual measures of vaccine efficacy

Experimental evidence suggests that BCG vaccination in cattle can provide both direct (reduction in susceptibility to infection $\varepsilon_d$) and indirect protection (reduction in infectiousness of infected vaccinates $\varepsilon_i$). We explore a range of model scenarios to quantify the relationship between defined values of $\varepsilon_d$ and $\varepsilon_i$ and standard estimates of the Direct, Indirect and Total efficacy of vaccination as defined below (Halloran et al., 1999; Smith et al., 1984).

Previous trials of BCG in a variety of contexts have suggested a direct efficacy in cattle of $\varepsilon_d \sim 60\%$ (Ameni et al., 2010; Hope et al., 2005, 2011; Lopez-Valencia et al., 2010; Suazo et al., 2003; Whelan et al., 2011). Vaccinated animals have also been demonstrated to have less extensive lesions after challenge with *M. bovis*, but there are no quantitative estimates of $\varepsilon_i$ (Ameni et al., 2010).

For all scenarios we compare the effect size and power for a range of assumed impacts of vaccination on susceptibility ($\varepsilon_d = 30\%, 60\%, 90\%$) and infectiousness ($\varepsilon_i = 0, 30\%, 60\%, 90\%$). Data from animal challenge studies suggests that protection by BCG is of limited duration of approximately one year. We model this “leakiness” of protection by a constant rate of loss of immunity corresponding to an average duration of protection of 1 year. Re-vaccination is assumed to provide the same protection as initial vaccination.

Unless otherwise stated statistical power is defined as the probability of estimating a protective effect of vaccination (i.e. a vaccine efficacy or effectiveness that is greater than zero).

For the purposes of estimating efficacy we define our study population as the sub-population of animals removed from trial herds during the period of study. The final (DIVA) test status and lesion status of each individual animal leaving a model herd is recorded. As previously stated, the probability of an infected animal having evidence of visible lesions depends on an empirical age-curve but not on vaccination status.

Individual measures of vaccine efficacy depend on the choice of a clinical endpoint to define cases and controls within the at-risk population. Unless otherwise stated, we take visible lesions as the endpoint for assessing efficacy.

**Attack rate** is defined as the ratio of the number of cases divided by the total at risk population. For our purposes the at-risk population is defined as the total population of animals removed from trial herds and cases can either be test-positive or TB lesioned animals.

**Direct Efficacy** compares the attack rate in vaccinated animals (ARV) against unvaccinated control animals (ARU). Depending on the study design, unvaccinated controls may either be
provided by unvaccinated animals within the same herd or by comparison to unvaccinated herds which share the same extrinsic background force of infection but a potentially greater burden of within-herd infection. Designs that include a mixture of herds with different target coverage of vaccination, direct efficacy is to be measured in both ways, using both within- and between-herd controls (Section 3.2).

**Indirect Efficacy** can only be measured within designs with whole herd controls and vaccinated herds with target vaccination coverage of <100% (Section 3.3). Indirect efficacy compares the attack rate in unvaccinated animals \((\text{ARU}_v)\) within a vaccinated herd and that from unvaccinated control herds (ARU).

**Total Efficacy** can also only be measured within designs with whole herd controls and compares the attack rate in all animals on a partially vaccinated herd (AR) to that within unvaccinated control herds (ARU). The estimated value of the Total Efficacy will therefore depend on both the direct and indirect protection afforded by vaccination and scaled with the proportion of the herd vaccinated.

**Direct Efficacy**

\[
1 - \frac{\text{ARV}}{\text{ARU}}
\]

**Indirect Efficacy**

\[
1 - \frac{\text{ARU}_v}{\text{ARU}}
\]

**Total Efficacy**

\[
1 - \frac{\text{AR}}{\text{ARU}}
\]

Figure A0.0 Definitions of direct, indirect and total efficacy measures and a schematic representation of the two populations being compared using each measure. Direct efficacy can be estimated through comparison of the attack rate in vaccinated animals (ARV) and unvaccinated animals (ARU) that may be held in the same herd to control for the background infectious pressure or through comparison of fully vaccinated and unvaccinated herds. Indirect efficacy measures the protection afforded to unvaccinated animals in a partially vaccinated herd. Indirect efficacy compares the attack rate in unvaccinated animals \((\text{ARU}_v)\) in a partially vaccinated herd to that in unvaccinated control herds (ARU). Total efficacy compares the attack rate of all animals in a partially vaccinated herd (AR) and unvaccinated control herds (ARU).

### 2.5 Herd level measures of vaccine effectiveness

The effectiveness of vaccination at the herd level can be quantified in terms of the risk of breakdown (herd level incidence), duration of breakdowns and the probability of recurrence. Note that due to the differences in the scheduling of testing during the proposed trials these measures are not directly comparable to those previously used to quantify within-herd persistence under the
current statutory regime of testing. Quantifying these herd level measures requires a design with both vaccinated and unvaccinated herds subject to the same (DIVA) testing protocol.

For the purposes of this report we define:

**Breakdown**: A period of short interval testing (at 60 day intervals) initiated by the disclosure of test-positive animals or slaughterhouse cases.

**Herd level incidence**: The proportion of study herds that have a breakdown over the fixed time horizon of the simulation (3 years unless otherwise stated).

**Prolonged breakdowns**: The proportion of herds that require more than 1 (DIVA) test in addition to the disclosing test to clear restrictions.

**Recurrent breakdowns**: The proportion of breakdowns that recur within the fixed time horizon of the simulation (3 years unless otherwise stated).

### Table A3.1: Herd level incidence for SORI model (3 Year trial period)

<table>
<thead>
<tr>
<th>Vaccine Coverage</th>
<th>εS (%)</th>
<th>εI (%)</th>
<th>Incidence (Herds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>92%</td>
</tr>
<tr>
<td>50%</td>
<td>30%</td>
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</tr>
<tr>
<td>50%</td>
<td>90%</td>
<td>0%</td>
<td>88%</td>
</tr>
<tr>
<td>100%</td>
<td>30%</td>
<td>0%</td>
<td>90%</td>
</tr>
<tr>
<td>100%</td>
<td>60%</td>
<td>0%</td>
<td>86%</td>
</tr>
<tr>
<td>100%</td>
<td>90%</td>
<td>0%</td>
<td>82%</td>
</tr>
</tbody>
</table>

### Table A3.2: Herd level incidence for SOR model (3 Year trial period)

<table>
<thead>
<tr>
<th>Vaccine Coverage</th>
<th>εS (%)</th>
<th>εI (%)</th>
<th>Incidence (Herds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>92%</td>
</tr>
<tr>
<td>50%</td>
<td>30%</td>
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</tr>
<tr>
<td>100%</td>
<td>90%</td>
<td>0%</td>
<td>83%</td>
</tr>
</tbody>
</table>

3 SIMULATION STUDIES TO SUPPORT TRIAL DESIGN

3.1 Model predictions for recruitment rates of visibly lesioned (VL) animals from Approved Finishing Units (AFU)

Finishing units have been proposed as candidate herds for DIVA validation due to the higher risk of infection among their intake populations. However, animals typically only have short residence times on finishing units of approximately 6 months. In this section we use our within-herd models to estimate the likely rate of recruitment of visibly lesioned (VL) animals from herds with demographics that are typical of finishing units. Given that such premises are not routinely flagged in the standard cattle tracing and bTB epidemiological data sets we were limited to using a list of 161 AFU herds from 2013 provided by AHVLA, from which a subset of 17 herds that are part of the study population of herds incorporated within the DDU within-herd transmission models. This subset of herds has a mean herd size of 467 (median size 241). All of the varieties of finishing herd considered in the AHVLA report have almost indistinguishable herd demographics to this subset of herds. As such the simulations presented in the report, and the
For the purposes of this exploration, each finishing unit was treated as belonging to a high incidence area with background force of infection sampled from the approximate posterior distributions.

We used this sub-population of herds to explore the number of herds per year necessary to recruit the target level of visibly lesioned (VL) vaccinates (300). We addressed this question using two distinct scenarios:

1) Vaccination under regular intake and management of AFU over a 1 year period
2) All in/All out design with a fixed number of animals residing on the AFU for a full year

WPP2.1, WPP2.2 and WPP2.3 correspond to scenario 2) and maximise the residence time of animals on the AFU at the expense of interfering with the management of the AFUs. Under option 1 there will a greater throughput of animals through the units over the course of the study, however the residence time of individual animals will be shorter. We found that the incidence rate of VLs is primarily driven by the period of study and the rate of extrinsic challenge and thus there is very little practical difference between these two scenarios. We therefore only present the results for Scenario 2 in this report (Figures A3.1 and A3.2).

Model simulations predict a linear increase in the number of recruited animals with visible lesions with AFUs with an average of 0.43 VL animals recruited per AFU per year. For a 50% coverage of vaccination and 60% efficacy ~ 1,000 herds would be necessary to recruit the target number of lesioned vaccinates (300). Increasing the level of vaccine coverage (as proposed in WPP 2.2) does not significantly affect the basic rate of recruitment of TB lesioned vaccinates (right hand columns Figure A3.2). Given the short duration of time that animals reside on the units and limited number of infectious animals per herd, results are largely insensitive to the assumed reduction in infectiousness ($\varepsilon$).

As a comparison to benchmark these predictions we estimated the same rate of recruitment of AFUs from historical data using the list of 161 AFU herds provided by AHVLA. The number of lesioned animals recorded in the national bTB databases from the 161 AFUs varies considerable by year, with the greatest being 45 lesioned animals in 2012 submitted from 15 herds with a median of 2 lesioned animals per herd. This gives a rate of 0.28 lesioned animals per AFU, even lower than the model predictions above. Numbers are considerably lower still for earlier years, however this is difficult to interpret as the herds are only known to have been trading with AFU status in 2013. Linearly extrapolating this rate would suggest needing 358 AFUs for 100 positives, 1071 for 300 positives given 100% vaccination coverage and a 0% efficacious vaccine. For 50% coverage this would rise to 716 AFUs for 100 positives and 2143 AFUs for 300 positives.
Figure A3.1: Predicted number of VLs for given number of AFU herds (SORI model)
Predicted number of VL animals generated by recruiting a given number of AFU herds using an all-in, all-out design with animals residing on the AFU for 1 year. Left column presents simulated output from 50% vaccination coverage; right column 75% vaccination coverage. Sensitivity to the assumed level of vaccine efficacy is explored in terms of susceptibility ($A \varepsilon_S = 30\%$, $B \varepsilon_S = 60\%$, $C \varepsilon_S = 90\%$) and infectiousness ($\varepsilon_I = 0, 30, 60, 90\%$). Herd sizes are sampled from subset of AFU herds within study population of model with mean herd size of 467 animals (median herd size 241). Each herd is subjected to an extrinsic infectious pressure as estimated from high incidence (PTI 1) areas and is assumed to be clear of infection at the beginning of the trial.
**Figure A3.2: Predicted number of VLs for given number of AFU herds (SOR model)**

Predicted number of VL animals generated by recruiting a given number of AFU herds using an all-in, all-out design with animals residing on the AFU for 1 year. Left column presents simulated output from 50% vaccination coverage; right column 75% vaccination coverage. Sensitivity to the assumed level of vaccine efficacy is explored in terms of susceptibility ($A \epsilon_s = 30\%$, $B \epsilon_s = 60\%$, $C \epsilon_s = 90\%$) and infectiousness ($\epsilon_i = 0, 30, 60, 90\%$). Herd sizes are sampled from subset of AFU herds within study population of model with mean herd size of 467 animals (median herd size 241). Each herd is subjected to an extrinsic infectious pressure as estimated from high incidence (PTI 1) areas and is assumed to be clear of infection at the beginning of the trial.
3.2 Estimating Direct Vaccine Efficacy

In this section we compare the utility of designs based upon within-herd and between-herd control groups for measuring the Direct Efficacy of vaccination. We achieve this by calculating the statistical power, defined as the probability of seeing a positive vaccine efficacy, as a function of the number of herds, for the two most basic trial designs to estimate direct efficacy:

1) 50% target vaccination overage within herd (using within-herd control animals)
2) 100% target vaccination coverage and whole herd (unvaccinated) controls

Power was estimated through simulation. For each parameter combination 5,000 model simulations were run with parameters sampled from the approximate posterior distributions of the relevant model. Each simulation represents a three year time period where the herd is subject to the DIVA testing regime as described above. The number of animals leaving the herd was recorded along with their vaccination and lesion status. Vaccine efficacy was then estimated using a subsample of these simulated herds for a given number of herds. The distribution of efficacies estimated by this procedure was then used to calculate the statistical power as the probability of the estimated efficacy being greater than 0.

The power to estimate direct efficacy depends on our ability to estimate the attack rate of bTB within both the vaccinated and unvaccinated subpopulations. As a consequence, for scenario 1) the maximum power to estimate efficacy is achieved for a 50% target vaccination coverage where the at-risk populations for both groups are balanced. In Figures A3.3 and A3.4 we compare the estimated value of the direct efficacy to assumed reductions in susceptibility (panels A, B, C) and infectiousness (linetype) for both the SOR and SORI models.

The predicted effect sizes and power for direct efficacy are consistent between the two alternative models. Reductions in susceptibility of $\varepsilon_S = 30\%, 60\%, 90\%$ correspond to predicted estimated Direct Efficacies of $\sim 20\%, 40\%$ and $60\%$. The Direct Efficacy is relatively insensitive to the value of $\varepsilon_S$ with a scatter of $< 10\%$ in the predicted mean efficacy over the range of values considered. This is a consequence of the limited duration of immunity (average of one year) relative to the within-herd transmission rate and rate of (annual) re-vaccination. This limited duration of immunity, extrinsic infection pressure and rapid turnover of animals within herds is also responsible for the measured, effective, efficacy to be consistently less than that of the “true” value of $\varepsilon_S$. From both models, a study population of at least 100 herds will provide $\sim 80\%$ power of estimating a 30% reduction in susceptibility and over 95% power of estimating a protective effect for a 60% reduction in susceptibility or greater.
Figure A3.3: Power to estimate direct efficacy using within-herd and between herd controls (SORI model)

Predicted value of direct efficacy for a range of assumed reductions in susceptibility (A $\epsilon_S = 30\%$, B $\epsilon_S = 60\%$, C $\epsilon_S = 90\%$) and infectiousness ($\epsilon_I = 0, 30, 60, 90\%$). For 50% vaccination coverage (red) efficacy is calculated using within-herd controls. For 100% coverage (blue) efficacy is calculated relative to whole herd controls. In both cases power and efficacy are plotted in terms of the total number of vaccinated and control herds. The SORI model, with herd level incidence (defined as at least one test positive or lesioned animal over trial period) as tabulated in Table A3.3, suggests a study population of at least 100 herds will provide ~ 80% power of estimating a 30% reduction in susceptibility and over 95% power of estimating a protective effect for a 60% reduction in susceptibility or greater.
Figure A3.4: Power to estimate direct efficacy using within-herd and between herd controls (SOR model)

Predicted value of direct efficacy for a range of assumed reductions in susceptibility (A $\varepsilon_S = 30\%$, B $\varepsilon_S = 60\%$, C $\varepsilon_S = 90\%$) and infectiousness ($\varepsilon_I = 0, 30, 60, 90\%$). For 50% vaccination coverage (red) efficacy is calculated using within-herd controls. For 100% coverage (blue) efficacy is calculated relative to whole herd controls. In both cases power and efficacy are plotted in terms of the total number of vaccinated and control herds. The SOR model, with herd level incidence (defined as at least one test positive or lesioned animal over trial period) as tabulated in Table A3.1, suggests a study population of at least 100 herds will provide ~ 80% power of estimating a 30% reduction in susceptibility and over 95% power of estimating a protective effect for a 60% reduction in susceptibility or greater.

3.3 Direct, Indirect and Total Vaccine Efficacy

Estimating the indirect effects of vaccination requires designs to recruit herds with variable levels of vaccination coverage. We consider the simplest such 2-level design composed of partially...
vaccinated herds and whole herd unvaccinated controls subject to the same regime of DIVA testing. The proportion of vaccinated to unvaccinated control herds impacts somewhat upon the predicted power for each measure, however the qualitative comparison is consistent. Here we specifically consider a design where 75% of recruited herds are vaccinated at a target coverage of 50% with the remaining herds as unvaccinated controls. We use this design to compare model predictions for the relative magnitude of the Direct, Indirect and Total vaccine efficacy.

**Direct Efficacy**

Direct efficacy compares the risk of infection of vaccinated animals relative to unvaccinated animals either within the same herd or a control herd. Assigning 25% of herds as unvaccinated controls has a modest impact on the power to estimate a protective effect of vaccination in terms of the direct efficacy. Note that in this design Direct Efficacy can be estimated relative to either within-herd (WH) or between-herd (BH) control animals. Estimated effect sizes and their corresponding power are comparable and in line with the simpler design considered in Section 3.2 (Figures A3.5 and A3.6). 100 herds will still provide > 90% power to estimate a protective direct efficacy for a true efficacy of 60%, however > 150 herds are required to achieve an 80% power to estimate a true efficacy of 30%.

**Indirect Efficacy**

Indirect efficacy compares the risk of infection of unvaccinated animals within a partially vaccinated herd to the risk of unvaccinated animals in an unvaccinated control herd. In contrast to the Direct efficacy, estimates of the Indirect Efficacy are sensitive to the choice of model with an Indirect Efficacy of ~ 0 predicted by the SORI model (Figure A3.5) and a positive Indirect Efficacy of up to 10% from the SOR model (Figure A3.6). This is a consequence of the different assumptions concerning the time from infection to infectiousness implicit in these two alternative models and their estimation from the same data set. For the SORI model, estimates of transmission rates are higher than for the SOR model, however animals must pass through a long period of latency before becoming infectious. For the SOR model animals have lower estimated transmission rates, but are immediately infectious upon infection. As a consequence the SOR model is more sensitive to the impact of vaccination on infectiousness and predicts a greater indirect benefit of vaccination.

**Total Efficacy**

Total efficacy compares the risk of infection of all animals within a partially vaccinated herd to that in unvaccinated control herds. Although this also depends on the level of vaccination coverage within the herd, the magnitude of indirect protection for both models is constrained by the extrinsic rate of infection that captures the risk of both animal movements and the unobserved environmental reservoir. These models assume that vaccination has no impact on this reservoir of infection, hence the small magnitude of the predicted indirect benefits of vaccination. As a consequence of this, the Total Efficacy is estimated to be approximately half that of the direct efficacy and the number of herds required to power a trial based on the Total Efficacy are correspondingly larger. Both models suggest that a 90% power of estimating a positive Total Efficacy would require ~ 300 herds for a true direct efficacy of $\varepsilon_S = 60\%$.

Reduction of the burden of infection within vaccinated herds is likely to impact upon the extrinsic rate of challenge assumed to be constant with the models. However, there is no empirical basis to predict the size of such an effect or the timescale over which it may be realized. In some sense, our model simulations provide a worst-case scenario. Given the predicted effect size of indirect protection from both models we would be unlikely to estimate a positive Indirect Efficacy and risk estimating a negative Total Efficacy even when the vaccine has a significant protective effect at the individual level. It should be emphasised that we only consider scenarios where vaccination has a protective effect and thus the “true” efficacy of vaccination will always be greater than or equal to zero. However, when the true efficacy is close to zero and the number of recruited herds is small a negative efficacy can be estimated due to stochastic effects. Our definition of statistical power is chosen to provide guidance on the number of herds necessary to mitigate the possibility of this significant risk to the success of a field trial.
Estimation of the indirect and total vaccine efficacy would provide important validation, or counterfactual evidence, for the robustness of our assumptions about the transmission between the cattle and environmental reservoirs. However, based on these results we do not recommend powering field trials based on either the indirect, or total vaccine efficacy. We strongly recommend that designs are flexible enough to allow these quantities to be estimated to assess the indirect benefits of vaccination. In the current designs this can be achieved by combining the analysis of the proposed efficacy trials WPP6.1 and WPP6.3 which taken together include a population of at least 100 herds with 50% vaccination coverage, 50 unvaccinated controls and 50 herds with 100% vaccination coverage.
Figure A3.5: Power to estimate Direct, Indirect and Total efficacy from 2-level design (SORI model)

Expected values of the Direct, Indirect and Total vaccine efficacies measured from a 2-level design with 75% of herds vaccinated at 50% and 25% of herds as unvaccinated whole-herd controls. We explore a range of assumed vaccine-induced reductions in susceptibility ($A_{\varepsilon_S} = 30\%$, $B_{\varepsilon_S} = 60\%$, $C_{\varepsilon_S} = 90\%$) and infectiousness (Linetype, $\varepsilon_I = 0, 30, 60, 90\%$). Note that in this design Direct Efficacy can be estimated relative to either within-herd (WH) or between-herd (BH) control animals. 100 herds provides > 90% power to estimate a protective direct efficacy for a true efficacy of 60%, however > 150 herds are required to achieve an 80% power to estimate a true efficacy of 30%. The indirect efficacy is predicted to be close to zero as the extrinsic force of infection acting on herds overwhelms the indirect protection provided by immunity within the herd. There is a ~ 50% probability of estimating a negative indirect efficacy of vaccination across the number of herds explored. As a consequence of the minimal indirect protection offered by vaccination, the Total Efficacy of vaccination with herds with 50% coverage is approximately half that of the direct efficacy. The model predicts that a 90% power of estimating a positive Total Efficacy would require > 300 herds for a true direct efficacy of 60%.
Figure A3.6: Power to estimate Direct, Indirect and Total efficacy from 2-level design (SOR model)

Expected values of the Direct, Indirect and Total efficacies of vaccination using a 2-level design with 75% of herds vaccinated at 50% and 25% of herds as unvaccinated whole-herd controls. We explore a range of assumed vaccine-induced reductions in susceptibility ($A_{\varepsilon_S} = 30\%$, $B_{\varepsilon_S} = 60\%$, $C_{\varepsilon_S} = 90\%$) and infectiousness ($\varepsilon_I = 0, 30, 60, 90\%$). Note that in this design Direct Efficacy can be estimated relative to either within-herd (WH) or between-herd (BH) control animals. 100 herds provides > 90% power to estimate a protective direct efficacy for a true efficacy of 60%, however > 150 herds are required to achieve an 80% power to estimate a true efficacy of 30%. For the SOR model the indirect efficacy is predicted to be ~ 10% as a consequence of the greater instantaneous number of infectious animals within herds as compared to the SORI model. This predicted effect size is still small and as such there is still only a 70% power of estimating a positive indirect efficacy for a study size of 300 herds. Power to estimate the Total Efficacy is likewise increased with a > 90% chance of estimating a positive Total Efficacy from 300 herds.
3.4 Herd level measures of vaccination effectiveness

In this section we consider the simplest possible randomised control trial design where whole herd vaccination is compared to unvaccinated controls (WPP6.3). Once again, motivated by balancing the size of the at risk population for vaccinates and controls we consider a 50:50 vaccination/control herd ratio. Herds from both WPP6.1 and WPP6.3 can be used to assess how the impact of vaccination at the herd level scales with vaccination coverage. We therefore consider the expected effect sizes based on both 50% target coverage (WPP6.1) and 100% (WPP6.3). For each measure effect sizes are presented as the % difference between in each measure as measured from vaccinated and unvaccinated herds.

For all three measures the predicted effect of vaccination is modest and variable, but with a power of > 80% for a study population with > 150 breakdowns for an assumed reduction in susceptibility of $\varepsilon_S = 60\%$. It should be noted that the predicted proportion of prolonged and recurrent breakdowns is smaller in trial simulations compared to that of historical breakdowns due to the assumed frequency of testing and improvement in test sensitivity and specificity of the DIVA test relative to tuberculin testing. These target test characteristics for a prospective DIVA test are based on limited data available from challenge studies (Conlan et al. 2014). Failing to meet these target values during DIVA validation would reduce this benefit and reduce the statistical power to estimate a positive efficacy of vaccination.

The predicted impact of vaccination on herd level incidence differs between the SORI (Figure A3.7) and SOR (Figure A3.8) models. For the SORI model and herds from high incidence areas there is an estimated ~ 10% reduction in incidence for 100% coverage and ~ 5% for 50% coverage. For the SOR model estimates are smaller and more sensitive to the assumed reduction in infectiousness $\varepsilon_I$, with an estimated reduction of ~ 5% for 100% coverage and ~ 2.5% for 50% coverage.

The predicted impact of vaccination on the duration of breakdowns is more consistent with an estimated ~ 10% reduction in the proportion of prolonged breakdowns for 100% coverage falling to ~ 5% for 50% coverage for both models (Figures A3.9 and A3.10). Estimates for the SOR model are once again more sensitive to the assumed reduction in infectiousness $\varepsilon_I$ (Figure A3.10).

The probability of recurrence follows the same pattern as the herd level incidence and differs between the SORI (Figure A3.11) and SOR (Figure A3.12) models. For the SORI model there is an estimated ~ 10% reduction in incidence for 100% coverage and ~ 5% for 50% coverage. For the SOR model estimates are smaller and more sensitive to the assumed reduction in infectiousness $\varepsilon_I$, with an estimated reduction of ~ 5% for 50% coverage and ~ 2.5% for 100% coverage.
**Figure A3.7: Predicted effect of vaccination on herd level incidence (SORI model)**

Simplest design to measure vaccine effectiveness with 50:50 mix of control and vaccinated herds and a target coverage of either 50% or 100% for a three year trial period. We explore a range of assumed vaccine-induced reductions in susceptibility (A $\epsilon_S=30\%$, B $\epsilon_S=60\%$, C $\epsilon_S=90\%$) and infectiousness (VEI $\epsilon_I=0, 30, 60, 90\%$). For an assumed vaccine efficacy of 60% (reduction in susceptibility) the SORI model predicts a ~10% reduction in herd level incidence (defined as the proportion of herds with at least one DIVA test positive animal or slaughterhouse case) for 100% vaccination coverage and ~5% for 50%. For this effect size 100 herds would provide an 80% power of estimating a protective effect of vaccination and 200 herds would be required to achieve 90%.
A

Figure A3.8: Predicted effect of vaccination on herd level incidence (SOR model)

B

Figure A3.8 contd.: Predicted effect of vaccination on herd level incidence (SOR model)

Description below.

C

Figure A3.8 contd. Predicted effect of vaccination on herd level incidence (SOR model)

Simplest design to measure vaccine efficiency with 50:50 mix of control and vaccinated herds and a target coverage of either 50,100% for a three year trial period. We explore a range of assumed vaccine-induced reductions in susceptibility (\(A\epsilon_s = 30\%, B\epsilon_s = 60\%, C\epsilon_s = 90\%\)) and infectiousness (Linetype, \(\epsilon_I = 0, 30, 60, 90\%\)). For an assumed vaccine efficacy of 60% (reduction in susceptibility) the SOR model predicts a ~ 5% reduction in herd level incidence (defined as the proportion of herds with at least one DIVA test positive animal or slaughterhouse case) for 100% vaccination coverage and ~ 2.5% for 50%. For this effect size at least 150 herds would be required to provide an 80% power of estimating a protective effect of vaccination and at least 300 herds would be required to achieve 90%.
Figure A3.9: Predicted effect of vaccination on duration of restrictions (SORI model)
Simplest design to measure vaccine effectiveness with 50:50 mix of control and vaccinated herds and a target coverage of either 50% or 100% for a three year trial period. We explore a range of assumed vaccine-induced reductions in susceptibility ($\varepsilon_S = 30\%$, $\varepsilon_S = 60\%$, $\varepsilon_S = 90\%$) and infectiousness ($\varepsilon_I = 0$, $\varepsilon_I = 30$, $\varepsilon_I = 60$, $\varepsilon_I = 90\%$). For an assumed vaccine efficacy of 60% (reduction in susceptibility) the SORI model predicts a ~ 10% reduction in prolonged breakdowns (defined as the proportion of herds that take more than 1 short interval test to clear restrictions) for 100% vaccination coverage and ~ 5% for 50%. For this effect size 100 herds would provide an 80% power of estimating a protective effect of vaccination and 200 herds would be required to achieve 90%.
Figure A3.10: Predicted effect of vaccination on duration of restrictions (SOR model)

Simplest design to measure vaccine effectiveness with 50:50 mix of control and vaccinated herds and a target coverage of either 50% or 100% for a three year trial period. We explore a range of assumed vaccine-induced reductions in susceptibility ($A_\varepsilon = 30\%$, $B_\varepsilon = 60\%$, $C_\varepsilon = 90\%$) and infectiousness ($VEI_\varepsilon = 0, 30, 60, 90\%$). For an assumed vaccine efficacy of 60% (reduction in susceptibility) the SOR model predicts a ~ 10% reduction in prolonged breakdowns (defined as the proportion of herds that take more than 1 short interval test to clear restrictions) for 100% vaccination coverage and ~ 5% for 50%. For this effect size > 100 herds would provide an 80% power of estimating a protective effect of vaccination and > 200 herds would be required to ensure a 90% power.
**Figure A3.11**: Predicted effect of vaccination on probability of recurrence (SORI model)

Simplest design to measure vaccine effectiveness with 50:50 mix of control and vaccinated herds and a target coverage of either 50,100% for a three year trial period. We explore a range of assumed vaccine-induced reductions in susceptibility ($\alpha_S = 30\%$, $\alpha_S = 60\%$, $\alpha_S = 90\%$) and infectiousness ($\epsilon_I = 0, 30, 60, 90\%$). For an assumed vaccine efficacy of 60% (reduction in susceptibility) the SORI model predicts a ~ 10% reduction in prolonged breakdowns (defined as the proportion of herds that take more than 1 short interval test to clear restrictions) for 100% vaccination coverage and ~ 5% for 50%. For this effect size 100 herds would provide an 80% power of estimating a protective effect of vaccination and 200 herds would be required to achieve 90%.
Figure A3.12: Predicted effect of vaccination on probability of recurrence (SOR model)

Simplest design to measure vaccine effectiveness with 50:50 mix of control and vaccinated herds and a target coverage of either 50, 100% for a three year trial period. We explore a range of assumed vaccine-induced reductions in susceptibility ($\varepsilon_S = 30\%$, $\varepsilon_S = 60\%$, $\varepsilon_S = 90\%$) and infectiousness ($\varepsilon_I = 0$, $\varepsilon_I = 30$, $\varepsilon_I = 60$, $\varepsilon_I = 90\%$). For an assumed vaccine efficacy of 60% (reduction in susceptibility) the SOR model predicts a ~10% reduction in prolonged breakdowns (defined as the proportion of herds that take more than 1 short interval test to clear restrictions) for 100% vaccination coverage and ~5% for 50%. For this effect size 100 herds would provide an 80% power of estimating a protective effect of vaccination and 200 herds would be required to achieve 90%.
3.5 Importance of DIVA specificity

The simulation studies in this report assume that a DIVA sensitivity of 73.3% and specificity of 99.85% can be achieved for both vaccinates and unvaccinated controls. As with the tuberculin skin test, the diagnostic characteristics of the proposed DIVA test can be calibrated by setting the cut-off values that classify animals as test positive or negative. Earlier estimates of DIVA specificity provided by AHVLA were based on applying cut-off values that maximises the specificity of the DIVA antigens in unvaccinated animals. These cut-offs, applied to a data-set of vaccinated animals only, provides an estimated DIVA sensitivity of 64.4% (95%CI: 48.8, 78.1%) and 99.4% specificity (95%CI: 96.9-100% CI) (Conlan et al. 2014). For a limited range of scenarios we use these estimates to explore the extent to which a DIVA test that falls below our recommended baseline specificity of 99.85% would affect the potential for trials to estimate a protective benefit of vaccination.

For the alternative DIVA the rate of false positives is such that close to 100% of vaccinated and control herds are predicted to have a breakdown, be prolonged and recur within the trial period. A specificity of less than 98.85% carries the risk that any protective individual benefit of vaccination will be masked by an excessive removal of false positive animals and imposition of “breakdown” status on trial herds (See Conlan et al. 2014 included as Appendix 14).

When DIVA test status is used as an endpoint for assessing vaccine efficacy a DIVA test with relatively low specificity will lead to an estimated efficacy of close to zero, even when there is a genuine reduction in susceptibility (Figure A3.13A). This is a consequence of seeing the same number of false positive test results in both the control and vaccinated cattle due to the low prevalence of disease. As lesions are assumed to be 100% specific, the power to estimate a protective effect of vaccination is not affected if this is our endpoint for measuring efficacy (Figure A3.13B). Thus, the rate of false positives would have a negative impact on the costs of the trial by increasing the number of animals sent to slaughter, but would not necessarily impact on the power to demonstrate a significant effect of vaccination.

In conclusion, the use of visible lesions as the endpoint for estimating vaccine efficacy mitigates against the potential for low DIVA specificity to reduce the power to estimate any direct individual level protection of vaccination. However, herd level measures of incidence and persistence are likely to be more sensitive to the performance of the DIVA test than the likely efficacy of BCG vaccination. We therefore recommend the use of visible lesions as the end-point for assessing vaccine efficacy. Furthermore, we emphasize that estimation of a protective herd level benefit of vaccination will depend on the appropriate validation of DIVA specificity before efficacy trials are started.
Figure A3.13: Power to estimate Direct Efficacy with less specific DIVA test
Predicted value of direct efficacy for an assumed reductions in susceptibility of $\varepsilon_S = 60\%$ and range of reductions in infectiousness ($\varepsilon_I = \text{VEI} = 0, 30, 60, 90\%$) using trial designs from Section 2.2 that compare within and between herd controls. Here we assume a DIVA sensitivity of 64.4% and specificity of 96.4%. For this specificity 100% of recruited herds will experience a bTB breakdown during the trial period of 3 years. As a consequence of the high rate of false positive test results the direct efficacy measured relative to DIVA test status collapses to close to zero (A). However, provided that efficacy is measured relative to confirmation of visible lesions (B), which are assumed to be 100% specific, the estimated efficacy and power of estimating a protective effect of vaccination is unaffected by the poorer performance of the alternative DIVA test (compared with Figure A3.3B).
4 REFERENCES


5 CONCLUSIONS AND RECOMMENDATIONS FROM EFSA OPINION RELEVANT TO SIMULATION STUDIES (QUOTES TAKEN FROM EFSA, 2013)

Conclusions

C1: “The strategy of establishing and attempting to simultaneously evaluate DIVA and vaccine performance in the same field trial poses considerable risks, because only limited conclusions about vaccine performance can be made if test performance is later found to be poor.”

C2: “Evaluation of DIVA test performance (sensitivity and specificity in vaccinated animals) under field conditions should be based on a representative sample of the target population. Any proposed deviations from such a design should be justified and the bias that may subsequently be introduced should be accounted for.”

C5: “The design of the trials for evaluating DIVA test and vaccine performance, with respect to numbers, duration and the sampling scheme of the animals, should be informed by modelling before the onset of the trials.”

C7: “Estimates of the reproductive number should be sufficiently precise to enable conclusions to be drawn about the performance of bTB vaccination, in conjunction with test and cull using the DIVA tests, for bTB eradication.”

C8: “The risk posed by transmission of bTB infection from wildlife to cattle enrolled in the field trials should be considered and accounted for in any trial design.”

C10: “Data collected should enable estimation of vaccine-induced reduction of bTB transmission, and not just reduction of bTB incidence, in order to reach a conclusion regarding the contribution of vaccination to eradication.”

Recommendations

R1: “A simulation analysis of potential trial results should be performed prior to the start of the trials to ensure that sufficient data are collected during the trials, appropriate to the analytical methods to be used. There is also a need to ensure that the data required for test validation and vaccine performance can indeed be obtained, and that the DIVA test and BCG vaccine performs according to expectations based on the laboratory experiments, while taking account of the many factors that may generate additional variation in the field.”

R2: “DIVA test performance should be analysed as early as possible in the trials, while continuing to generate more data for analysis of vaccine performance in greater detail. Once the results from test validation are known, the analysis for the power of the field trials for vaccine performance can be repeated, using the updated results.”

R3: “The use of the tuberculin skin test should be avoided if possible as its use would limit the ability to double-blind during the field trials.”

R4: “Matched-pairing of herds should be considered if herd or region is included as the unit of interest, to deal with the confounding effect of wildlife exposure.”
APPENDIX 4 WPP2.1 – STUDY FOR BLOOD IFN-γ DIVA TEST VALIDATION WITH CONCURRENT SICCT TESTING: PROPOSED PROTOCOL

Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Pre-vaccination with BCG Vaccine then Exposed to *M. bovis* Challenge

WPP 2, Design 1
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1 TITLE
Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Pre-vaccination with BCG Vaccine then Exposed to M. bovis Challenge.

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
This study is intended to be run in conjunction with other studies in the WPP2 series which together will form a data package to determine and validate the fitness of the developed, optimised and standardised blood IFN-γ DIVA test for the intended purpose as a stand-alone test with concurrent SICCT testing appropriate for the diagnosis of bTB infection in cattle that have been vaccinated with the BCG vaccine.

The primary objective of this trial is to generate blood samples from animals which are bTB negative pre-vaccination, vaccinated and then exposed to natural challenge in AFUs become positive.

The secondary objectives for the study are:
- To generate data required to meet EU veterinary medicines legislation requirements on injection site observations and lung lesions.
- To generate supportive data (for the efficacy claim) as recommended by EFSA to provide power calculations with new additional data upon which to recalculate animal numbers for the pivotal field studies (WPP6).
- To generate reference samples for correlate markers if appropriate for APHA projects.
- To confirm if vaccination influences the SICCT test result approximately one year post-vaccination. This helps to inform on the scope for use of SICCT post-vaccination.

5 STUDY DESIGN

5.1 General
A negative controlled, multicentre study to be carried out in different geographical regions within England and Wales.

Animals will be recruited onto the study at a ratio of 1:1, IVP:CP respectively, based on the following requirements:

Group 1: ½ animals negative for bTB (from a low risk area), vaccinated
Group 2: ½ animals from a high risk area, unvaccinated

Assessments will be made at the individual animal level.

5.1.1 Study Blinding
This study will be at least partially blinded. At a minimum it is recommended that personnel performing the post mortems and any laboratory personnel are blinded to vaccine status. The study may also involve the use of a placebo (instead of a negative control group). The decision to use a placebo will be determined in the final study protocols. A placebo may be
used to mirror that which may be used during the field pivotal safety and efficacy studies. It may be considered useful to include a placebo in the DIVA validation studies as these could be both supportive to efficacy studies and an opportunity to develop an essential tool for maintaining blinding of study personnel in specific efficacy studies. Ideally the animal owners and all study personnel treating animals and collecting data will be blinded to the treatment received by individual animals. This will only be possible if the placebo induces a site reaction equivalent to that when the BCG vaccine is used, otherwise blinding will not be possible for the collection of samples whilst injection site reactions are still in evidence.

Technicians undertaking blood sample analysis will be blind to the treatment received by the animals as will technicians undertaking post-mortem examinations or any analyses on collected samples.

5.2 Farms/Sites suitable for inclusion

Approximately 50% of animals will be deemed bTB negative being recruited from a bTB free source farm. This will be defined as a farm which has operated a closed herd with no history of bTB in the preceding 5 years at least and if possible, one for which all adjoining farms are of a similar history status.

The balance of animals will be sourced from farm sites which are located in high risk areas and with a farm history of bTB in the past 12 months, i.e. "breakdown" farms.

The study site (destination) AFUs will be established in the business of accepting animals from high risk farms/areas and based on reported experience these are expected to generate a higher rate of bTB challenge. If possible AFUs will be selected in different geographical locations so that vaccine is exposed to different environmental Mycobacterium i.e. different strains and incidences of M. bovis and M. avium.

5.3 Animals suitable for inclusion

Cattle of any breed, weight or gender will be suitable for the study. Inclusion criteria include an age of greater than 6 months and being neither pregnant nor lactating. Animals must also have a negative SICCT result prior to vaccination.

5.4 Number of animals

The number of samples (300) required from animals which are vaccinated and then determined to become positive for bTB (by post mortem inspection) is advice adopted by APHA (based on OIE guidance) and as supported by BioSS statistical analysis (Appendix 2).

Factors which impact the number of animals required for this study include vaccine efficacy. The predicted vaccine efficacy is 60%. Therefore approximately 40% of the vaccinated population will be susceptible to challenge. It should be noted that if the efficacy of the vaccine is higher than anticipated then the number of animals required to generate the 300 samples will also increase.

Another factor that will influence the number of animals required is transmission rate. AFUs have been selected for inclusion as these farm sites have the highest incidence rate of bTB, in comparison to conventional farm sites. Based on available literature (Downs, et. al, 2013) it is suggested that AFUs can have a transmission rate of ca. 20% which is about 4 times higher than background rate.

In addition to this a further factor to consider is the sensitivity and specificity of the DIVA test. Mathematical modelling performed by Cambridge University predicts that 1000 AFUs will be required, with animal residency on the individual AFU of one year to achieve the 300 samples required. Based on the average capacity of an AFU of 300 animals, this would involve a total of 300,000 animals. High risk non-AFU farm sites have been considered, however, the difficulties associated with enrolling such a site, the ethical implications and associated costs make these a less favourable option in the opinion of the Consortium.

Based on modelling and information available at the time of writing this report, consideration should be given to the prudence of conducting this study for an initial period of two years with 100 AFUs recruited onto the study for one year each. This approach would allow an initial
assessments of the factors that influence the rate of bTB positive sample generation. It is recommended that Defra and WG should take these results and those of other studies performed to date and seeking scientific advice and further guidance from e.g. OIE, on sample numbers.

5.5 Study treatments and allocation
Animals recruited from bTB free farm sites will be vaccinated. Animals recruited from high risk areas will be unvaccinated. Animals from both sources will be transferred and mixed at the AFU at an approximate ratio of 1:1.

The bTB negative animals will be treated with IVP on the source farms and transferred to the study AFU in the high risk area after about 30 days. This period is suggested as a time frame in which maximum t-cell response will occur thus ensuring that the protective immune response will be induced and established prior to bTB challenge at the AFU. Note that a select number of animals at the bTB negative source farm may be treated with prospective placebo with a view to determining if attending animal care personnel can tell true vaccinated animals apart from placebo treated.

Animals on the bTB negative source farm will be vaccinated in batches as they become available. The animals will remain on their source farm for 30 days to allow immunity to be established and will then be transferred, in the same batches, to the AFU test site. It is anticipated that for a period of at least one year all of the animals arriving at a given AFU test site will be exclusively study animals.

5.6 Study schedule

<table>
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<th>Data Collection</th>
<th>Procedure</th>
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</tr>
<tr>
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<td>✓</td>
</tr>
</tbody>
</table>

a: Transfer to the AFU. b: pre-vaccination for confirmation of -ve status. PM = Post Mortem. c: Blood sample will be taken prior to administration of the SICCT test. d: SICCT reading. Flexibility in the study schedule (± x days) will be determined in the final study protocol and the design is presented in this way for pragmatic practical reasons.

In respect of secondary objectives, pre-vaccination blood samples will be collected for DIVA testing as it is considered best practice to collect this data should these studies be used as supportive efficacy studies.

It is currently unclear whether the use of the SICCT pre-vaccination will affect the DIVA result from the sample collected on Study Day 0. This may impact whether or not the SICCT is used in the final study design (it may be required to allow the transport of animals from source farm sites to test site AFUs, i.e. pre-movement test) or alternatively if no pre-vaccination blood sample is obtained whether there will be sufficient sampling points within this study design to use this data as supportive efficacy data (towards the MA).

A further SICCT will be performed at the time of slaughter to obtain data on the likelihood of an animal testing positive for a SICCT approximately 12 months post-vaccination.
5.7 Treatment administration
Vaccination with either IVP or CP will be on Study Day 0.

The IVP or CP will be administered at a dose rate of 0.5mL per animal. This is the dose rate which, based on previous clinical studies, has been shown to be safe and effective for the control of bTB in cattle.

The IVP or CP will be administered subcutaneously into the neck region on the left hand side. The side of vaccination is specified to facilitate subsequent injection site observations.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.8 Clinical examinations
Clinical examinations will be performed as per the study schedule (see Section 5.6).

The clinical examination will include an examination of the general health status of the study animals, any abnormalities will be recorded.

5.9 Injection site observations
All animals will be monitored for injection site observations for a period of at least 30 days post-vaccination.

In addition a remote assessment will be made by the assessor/farmer as to whether the injection site is visible and to whether they would consider the animal to have received IVP or CP.

5.10 Post mortem examination
A PM will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death, results from existing blood samples from any such animals will be excluded from subsequent validation tests.

- Animals which are despatched to slaughter as per standard farm practice (finished production) will undergo a routine PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5 to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

The detailed method for enhanced PM and histopathological examination has been developed by APHA. This is considered to be suitable methodology for the conduct of these studies and should be performed, at a minimum, for the 300 animals determined as positive and a corresponding number of bTB negative cattle. The remainder of the study animals should undergo PM using a method consistent with slaughterhouse inspection of reactor cattle.

5.11 Samples and analysis
SICCT testing will be undertaken prior to vaccination and at one year post-vaccination.
For the purpose of acceptance onto the study as being bTB negative, animals must be negative to the SICCT pre-vaccination.

To be considered as having tested positive an animal must give a positive result to the blood IFN-DIVA blood test. To be considered as a confirmed positive animal a test-positive animal must show unequivocal symptoms of bTB at PM (including histo-pathological and bacteriological results if these analyses have been undertaken). The precise method of both PM and end point parameters will need to be agreed with EFSA and, as applicable other interested bodies, e.g. OIE.

Blood samples collected prior to vaccination and at the end of the study may have additional value as sources of biomarkers used to predict vaccine success.

The analytical method for DIVA analysis is provided separately to this study outline.

5.12 Adverse events

Adverse events will be recorded throughout the study period for the collection of safety data.

5.13 Summary of trial details required for ATC

Taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product'; VMD/L4/Authorisations/0321/C - #713143.


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications:

For the active immunisation of cattle to reduce infection with Mycobacterium bovis and to prevent lesions of tuberculosis caused by Mycobacterium bovis.

Estimated duration of trial: approximately 1 year/animal.

Maximum no. of animals:

i. Treated (with the test product): 150,000 (from bTB free source farm)
ii. Positive controls: not applicable
iii. Negative controls: 150,000 (from farms in high risk area with history of bTB)
iv. Placebo treated controls: maybe included at the discretion of Defra and WG (and would be instead of the negative control group).

Inclusion criteria: See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: Animals which are less than 6 months of age, lactating or pregnant will not be included. Also animals with a positive SICCT result prior to vaccination will not be included.

Description of safety monitoring: Clinical examinations will be carried out on Study Days -3 and 357 and will include an assessment of the general health status of the study animals.

Injection site observations will include an assessment of erythema, heat and swelling and will be monitored for a period of at least 30 days post-vaccination.

SICCT testing will be carried out on Study Days -3, 0, 357 and 360.

Blood samples will be taken on Study Day 0 and 357 to validate the DIVA blood test.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortems will be performed either following death during the study where the cause of death is unknown or when animals are despatched to slaughter as per standard farm practice (finished production).

(For further details see 5.6 Study schedule, 5.8 Clinical examinations, 5.9 Injection site observations, 5.10 Post mortem examination, 5.11 Samples and analysis.)
Method of administration / dose rate/ duration of administration:
i. Treated (with the test product): 0.5mL reconstituted vaccine containing $1-4 \times 10^6$ cfu, by subcutaneous injection.
i. Positive controls: Not applicable
iii. Negative controls: Not applicable
iv. Placebo treated controls: If a placebo is used, the method of administration should mirror that of the treated group.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end on Study Day 360 (12 months post-vaccination).

An SICCT test will be conducted at the end of the study to confirm if the vaccination on Study Day 0 influences the SICCT result on Study Day 360. In addition to this the SICCT result will be compared to the PM result (bTB positive/negative status) to confirm if the SICCT test can be used within the scope of WPP6 to ‘sign the animal off the study’ without further need for derogations post-study.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made. All animals will be sent for PM to generate lung lesion data.

It should be ensured that details of the appropriate withdrawal period (for any veterinary medicinal product) are observed for all animals destined for the food chain.

Further to this it should be noted that no other industry standard testing for bTB should be permitted during the study period and this will form part of the derogation.

7 ASSESSMENT OF VALIDATION

This study will provide bTB positive reference samples for the purpose of DIVA validation.

The DIVA test results will be compared to the results obtained at PM as a part of the validation process along with samples generated under other WPP2 protocols. It has been suggested by APHA that a blood sample and SICCT performed one week prior to slaughter, with a further blood sample at the time of slaughter at Study Day 360, would provide data for sensitivity testing. This is an alternative option within this study design and would assess the performance of the DIVA test against the SICCT test for animals confirmed as bTB positive (lesions at PM or culture data).

Data generated from this study could contribute towards the assessment for the supportive efficacy (e.g. reduction in lung lesions) and safety (e.g. injection site observations) of the vaccine.

Due to the number of animals required to generate 300 samples from vaccinated bTB positive animals, data from all samples may contribute to latent class analysis.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and
protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.

9 REFERENCES

Downs, S (2013). The potential of cattle in Approved Finishing Units, Exempt Finishing Units and/or Approved Quarantine Units as populations for validating defined antigen blood tests for bovine tuberculosis. APHA.
APPENDIX 5  WPP2.2 – STUDY FOR BLOOD IFN-γ DIVA TEST VALIDATION WITH CONCURRENT SICCT TESTING: PROPOSED PROTOCOL

Project No.: PN1567

Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Pre-vaccination BCG Vaccine then Exposed to M. bovis Challenge

WPP 2, Design 2
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1 TITLE
Blood IFN-γ DIVA Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Pre-vaccination with BCG Vaccine then Exposed to M. bovis Challenge.

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
This study is intended to be run in conjunction with other studies in the WPP2 series which together will form a data package to determine and validate the fitness of the developed, optimised and standardised blood IFN-γ DIVA test for the intended purpose as a stand-alone test with concurrent SICCT testing appropriate for the diagnosis of bTB infection in cattle that have been vaccinated with the BCG vaccine.

The primary objective of this trial is to generate blood samples from animals which are bTB negative pre-vaccination, vaccinated and then exposed to natural challenge in AFUs which become positive.

The secondary objectives for the study are:

- To generate data required to meet EU veterinary medicines legislation requirements on injection site observations and lung lesions.
- To generate supportive data (for the efficacy claim) as recommended by EFSA to provide power calculations with new additional data upon which to recalculate animal numbers for the pivotal field studies (WPP6).
- To generate reference samples for correlate markers if appropriate for APHA projects.
- To confirm if vaccination influences the SICCT test result approximately one year post-vaccination. This helps to inform on the scope for use of SICCT post-vaccination.

5 STUDY DESIGN

5.1 General
A negative controlled, multicentre clinical field efficacy and safety study to be carried out in different geographical regions within England and Wales.

Animals will be recruited onto the study at a ratio of 2:1, IVP:CP respectively, based on the following requirements:

- Group 1: ⅓ animals negative for bTB (from a low risk area), vaccinated
- Group 2: ⅓ animals from a high risk area, vaccinated
- Group 3: ⅓ animals from a high risk area, unvaccinated

Assessments will be made at the individual animal level.

This study design is proposed to fulfil one of the requirements of EU veterinary medicines legislation (V6 in Section 1.1.1 of the main report) that the vaccine (and hence its associated DIVA) should be tested in animals that are potentially pre-exposed to bTB.
5.1.1 Study Blinding

This study will be at least partially blinded. At a minimum it is recommended that personnel performing the post mortems and any laboratory personnel are blinded to vaccine status. The study may also involve the use of a placebo (instead of a negative control group). The decision to use a placebo will be determined in the final study protocols. A placebo may be used to mirror that which may be used during the field pivotal safety and efficacy studies. It may be considered useful to include a placebo in the DIVA validation studies as these could be both supportive to efficacy studies and an opportunity to develop an essential tool for maintaining blinding of study personnel in specific efficacy studies. Ideally the animal owners and all study personnel treating animals and collecting data will be blinded to the treatment received by individual animals. This will only be possible if the placebo induces a site reaction equivalent to that when the BCG vaccine is used, otherwise blinding will not be possible for the collection of samples whilst injection site reactions are still in evidence.

Since Group 3 will remain unvaccinated blinding will not be possible for the collection of samples whilst injection site reactions are still visible.

Technicians undertaking blood sample analysis will be blind to the treatment received by the animals as will technicians undertaking post mortem examinations or any analyses on collected samples.

5.2 Farms/Sites suitable for inclusion

Farm sites will be used from both low and high risk bTB areas, the farm sites will be representative of standard farming practices for the region. Dairy and beef farms will be recruited in an approximately equal split.

Farms from the low risk area should be bTB free. This will be defined as farms which have operated as closed herds with no history of bTB in the preceding 5 years at least and if possible, those for which all adjoining farms are of a similar history status.

The balance of animals will be sourced from farm sites which are located in high risk areas and with a farm history of bTB in the past 12 months, i.e. “breakdown” farms.

The study site (destination) AFUs will be established in the business of accepting animals from high risk farms/areas and based on reported experience these are expected to generate a higher rate of bTB challenge. If possible AFUs will be selected in different geographical locations so that vaccine is exposed to different environmental *Mycobacterium* i.e. different strains and incidences of *M. bovis* and *M. avium*.

5.3 Animals suitable for inclusion

Cattle of any breed, weight and gender above 6 months of age at vaccination/enrolment will be suitable for study. Inclusion criteria include an age of greater than 6 months and being neither pregnant nor lactating. Animals in groups 1 and 2 must also have a negative SICCT result prior to vaccination. The bTB status of animals in Group 3 (unvaccinated) will not be confirmed. The number of animals required will be confirmed following statistical advice.

5.4 Number of animals

The number of samples (300) required from animals which are vaccinated and then determined to have become positive for bTB (by post mortem inspection) is advice adopted by APHA (based on OIE guidance) and as supported by BioSS statistical analysis (Appendix 2).

Factors which impact the number of animals required for this study include vaccine efficacy. The predicted vaccine efficacy is 60%. Therefore approximately 40% of the vaccinated population will be susceptible to challenge. It should be noted that if the efficacy of the vaccine is higher than anticipated then the number of animals required to generate the 300 samples will also increase.

Another factor that will influence the number of animals required is transmission rate. AFUs have been selected for inclusion as these farm sites have the highest incidence rate of bTB, in comparison to conventional farm sites. Based on available literature (Downs, *et. al*, 2013) it is
suggested that AFUs can have a transmission rate of ca. 20% which is about 4 times higher than background rate.

In addition to this a further factor to consider is the sensitivity and specificity of the DIVA test. Mathematical modelling performed by Cambridge University predicts that 1000 AFUs will be required, with animal residency on the individual AFU of one year to achieve the 300 samples required. Based on the average capacity of an AFU of 300 animals, this would involve a total of 300,000 animals. High risk non-AFU farm sites have been considered, however, the difficulties associated with enrolling such a site, the ethical implications and associated costs make these a less favourable option in the opinion of the Consortium.

Based on modelling and information available at the time of writing this report, consideration should be given to the prudence of conducting this study for an initial period of 2 years with 100 AFUs recruited onto the study for one year each. This approach would allow an initial assessment of the factors that influence the rate of bTB positive sample generation. It is recommended that Defra and WG should take these results and those of other studies performed to date and seeking scientific advice and further guidance from e.g. OIE, on sample numbers.

5.5 Study treatments and allocation

Animals will be recruited to the study at a ratio of 1:1:1, for Groups 1 to 3 respectively.

The bTB negative animals will be treated with IVP on the source farms and transferred to the study AFU in the high risk area after about 30 days. This period is suggested as a time frame in which maximum t-cell response will occur thus ensuring that the protective immune response will be induced and established prior to bTB challenge at the AFU. Note that a select number of animals at the bTB negative source farm may be treated with prospective placebo with a view to determining if attending animal care personnel can tell true vaccinated animals apart from placebo treated.

Animals on the bTB negative source farm will be vaccinated in batches as they become available. The animals will remain on their source farm for 30 days to allow immunity to be established and will then be transferred, in the same batches, to the AFU test site. It is anticipated that for a period of at least one year all of the animals arriving at a given AFU test site will be exclusively study animals.

5.6 Study schedule

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Data Collection</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm Details</td>
<td>Animal Details</td>
</tr>
<tr>
<td>Pre-Vacc. -30 to 0</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>0</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>1 - 30</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>30</td>
<td>Transfer to AFU and mix with AFU stock</td>
<td>✓</td>
</tr>
<tr>
<td>357</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>360</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

a: Groups 1 and 2 only for confirmation of –ve status. For group 3 ‘pre-vaccination’ procedures will take place prior to mixing of the animals on Day 30.b: SICCT reading. Flexibility in study schedule (± x days) will be determined in the final study protocol and the design is presented in this way for pragmatic practical reasons.

In respective of secondary objectives, pre-vaccination blood samples will be collected for DIVA testing as it is considered best practice to collect this data should these studies be used as supportive efficacy studies.

It is currently unclear whether the use of the SICCT pre-vaccination will affect the DIVA result from the sample collected on Study Day 0. This may impact whether or not the SICCT is used in the final study design (it may be required to allow the transport of animals from source farm sites
to test site AFUs, i.e. pre-movement test) or alternatively if no pre-vaccination blood sample is obtained whether there will be sufficient sampling points within this study design to use this data as supportive efficacy data (towards the MA).

A further SICCT will be performed at the time of slaughter to obtain data on the likelihood of an animal testing positive for a SICCT approximately 12 months post-vaccination.

5.7 Treatment administration
Vaccination will be on Study Day 0 for animals in Groups 1 and 2. If CP is used it will also be administered at the source site of Group 1 at this time.

The IVP will be administered at a dose rate of 0.5mL per animal. This is the dose rate which, based on previous clinical studies, has been shown to be safe and effective for the control of bTB in cattle.

The IVP or CP will be administered subcutaneously into the neck region on the left hand side. The side of vaccination is specified to facilitate subsequent injection site observations.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.8 Clinical examinations
Clinical examinations will be performed as per the study schedule (see Section 5.6).

The clinical examination will include an examination of the general health status of the study animals, any abnormalities will be recorded.

5.9 Injection site observations
All vaccinated animals will be monitored for injection site observations for a period of at least 30 days post-vaccination.

Injection site observations will include an assessment of erythema, heat and swelling.

In addition a remote assessment will be made by the assessor/farmer as to whether the injection site is visible and if they would consider the animal to have received IVP or CP.

5.10 Post mortem examination
During the study period a post mortem (PM) will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death, results from existing blood samples from any such animals will be excluded from subsequent validation tests.
- Animals which are despatched to slaughter as per standard AFU practice (finished production) will undergo a routine PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

The detailed method for enhanced PM and histo-pathological examination has been developed by APHA. This is considered to be suitable methodology for the conduct of these studies and should be performed, at a minimum, for the 300 animals determined as positive and a corresponding number of bTB negative cattle. The remainder of the study animals should undergo PM using a method consistent with slaughterhouse inspection of reactor cattle.
5.11 Samples and analysis

SICCT testing will be undertaken prior to vaccination and at one year post-vaccination.

For the purpose of acceptance onto the study as being bTB negative, animals must be negative to the SICCT pre-vaccination.

To be considered as having tested positive an animal must give a positive result to the blood IFN-DIVA blood test. To be considered as a confirmed positive animal a test-positive animal must show unequivocal symptoms of bTB at PM (including histo-pathological and bacteriological results if these analyses have been undertaken). The precise method of both PM and end point parameters will need to be agreed with EFSA and, as applicable other interested bodies, e.g. OIE.

Blood samples collected prior to vaccination and at the end of the study may have additional value as sources of biomarkers used to predict vaccine success.

The analytical method for DIVA analysis is provided separately to this study outline.

5.12 Adverse events

Adverse events will be recorded throughout the study period for the collection of safety data.

5.13 Summary of trial details required for ATC

Taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143.


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications: For the active immunisation of cattle to reduce infection with Mycobacterium bovis and prevent lesions of tuberculosis caused by Mycobacterium bovis.

Estimated duration of trial: Approximately 1 year/Animal.

Maximum no. of animals:

  i. Treated (with the test product): 200,000
  ii. Positive controls: not applicable
  iii. Negative controls: 100,000
  iv. Placebo treated controls: May be included at the discretion of Defra and WG (and would be instead of the negative control group).

Inclusion criteria: See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: Animals which are less than 6 months of age, lactating or pregnant will not be included. Also animals with a positive SICCT result prior to vaccination will not be included in groups 1 and 2.

Description of safety monitoring: Clinical examinations will be carried out prior to vaccination/enrolment, at 6 months after the start of the study and at the end of the study and will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be monitored for a period of at least 30 days post-vaccination.

SICCT testing will be carried out prior to vaccination and at 1 year post-vaccination.

Blood samples will be collected prior to vaccination and at 1 year post-vaccination to validate the blood test.

Adverse events will be recorded throughout the study period for the collection of safety data.
Post mortems will be performed either following death during the study where the cause of death is unknown or when animals are despatched to slaughter as per standard farm practice (finished production).

(For further details see 5.6 Study schedule, 5.8 Clinical examinations, 5.9 Injection site observations, 5.10 Post mortem examination, 5.11 Samples and analysis.)

**Method of administration / dose rate/ duration of administration:**

i. Treated (with the test product): 0.5mL reconstituted vaccine containing $1 \times 10^6$ cfu, by subcutaneous injection.

ii. Positive controls: Not applicable

iii. Negative controls: Not applicable

iv. Placebo treated controls: If a placebo is used, the method of administration should mirror that of the treated group.

**Disposal of unused product and empty containers:** to be advised.

**Disposal or fate of test food producing animals (not intended to enter the human food chain for food):** see 6.1 Fate of study animals.

### 6 STUDY END

The study will end on Study Day 360 (12 months post-vaccination/enrolment).

An SICCT test will be conducted at the end of the study; this is to confirm if the vaccination on Study Day 0 influences the SICCT result on Study Day 360. In addition to this the SICCT result will be compared to the PM result (bTB positive/negative status) to confirm if the SICCT test can be used within the scope of WPP6 to ‘sign the animal off the study’ without further need for derogations post-study.

#### 6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made. All animals will be sent for PM to generate lung lesion data.

It should be ensured that details of the appropriate withdrawal period (for any veterinary medicinal product) are observed for all animals destined for the food chain.

It should be noted that no other industry standard testing for bTB should be permitted during the study period and a derogation may be required for this.

### 7 ASSESSMENT OF VALIDATION

This study will provide bTB positive reference samples for the purpose of DIVA validation.

The DIVA test results will be compared to the results obtained at PM as a part of the validation process along with samples generated under other WPP2 protocols. It has been suggested by APHA that a blood sample and SICCT performed one week prior to slaughter, with a further blood sample at the time of slaughter at Study Day 360, would provide data for sensitivity testing. This is an alternative option within this study design and would assess the performance of the DIVA test against the SICCT test for animals confirmed as bTB positive (lesions at PM or culture data).

Data generated from this study could contribute towards the assessment for the supportive efficacy (e.g. reduction in lung lesions) and safety (e.g. injection site observations) of the BCG vaccine.

Due to the number of animals required to generate 300 samples from vaccinated bTB positive animals, data from all samples may contribute to latent class analysis.

### 8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.
From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinary practices acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.

9 REFERENCES

Downs, S (2013). The potential of cattle in Approved Finishing Units, Exempt Finishing Units and/or Approved Quarantine Units as populations for validating defined antigen blood tests for bovine tuberculosis. APHA (formally AHVLA).
APPENDIX 6  WPP2.3 – STUDY FOR BLOOD IFN-γ DIVA TEST VALIDATION WITH CONCURRENT SICCT TESTING: PROPOSED PROTOCOL

Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Pre-vaccination with BCG Vaccine then Exposed to *M. bovis* Challenge

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1 TITLE

Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: DIVA Validation Study: Generation of Blood Samples from Cattle which are bTB Negative Pre-vaccination with BCG vaccine then Exposed to M. bovis Challenge.

2 REGULATORY GUIDANCE

It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE

This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES

This study is intended to be run in conjunction with other studies in the WPP2 series which together will form a data package to determine and validate the fitness of the developed, optimised and standardised blood IFN-γ DIVA test for the intended purpose as a stand-alone test with concurrent SICCT testing appropriate for the diagnosis of bTB infection in cattle that have been vaccinated with the BCG vaccine.

The primary objective of this trial is to generate blood samples from animals which are bTB negative pre-vaccination, vaccinated and then exposed to natural challenge in AFUs and which become positive.

The secondary objectives for the study are:

- To generate data required to meet EU veterinary medicines legislation requirements on injection site observations and lung lesions.
- To generate secondary supportive data (for efficacy claim) as required by EFSA to re-calculate animal numbers for the pivotal field studies (WPP6).
- To generate reference samples for correlate markers.
- To confirm if vaccination influences the SICCT test result approximately one year post-vaccination. This helps to inform on the scope for use of SICCT post-vaccination.

5 STUDY DESIGN

5.1 General

A randomised, negative-controlled, multicentre study to be carried out in different geographical regions within England and Wales.

Animals will be recruited onto the study at a ratio of 1:1, IVP:CP respectively, based on the following requirements:

Group 1: ½ animals negative for bTB (from a high risk area), vaccinated
Group 2: ½ animals from a high risk area, unvaccinated

Assessments will be made at the individual animal level.

5.1.1 Study Blinding

This study will be at least partially blinded. At a minimum it is recommended that personnel performing the post mortems and any laboratory personnel are blinded to vaccine status. The study may also involve the use of a placebo (instead of a negative control group). The decision to use a placebo will be determined in the final study protocols. A placebo may be
used to mirror that which may be used during the field pivotal safety and efficacy studies. It may be considered useful to include a placebo in the DIVA validation studies as these could be both supportive to efficacy studies and an opportunity to develop an essential tool for maintaining blinding of study personnel in specific efficacy studies. Ideally the animal owners and all study personnel treating animals and collecting data will be blinded to the treatment received by individual animals. This will only be possible if the placebo induces a site reaction equivalent to that when the BCG vaccine is used, otherwise blinding will not be possible for the collection of samples whilst injection site reactions are still in evidence.

Technicians undertaking blood sample analysis will be blind to the treatment received by the animals as will technicians undertaking post mortem examinations or any analyses on collected samples.

5.2 Farms/Sites suitable for inclusion
AFUs located in high risk areas will be selected for inclusion. The selected AFUs should be able to demonstrate historical base line bTB transmission rates. AFUs have been selected for the study as AFUs are likely to have a greater transmission rate than standard farm sites. If possible AFUs will be selected in different geographical locations so that vaccine is exposed to different environmental *Mycobacterium* i.e. different strains and incidences of *M. bovis* and *M. avium*.

5.3 Animals suitable for inclusion
Cattle of any breed, weight or gender will be suitable for the study. Inclusion criteria include an age of greater than 6 months and being neither pregnant nor lactating. Animals must also have a negative SICCT result prior to vaccination.

5.4 Number of animals
The number of samples (300) required from animals which are vaccinated and then determined to become positive for bTB (by post mortem inspection) is advice adopted by APHA (based on OIE guidance) and as supported by BioSS statistical analysis (Appendix 2).

Factors which impact the number of animals required for this study include vaccine efficacy. The predicted vaccine efficacy is 60%. Therefore approximately 40% of the vaccinated population will be susceptible to challenge. It should be noted that if the efficacy of the vaccine is higher than anticipated then the number of animals required to generate the 300 samples will also increase.

Another factor that will influence the number of animals required is transmission rate. AFUs have been selected for inclusion as these farm sites have the highest incidence rate of bTB, in comparison to conventional farm sites. Based on available literature (Downs, *et al.*, 2013) it is suggested that AFUs can have a transmission rate of ca. 20% which is about 4 times higher than background rate.

In addition to this a further factor to consider is the sensitivity and specificity of the DIVA test. Mathematical modelling performed by Cambridge University predicts that 1000 AFUs will be required, with animal residency on the individual AFU of one year to achieve the 300 samples required. Based on the average capacity of an AFU of 300 animals, this would involve a total of 300,000 animals. High risk non-AFU farm sites have been considered, however, the difficulties associated with enrolling such a site, the ethical implications and associated costs make these a less favourable option in the opinion of the Consortium.

Based on modelling and information available at the time of writing this report, consideration should be given to the prudence of conducting this study for an initial period of 2 years with 100 AFUs recruited onto the study for one year each. This approach would allow an initial assessment of the factors that influence the rate of bTB positive sample generation. It is recommended that Defra and WG should take these results and those of other studies performed to date and seeking scientific advice and further guidance from *e.g.* OIE, on sample numbers.

5.5 Study treatments and allocation
Animals will be randomly allocated to treatment at a ratio of 1:1, IVP:CP.

Animals on a given AFU will be vaccinated or treated at a single time point.
5.6 Study schedule

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Data Collection</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm Details</td>
<td>Animal Details</td>
</tr>
<tr>
<td>Pre-Vacc.</td>
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<td>✓</td>
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a: Testing performed as per standard practice, i.e. performed prior to transfer to AFU, not undertaken specifically for the study as a study procedure per se, but results made available in the Clinical Study Files. b: SICCT reading. Flexibility in the study schedule (± x days) will be determined in the final study protocol and the design is presented in this way for pragmatic practical reasons. c: pre-vaccination. d: Blood sample taken prior to SICCT testing.

In respect of secondary objectives, pre-vaccination blood samples will be collected for DIVA testing as it is considered best practice to collect this data should these studies be used as supportive efficacy studies.

It is currently unclear whether the use of the SICCT pre-vaccination will affect the DIVA result from the sample collected on Study Day 0. This may impact whether or not the SICCT is used in the final study design (it may be required to allow the transport animals from source farm sites to test site AFUs, i.e. pre-movement test) or alternatively if no pre-vaccination blood sample is obtained whether there will be sufficient sampling points within this study design to use this data as supportive efficacy data (towards the MA).

A further SICCT will be performed at the time point of slaughter to obtain data on the likelihood of an animal testing positive for a SICCT approximately 12 months post-vaccination.

5.7 Treatment administration

Vaccination with either IVP or CP will be on Study Day 0. The IVP or CP will be administered at a dose rate of 0.5mL per animal. This is the dose rate which, based on previous clinical studies, has been shown to be safe and effective for the control of bTB in cattle.

The IVP or CP will be administered subcutaneously into the neck region on the left hand side. The side of vaccination is specified to facilitate subsequent injection site observations.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.8 Clinical examinations

Clinical examinations will be performed as per the study schedule (see Section 5.6).

The clinical examination will include an examination of the general health status of the study animals, any abnormalities will be recorded.

5.9 Injection site observations

All animals will be monitored for injection site observations for a period of at least 30 days post-vaccination.

Injection site observations will include an assessment of e.g. erythema, heat and swelling.

5.10 Post mortem examination

A post mortem (PM) will be performed for the following reasons:
Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death. Results from existing blood samples from any such animals will be excluded from subsequent validation tests.

Animals which are despatched to slaughter as per standard farm practice (finished production) will undergo a routine PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

The detailed method for enhanced PM and histo-pathological examination has been developed by AHPA. This is considered to be suitable methodology for the conduct of these studies and should be performed, at a minimum, for the 300 animals determined as positive and a corresponding number of bTB negative cattle. The remainder of the study animals should undergo PM using a method consistent with slaughterhouse inspection of reactor cattle.

5.11 Samples and analysis

SICCT testing will be undertaken prior to vaccination and at one year post-vaccination.

For the purpose of acceptance onto the study as being bTB negative, animals must be negative to the SICCT pre-vaccination.

To be considered as having tested positive an animal must give a positive result to the blood IFN-DIVA blood test. To be considered as a confirmed positive animal a test-positive animal must show unequivocal symptoms of bTB at PM (including histo-pathological and bacteriological results if these analyses have been undertaken). The precise method of both PM and end point parameters will need to be agreed with EFSA and, as applicable other interested bodies, e.g. OIE.

Blood samples collected prior to vaccination and at the end of the study may have additional value as sources of biomarkers used to predict vaccine success.

The analytical method for DIVA analysis is provided separately to this study outline.

5.12 Adverse events

Adverse Events will be recorded throughout the study period for the collection of safety data.

5.13 Summary of trial details required for ATC

Taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143.


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications: For the active immunisation of cattle to reduce infection with Mycobacterium bovis and prevention of lesions of tuberculosis caused by Mycobacterium bovis.
Estimated duration of trial: Approximately 1 year/Animal.

Maximum no. of animals:
1. Treated (with the test product): 150,000
2. Positive controls: not applicable
3. Negative controls: 150,000
4. Placebo treated controls: Maybe included at the discretion of Defra and WB (and would be instead of the negative control group).

Inclusion criteria: See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: Animals which are less than 6 months of age, lactating or pregnant will not be included. Also animals with a positive SICCT result prior to vaccination will not be included.

Description of safety monitoring: Clinical examinations will be carried out prior to vaccination/enrolment and at the end of the study and will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be monitored for a period of at least 30 days post-vaccination.

SICCT testing will be carried out prior to vaccination and at one year post-vaccination.

Blood samples will be collected prior to vaccination and at one year post-vaccination to validate the blood test.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortem will be performed either following death during the study where the cause of death is unknown or when animals are despatched to slaughter as per standard farm practice (finished production).

(For further details see 5.6 Study schedule, 5.8 Clinical examinations, 5.9 Injection site observations, 5.10 Post mortem examination, 5.11 Samples and analysis.)

Method of administration / dose rate / duration of administration:
1. Treated (with the test product): 0.5mL reconstituted vaccine containing 1-4 x 10^6 cfu, by subcutaneous injection.
2. Positive controls: Not applicable
3. Negative controls: Not applicable
4. Placebo treated controls: If a placebo is used, the method of administration should mirror that of the treated group.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end on Study Day 360 (12 months post-vaccination).

An SICCT test will be conducted at the end of the study to confirm if the vaccination on Study Day 0 influences the SICCT result on Study Day 360. In addition to this the SICCT result will be compared to the PM result (bTB positive/negative status) to confirm if the SICCT test can be used within the scope of WPP6 to ‘sign the animal off the study’ without further need for derogations post-study.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

It should be noted that no other industry standard testing for bTB should be permitted during the study period, this will form part of the derogation.
7 ASSESSMENT OF VALIDATION

This study will provide bTB positive reference samples for the purpose of DIVA validation.

The DIVA test results will be compared to the results obtained at PM as a part of the validation process along with samples generated under other WPP2 protocols. It has been suggested by APHA that a blood sample and SICCT performed one week prior to slaughter, with a further blood sample at the time of slaughter at Study Day 360, would provide data for sensitivity testing. This is an alternative option within this study design and would assess the performance of the DIVA test against the SICCT test for animals confirmed as bTB positive (lesions at PM or culture data).

Data generated from this study could contribute towards the assessment for the supportive efficacy (e.g. reduction in lung lesions) and safety (e.g. injection site observations) of the vaccine.

Due to the number of animals required to generate 300 samples from vaccinated bTB positive animals, data from all samples may contribute to latent class analysis.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.

9 REFERENCES

Downs, S (2013). The potential of cattle in Approved Finishing Units, Exempt Finishing Units and/or Approved Quarantine Units as populations for validating defined antigen blood tests for bovine tuberculosis. APHA.
APPENDIX 7 WPP2.4 – STUDY FOR BLOOD IFN-γ DIVA TEST VALIDATION WITH CONCURRENT SICCT TESTING: PROPOSED PROTOCOL

Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Following Vaccination with BCG Vaccine

WPP 2, Design 4
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1 TITLE
Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are bTB Negative Following Vaccination with BCG Vaccine.

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
This study is intended to be run in conjunction with other studies in the WPP2 series which together will form a data package to determine and validate the fitness of the developed, optimised and standardised blood IFN-γ DIVA test for the intended purpose as a stand-alone test with concurrent SICCT testing appropriate for the diagnosis of bTB infection in cattle that have been vaccinated with the BCG vaccine.

The primary objective for this trial is to generate samples from animals which are bTB negative pre-vaccination, vaccinated and then remain bTB negative.

The secondary objective for the study is to generate reference samples for correlate markers.

5 STUDY DESIGN
5.1 General
As this study is designed to generate samples from bTB negative animals, all animals will receive IVP. This decision has been made so that only the minimum number of animals (plus a small overage) is used to generate the required samples.

A multi-centred clinical field study to be carried out in different geographical regions within the United Kingdom.

5.2 Farms/Sites suitable for inclusion
Animals will be recruited from farms located in low bTB risk areas, which have been bTB free for a minimum of 10 years. If possible only closed herd farm sites/AFUs with adjoining neighbours of similar herd health history will be recruited for the study. Ideally the farms should have undergone routine bTB testing within 12 months of the study start.

5.3 Animals suitable for inclusion
Cattle of any breed, gender or weight at vaccination will be suitable for study. Cattle must be greater than 6 months of age and must be neither pregnant nor lactating. Animals considered suitable for the study will be confirmed as bTB negative by SICCT within the 30 days prior to vaccination.

The number of animals required for recruitment will be confirmed following statistical input. It should be noted that the OIE require 1000 samples from vaccinated animals which remain bTB negative. As the specificity of the DIVA test is projected to be 99.85% there may be a small number of animals which test positive (“false positives”). Therefore it is advisable that an overage of animals is included within this study population (1100 animals).
5.4 Study treatments and allocation

Animals will be recruited onto the study and will all receive IVP.

Animals on a single farm site will be vaccinated at a single time point.

5.5 Study schedule

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<th>Procedure</th>
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a: Flexibility for reading the SICCT or Skin-DIVA tests (± x days) will be determined in the final study protocol.

On Study Day 56, any animals which test positive will be subject to PM and confirmation of bTB status obtained (enhanced PM with bacterial culture).

A possible suggested addition is to include a small number of animals (approximately 10), from a bTB negative source, which are treated with CP. This would be to confirm that the suggested CP e.g. placebo demonstrates the same presentation, magnitude and resolution course of injection site reactions induced by the IVP group. This option is not currently covered in the study design and associated costings.

5.6 Treatment administration

Vaccination with IVP will be on Study Day 0.

The IVP will be administered at a dose rate of 0.5mL per animal. This is the dose rate which, based on previous clinical studies, has been shown to be safe and effective for the control of bTB in cattle.

The IVP will be administered subcutaneously into the neck region on the left hand side. The side of vaccination is specified to allow any adverse events specific to injection site reactions to be recorded.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.7 Clinical examinations

Prior to vaccination a clinical examination will be performed by a veterinarian on all animals to ensure that the animal is suitable for inclusion in the study. At the end of the study a final clinical examination will be performed to ensure the animal has remained in good health and can be signed off the study (returning to the commercial herd).

The clinical examination will include an examination of the general health status of the study animals by observing the main body systems, and any abnormalities will be recorded.

5.8 Injection site observations

As all animals will receive the IVP, no specific injection site observations will be recorded. However, should the attending veterinarian observe an adverse reaction relating to the site of injection then this will be recorded as an adverse event.

5.9 Post mortem examination

A post mortem (PM) will be performed for the following reasons:
• Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death.

• Animals which are DIVA test positive, results from existing blood samples from any such animals will be excluded from subsequent validation tests.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes, and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

The detailed method for enhanced PM and histo-pathological examination has been developed by APHA. This is considered to be suitable methodology for the conduct of these studies and should be performed, at a minimum, for the 300 animals determined as positive and a corresponding number of bTB negative cattle. The remainder of the study animals should undergo PM using a method consistent with slaughterhouse inspection of reactor cattle.

5.10 Samples and analysis

Blood samples will be collected prior to vaccination and at 8 weeks post-vaccination for use in the validation testing of the DIVA analytical method.

5.11 Adverse events

Adverse events will be recorded throughout the study period for the collection of safety data.

5.12 Summary of trial details required for ATC

(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications: To be advised

Estimated duration of trial: 56 days.

Maximum no. of animals:

i. Treated (with the test product): 1100
ii. Positive controls: not applicable
iii. Negative controls: not applicable
iv. Placebo treated controls: not applicable

Inclusion criteria: see 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: animals which are less than 6 months of age, lactating or pregnant will not be included. Also animals with a positive SICCT result prior to vaccination will not be included.

Description of safety monitoring: clinical examinations will be carried out prior to vaccination and at the end of the study and will include an assessment of the general health status of the study animals.
SICT testing will be carried out within 30 days prior to vaccination.

Blood samples will be collected prior to vaccination and at 56 days post-vaccination to validate the blood test.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortems will be performed either following death during the study where the cause of death is unknown or when animals are DIVA test positive).

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Injection site observations, 5.9 Post mortem examination, 5.11 Samples and analysis.)

Method of administration / dose rate / duration of administration:

i. Treated (with the test product): To be advised
ii. Positive controls: Not applicable
iii. Negative controls: Not applicable
iv. Placebo treated controls: Not applicable

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end on Study Day 56.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study will be made.

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (e.g. animals that are to be sold on).

In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will form part of the derogation.

It is anticipated that approximately 9-12 months following the end of the study period animals will undergo an industry standard SICT test. Animals which are positive will then undergo a blood gamma test and PM. If both results from the blood gamma test and PM show the animal as being bTB negative, the farm site will have no restriction/further testing. This additional testing is considered to be outside the scope of the schedule in Section 5.5 and is suggested to provide a reassurance to the farm sites involved.

7 ASSESSMENT OF VALIDATION

This study will provide samples from bTB negative cattle that are vaccinated and then test negative for bTB infection. These samples will be used in the validation testing of the blood IFN-γ DIVA test method.

PM results from animals that tested DIVA positive during the study will be used to either confirm or question the accuracy of the DIVA result.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and
protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 8  WPP2.5 – STUDY FOR BLOOD IFN-γ DIVA TEST VALIDATION WITH CONCURRENT SICCT TESTING: PROPOSED PROTOCOL

Project No.: PN1567

Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are either bTB Positive or bTB Negative

WPP 2, Design 5
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1 TITLE
Blood IFN-γ DIVA Test Validation with Concurrent SICCT Testing: Generation of Blood Samples from Cattle which are either bTB Positive or bTB Negative.

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
This study is intended to be run in conjunction with other studies in the WPP2 series which together will form a data package to determine and validate the fitness of the developed, optimised and standardised IFN-γ DIVA blood test assay for the intended purpose as a stand-alone test with concurrent SICCT testing.

The primary objective of this trial is to generate blood samples from unvaccinated animals which are either test positive or test negative for bTB.

The secondary objective is to generate data to support the proposed minimum age by collecting data from animals which are less than 6 months of age as well as over 6 months of age.

5 STUDY DESIGN

5.1 General
A multicentre, clinical field study to be carried out in different geographical regions within England and Wales.

5.2 Farms/Sites suitable for inclusion
Farm sites will be recruited from high risk and edge bTB areas, the farm sites will be representative of standard farming practices for the region. Dairy and beef farms will be recruited in an approximately equal split.

5.3 Animals suitable for inclusion
Cattle of any breed, weight or gender and above 15 days of age at enrolment will be suitable for the study.

The number of animals required will be confirmed following statistical advice. It should be noted that the OIE require ca. 1000 samples from vaccinated animals which remain bTB negative and 300 samples from vaccinated animals which become bTB positive, therefore it may be necessary to match this number of samples in unvaccinated animals.

5.4 Study treatments and allocation
Not applicable, animals left unvaccinated.
5.5 Study schedule

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PM = Post Mortem. a: Pre-SICCT Flexibility (± x days) will be determined in the final study protocol. b: SICCT reading. c: Animals which are positive to SICCT test and/or DIVA.

5.6 Treatment administration

Not applicable as animals will be unvaccinated.

5.7 Clinical examinations

No study clinical examinations will be performed. Animals will be monitored by the farm sites own veterinarian as per standard farm practice.

5.8 Injection site observations

Not applicable as no vaccine will be administered.

5.9 Post mortem examination

Any animal which is positive according to the blood IFN-γ DIVA test of the blood sample and/or the SICCT skin test (i.e. reacting as being bTB positive) will undergo a routine PM to confirm the accuracy of the DIVA test result.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes, and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

5.10 Samples and analysis

Blood samples will be collected on Study Day 0 for use in the validation testing of the DIVA analytical method.

5.11 Adverse events

Any adverse events which occur during the study period will be recorded.

5.12 Summary of trial details required for ATC

(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)


Pharmaceutical form: not applicable.

Target species: *Bos taurus*
Indications: Not applicable

Estimated duration of trial: 7 days.

Maximum no. of animals:

i. Treated (with the test product): not applicable
ii. Positive controls: not applicable
iii. Negative controls: to be advised following statistical advice
iv. Placebo treated controls: not applicable

Inclusion criteria: see 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: animals which are less 15 days of age will not be included.

Description of safety monitoring:

Blood samples will be collected on Study Day 0 to validate the blood IFN-γ DIVA test.

Adverse events will be recorded throughout the study period.

Post mortems will be performed on any animal which is positive to the DIVA blood test and/or the SICCT skin test.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Injection site observations, 5.9 Post mortem examination, 5.11 Samples and analysis.)

Method of administration / dose rate/ duration of administration:

i. Treated (with the test product): Not applicable
ii. Positive controls: Not applicable
iii. Negative controls: Not applicable
iv. Placebo treated controls: Not applicable

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end following receipt of the blood IFN-γ DIVA test results, the reading of the SICCT skin tests and the results of any subsequent post mortems.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made. It is anticipated that study animals will return to farm stock at the test site.

7 ASSESSMENT OF VALIDATION

The study will provide samples for bTB negative and positive cattle for use in the validation testing of the blood IFN-γ DIVA test.

SICCT and DIVA positive test results will be confirmed by the results obtained at post mortem.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.
In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 9  WPP2.6 – STUDY FOR DIVA VALIDATION, PROPOSED PROTOCOL; MULTIPLE SICCT TESTING

Generation of Blood Samples from Cattle which are bTB Negative Pre- and Post-vaccination with BCG Vaccine that also are Exposed to Multiple Standard SICCT Testing

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1 TITLE
Generation of Blood Samples from Cattle which are bTB Negative Pre- and Post-vaccination with BCG Vaccine that also are Exposed to Multiple Standard SICCT Testing.

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
The objective is to generate blood samples (BS) from cattle which are bTB negative pre- and post-vaccination with BCG vaccine which are also exposed to multiple standard Single Intradermal Comparative Cervical Tuberculin (SICCT) tests, to show any potential effect of the SICCT on the blood IFN-γ DIVA test results.

The decision to conduct this study will be taken following the advice of the regulatory authorities.

5 STUDY DESIGN

5.1 General
A randomised, blinded, placebo-controlled, multicentre study to be carried out in different geographical regions within the United Kingdom.

Assessment will be made at the individual animal level, comparing results to the individual animal’s baseline blood IFN-γ DIVA test over the study period.

5.2 Farms/Sites suitable for inclusion
Farm sites in low risk areas will be selected for inclusion. A maximum of two farm sites will be selected for the study; each farm site should provide ca. 50 cattle for vaccination.

5.3 Animals suitable for inclusion
Cattle of any breed, weight or gender will be suitable for the study. Cattle must be greater than 6 months of age but must not be pregnant or lactating. Animals considered suitable for the study will be confirmed as bTB negative by SICCT prior to vaccination. Alternatively if the blood DIVA test has been validated prior to the start of the study, then the blood DIVA test may be used to confirm the status.

5.4 Study treatments and allocation
Animals will be randomly allocated to treatment at a ratio of 1:1, IVP:CP respectively.

Animals on an individual farm site will be vaccinated at a single time point.
5.5 Study schedule

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Data Collection</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm Details</td>
<td>Animal Details</td>
</tr>
<tr>
<td>Pre-Vacc.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
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</tr>
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<tr>
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</tr>
<tr>
<td>123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. If blood DIVA validation has been completed prior to study start, then confirmation of animals negative status will be confirmed via this method and not SICCT. b. BS collected prior to SICCT. c. SICCT result. Flexibility (± x days) will be determined in the final study protocol.

5.6 Treatment administration

Vaccination with either IVP or CP will be on Study Day 0.

The IVP or CP will be administered at a dose rate of 0.5mL per animal. This is the dose rate which, based on previous clinical studies, has been shown to be safe and effective for the control of bTB in cattle.

The IVP or CP will be administered subcutaneously into the neck region.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.7 Clinical examinations

Clinical examinations will be performed as per the study schedule (see Section 5.5).

The clinical examination will include an assessment of the general health status of the study animals, any abnormalities will be recorded.

5.8 Post mortem examination

A post mortem will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death.
- Animals which are despatched to slaughter as per standard farm practice for reasons relating to animal welfare will undergo a routine PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection as per standard slaughterhouse methodology.

5.9 Samples and analysis

Blood samples will be collected as per the schedule in 5.5, for use in the validation testing of the blood IFN-γ DIVA test analytical method.

5.10 Adverse events

Adverse events will be recorded throughout the study period for the collection of safety data. Prior to farm recruitment onto the study, base line data on the farms disease history will be collated and agreed with the farm site’s own veterinarian. Adverse events will be reported as those outside of this baseline ‘normal’ for the farm site.
5.11 Summary of trial details required for ATC
(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: *Bos taurus*

Indications: To be advised

Estimated duration of trial: 180 days.

Maximum no. of animals:
  i. Treated (with the test product): 50
  ii. Positive controls: not applicable
  iii. Negative controls: not applicable
  iv. Placebo treated controls: 50

Inclusion criteria: see 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: animals which are less than 6 months of age, lactating or pregnant will not be included. Also animals with a positive SICCT result prior to vaccination will not be included.

Description of safety monitoring: clinical examinations will be carried out prior to vaccination and at the end of the study and will include an assessment of the general health status of the study animals.

SICCT testing will be carried out prior to vaccination.

Blood samples will be collected at 60, 123 and 180 days post-vaccination to contribute analysis data to validate the blood IFN-γ DIVA test.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortems will be performed either following death during the study where the cause of death is unknown or when animals are despatched to slaughter as per standard farm practice.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Post mortem examination, 5.9 Samples and analysis.)

Method of administration / dose rate/ duration of administration:
  i. Treated (with the test product): To be advised
  ii. Positive controls: Not applicable
  iii. Negative controls: Not applicable
  iv. Placebo treated controls: Not applicable

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end on Study Day 180.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (e.g. animals that are to be sold on).
In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.

7 ASSESSMENT OF VALIDATION

This study will provide supportive evidence that:

- SICCT test results are influenced by the presence of the BCG vaccine.
- DIVA results are not influenced by the presence of the SICCT tuberculin (administered on multiple occasions).

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces etc.).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 10 THE KEY PRINCIPLES OF CONDUCTING WORK TO VICH GCP STANDARD

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1 INTRODUCTION
The principles of Good Clinical Practice (VICH GCP) are outlined in the “International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)” guideline CVMP/VICH/595/98 “VICH GL9 Step 7 - Guideline on Good Clinical Practices (CVMP approved July 2000)”. VICH GCP is the standard for the design, conduct, monitoring, recording, auditing, analysis, and reporting of clinical studies. Compliance with this standard ensures that data and reported results are complete and accurate.

2 DEFINED ROLES
For large clinical studies it is usual to have multiple sites (known as multicentre studies) involved. Each site has an individual known as the ‘Investigator’ who is responsible for all aspects of the conduct of the study at the site. Investigators are selected for involvement in a clinical study based on their field of expertise, exposure to the disease of interest and the ability to perform the study. Typically once a Mutual Confidentiality Agreement has been obtained from the Investigator, they are provided with an ‘Investigator Brochure’ which details the background of the product under test: Investigational Veterinary Product (IVP), as well as the requirements of the study, inclusion of the sites and of the study animals. This is a key document introducing the project and identifying all areas of significance within the proposal including matters of risk.

To support the Investigator, there is the Monitor. A Monitor is an individual who is responsible for overseeing a clinical study and ensures that it is conducted, recorded, and reported in accordance with the Study Protocol, Standard Operating Procedures (SOPs), VICH GCP and to any applicable regulatory requirements. The Monitor supports the Investigator by the provision of training, instructions on technical procedures and by the provision of tools to ensure compliance to the Study Protocol (e.g. ‘Study Schedules’ which track individual study animals and notify the Investigator when visits are due or track samples collected, despatched to and received at the laboratory and also to confirm results).

3 STUDY ELEMENTS
For every study there is a VICH GCP compliant Study Protocol. The Study Protocol details the design of the clinical study which is used to determine the efficacy and field safety in the target species by generating statistically relevant results. The Study Protocol will be peer reviewed (including quality control) by the scientific team.

The Study Protocol and the requirements of the study design form the basis of the Clinical Study Files (CSF) which are compiled in a logical and systematic format to ensure that consistent information is captured across all sites. The CSF include, but are not limited to the provision of an Investigator File, Treatment Administer File (including treatment randomisations and instruction for breaking of blinding), Farm Site Files and any Data Capture Forms (DCFs).

In addition to the CSF, detail is provided on all study equipment necessary for the study to be performed. This includes the IVP and any related Control Products (CP) if applicable. If the IVP/CP...
requires storage at specific temperatures, sites should have adequate secure facilities to maintain temperature. Calibrated thermometers/data loggers to monitor the temperature of the IVP/CP both during storage at the Investigator site and during transit e.g. to and from a farm are typically provided. Third party suppliers e.g. laboratories, are engaged and integrated as applicable. They supply the Investigator with the correct sample packs (including e.g. sampling tubes, packaging material, pre-paid courier consignment notes) to ensure that samples are collected in accordance with the protocol and to ensure validity of the sample once received by the laboratory. This support may also include providing courier services to ensure expedient delivery of samples from the Investigator to the laboratory. Any additional equipment supplied to the Investigator e.g. weigh scales; will be fully calibrated and deemed ‘fit for purpose’ prior to use on the study.

4 TRAINING

Full and documented training of Investigators is imparted by Monitors in the use of the CSF as well as the Study Protocol. This is either given centrally to multiple Investigators (to allow for a unified approach between Investigators) or on an Investigator site basis using a standard set of presentations for training as well as Monitor checklists. Additional training is provided to any attending veterinarians, treatment administrators and farm site personnel. Training is performed according to SOPs prior to study start and usually delivered during the Monitor set-up visit.

Training covers, but is not limited to the following:

VICH GCP (provision of the Investigator handbook) and the responsibilities of the Investigator, data recording (to permit reconstruction of study), study procedures and sampling, adverse event reporting, IVP/CP use/accountability and fate, the importance of obtaining owner informed consent prior to animal involvement in a study, health and welfare of the study animals (including withdrawal/disposal), etc.

5 DATA COLLECTION

Once the study is underway at a site, the Monitor will continue to support the Investigator for the duration. The Monitor will perform ‘in-phase’ visits to site. At these visits the Monitor will review all data collected to date and raise any queries, and make copies of any source data applicable to the study (e.g. Animal Passports). The Monitor will inspect the IVP/CP store and ensure that IVP/CP supplies are sufficient for the study duration and that the storage temperatures are within specification. The Monitor will report and summarise any adverse events recorded, any deviations (departure from the procedure described in the Study Protocol) and also assess if any Study Protocol amendments (modification to the Study Protocol) are necessary. The Monitor will deliver any further training required or train any new study personnel. At the end of the study, the Monitor will perform a ‘close out’ visit and will ensure that all data has been verified and confirmed to be accurate according to the Study Protocol. The Monitor will collect all documentation and equipment during this visit, ensure that the Investigator has completed a statement of compliance (for inclusion in the Final Study Report (FSR)) and has confirmation in writing that the original documents were collected and states the archive location.

Once the data collected during the study has been verified and validated by the Monitor, the data can be released for data entry (if not captured using an electronic data capture system), statistical analysis and to the report author. Each VICH GCP clinical study results in a corresponding FSR. The FSR is a comprehensive description of the study and includes all raw data, description of the objectives, material, methods (including statistical analysis), presents the study results and contains a critical evaluation of these results. As with the Study Protocol the FSR is peer reviewed (including quality control) by the clinical team prior to being issued as final and submitted as part of the regulatory dossier.

3.1 Quality Assurance

The assurance of quality of every aspect of a study is a fundamental component of sound scientific practices. The principles of VICH GCP support the use of Quality Assurance (QA) procedures for clinical studies.

QA is a planned systematic process established to ensure that a study is performed and the data are collected, documented (recorded) and reported in compliance with the guidance of CVMP/VICH/595/98 “VICH Topic GL9 step 7 — Guidelines on good clinical practices (CVMP approved July 2000)” (i.e. “GCP”) and the applicable regulatory requirements.
Quality Assurance audits should be carried out on critical phases in the execution of a clinical study by trained personnel. The following phases may be inspected:

- Protocol review – including an assessment for accuracy, consistency, formatting, compliance with SOPs and any applicable regulatory requirements for the study being performed.
- Study documentation (CSF)
- Investigator site audits at pre-selected critical phases, defined at the start of the study.
- Report audit – draft
- Report audit – final

Additionally, any number of audits may be scheduled, including for example, but not limited to, the following:

- IVP/CP re-labelling, randomisation, distribution and records
- IVP/CP incorporation into vehicle and analysis
- Treatment administration
- Specific animal phases
- Laboratory phase
- Data Audit

For large clinical studies which generate a high volume of data it would be beneficial to schedule a process audit of the data handling procedures.

Inspections and audits are carried out by Quality Assurance personnel independent of the staff involved in the clinical study. An audit is a systematic and independent examination of study related activities and documentation to determine whether the study being evaluated is or was properly conducted and whether the data are or were recorded, analysed and accurately reported according to the study protocol, study related standard operating procedures (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirements.

Any organisation contracted to perform services as part of a clinical study should be chosen on the basis of reputation, quality, price, location and viability. Quality Assurance may “approve” the use of subcontractors, possibly as a result of inspection of facilities. An example of a subcontractor used on a clinical study is an analytical laboratory where staff are responsible for the quality of the work conducted.
APPENDIX 11 WPP6.1 – FIELD EFFICACY AND SAFETY OF BCG VACCINE, PROPOSED PROTOCOL

Project No.: PN1567

Field Efficacy and Safety of BCG Vaccine When Used in Cattle to Reduce Infection with *Mycobacterium bovis* (bTB)

WPP 6, Design 1
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1 TITLE
Field Efficacy and Safety of BCG Vaccine When Used in Cattle to Reduce Infection with *Mycobacterium bovis* (bTB).

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(S)P may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
The primary objective is to generate data to support field safety and efficacy of the BCG vaccine when used to reduce infection of bTB in cattle. This study will focus on generating data on the effect at the individual animal level.

The secondary objectives for the study are listed below:

- To generate data associated with Injection Site Observations (ISO) for a sub-population of animals which include pregnant and lactating animals. This data has not been collected in any previous studies and is specifically requested by the regulators unless covered by data generated as part of WPP8.
- To generate data on the reduction of lung lesions for animals which die during the study period and are positive at Post Mortem (PM).
- To generate blood samples from animals within a range of ages, including the minimum age for vaccination. Blood samples will be for analysis via the differentiating infected from vaccinated animals (DIVA) test. This objective is intended to support the proposed minimum age through assessment of DIVA in neonates.
- To generate pre- and post-vaccination farm site data on yield and milk composition (according to standard practice) to assess the safety of vaccine administration in dairy cattle.
- To generate pre- and post-vaccination farm site data on fertility (including abortion/birth rate) to assess the safety of vaccine.

For a sub-population of animals (approximately 11,000) collection of blood samples to confirm blood IFN-γ DIVA test validation using latent class analysis, unless completed as part of WPP2.

5 STUDY DESIGN

5.1 General
A randomised, blinded, placebo-controlled, multicentred clinical field efficacy study to be carried out in different geographical regions within England and Wales. Animals will be recruited onto the study at a ratio of 1:1, IVP:CP. Assessment will be made at the individual animal level.

5.2 Farms/Sites suitable for inclusion
One hundred (100) farm sites will be recruited; the farm sites will be representative of standard farming practices for the region. Dairy and beef farms will be recruited in an approximately equal split. Farms will be recruited in high bTB risk areas in England and Wales.
Prior to vaccination all animals at the farm site will be subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) testing. All animals which are negative will be considered suitable for the study. All animals which are positive will be despatched to slaughter prior to the study start and as per standard practice.

5.2.1 Farm site data

For farm sites which are dairy herds, pre- and post-vaccination farm site data on yield and milk composition (according to standard practice) will be collected. This will include three months prior to and three months post V1 and V2. The collection of this data will be via the farm sites' standard method of recording (e.g. National Milk Record).

For all farm sites, pre- and post-vaccination farm site data on fertility (including abortion/birth rate) will be collected.

5.3 Animals suitable for inclusion

Cattle of any breed, weight and gender above 15 days of age at vaccination will be suitable for the study. Pregnant and lactating animals should be included. All animals on the farm at the time point of vaccination, which were test negative via SICCT will be vaccinated.

Based on the average herd size in England and Wales, a minimum of twelve thousand, two hundred (12,200) animals will be recruited on to the study.

5.4 Study treatments and allocation

Animals will be recruited on to the study at a ratio of 1:1, IVP:CP.

Animals on a single farm site will be vaccinated at a single time point. Animals which are then either born on or are brought onto the farm site will be vaccinated at the next scheduled vaccination time point. In the interim between birth/arrival and vaccination, these unvaccinated animals will undergo study procedures e.g. blood sampling/PM (as applicable) as this data may provide background information on transmission rate of bTB on the farm site. These animals will effectively be ‘in-contact negative controls’ up until the time point of vaccination.

5.5 Study schedule

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Data Collection</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm Details</td>
<td>Animal Details</td>
</tr>
<tr>
<td>Pre-Vacc.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>0 (V1)</td>
<td>180</td>
<td>✓</td>
</tr>
<tr>
<td>360 (V2)</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>540</td>
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<tr>
<td>720 (V3)</td>
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<td>900</td>
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<td>1080 (V4)</td>
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<td>✓</td>
</tr>
<tr>
<td>1800</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

Notes: V1: Vaccination 1 (primary vaccination) V2-5: Re-vaccination, a. Pre-vaccination, b. Samples may also form part of the latent class analysis. Flexibility (± x days) will be determined in the final study protocol.

5.6 Treatment administration

The first injection of IVP/CP will be on Study Day 0. Re-vaccination will then take place on Study Days 360, 720, 1080 and 1440.

The IVP/CP will be administered at a dose rate of 0.5mL per animal per administration. This is the dose rate which, based on previous clinical studies, has been shown to be safe and effective for the control of bTB in cattle.
The IVP/CP will be administered subcutaneously into the neck region.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.7 **Clinical examinations**

Prior to each vaccination a clinical examination will be performed by a veterinarian on all animals to ensure that the animal is suitable for inclusion or for continuation in the study. At the end of the study a final clinical examination will be performed to ensure the animal has remained in good health and can be signed off the study (returning to the commercial herd).

The clinical examination will include an examination of the general health status of the study animals by observing the main body systems and any abnormalities will be recorded.

5.8 **Injection site observations**

For a sub-population of animals which are pregnant and lactating, injection site observations will be monitored daily for a period of at least 30 days post-vaccination.

Additional Schedule of Events (monitoring injection site observations in a sub-population of pregnant/lactating animals):

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injection Site Observation</td>
</tr>
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</tr>
<tr>
<td>1 - 30</td>
<td>✓</td>
</tr>
<tr>
<td>Pre-V2 (Day 359)</td>
<td>✓</td>
</tr>
<tr>
<td>361 - 390</td>
<td>✓</td>
</tr>
</tbody>
</table>

Injection site observations will include an assessment of erythema, heat and swelling.

5.9 **Post mortem examination**

A post mortem (PM) will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death.
- Animals which are despatched to slaughter as per standard farm practice (finished production) will undergo a routine PM.
- Animals which are positive to DIVA testing (i.e. considered to be bTB positive) will undergo an enhanced PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes, and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

The detailed method for enhanced PM and histopathological examination has been developed by APHA. This is considered to be suitable methodology for the conduct of these studies and should
be performed, at a minimum, for the 300 animals determined as positive and a corresponding number of bTB negative cattle. The remainder of the study animals should undergo PM using a method consistent with slaughterhouse inspection of reactor cattle.

5.10 Samples and analysis
Blood samples will be collected as per the study schedule for DIVA testing.

The blood sample collected on Study Day 360 will also be used to complete blood IFN-γ DIVA test validation using latent class analysis (if not completed as part of WPP2). A proportion of these samples (as advised by the project statistician) should be obtained from animals of minimum age (15 days).

5.10.1 Blood samples for monitoring herds post-vaccination (sub-population)

It would be option, for a sub-population of herds (as advised by the project statistician), to have follow-up blood samples collected post Study Day 1800; this will generate data on the transmission rate of bTB post vaccination. These blood samples will be collected on Study Day 1980, 2160, 2340 and 2520 (i.e. for a follow-up period of 2 years following the last vaccination).

5.11 Adverse events
Adverse Events will be recorded throughout the study period for the collection of safety data. Prior to farm recruitment onto the study, baseline data on the farms’ disease history will be collated and agreed with the farm sites’ own veterinarian e.g. background abortion rates. Adverse events will be reported as those outside of this baseline ‘normal’ for the farm site.

5.12 Summary of trial details required for ATC (taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)

**Nature and purpose of the test (objectives):** See section 4. Objectives.

**Pharmaceutical form:** Powder and solvent for suspension for injection.

**Target species:** *Bos taurus*

**Indications:** To be advised

**Estimated duration of trial:** initial period of 2 years with expected duration of 5-7 years after primary vaccination.

**Maximum no. of animals:**
- i. Treated (with the test product): minimum of 6,100
- ii. Positive controls: not applicable
- iii. Negative controls: not applicable
- iv. Placebo treated controls: minimum of 6,100

**Inclusion criteria:** see 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

**Exclusion criteria:** animals which are less than 15 days of age will not be included. Animals with a positive SICCT result prior to vaccination will not be included.

**Description of safety monitoring:** clinical examinations will be carried out on all animals prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be monitored for a period of at least 30 days post-vaccination, for a sub-population of study animals which are pregnant and lactating.

Blood samples will be collected as per 5.5 Study schedule for blood IFN-γ DIVA testing.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortem examination is described in 5.9.
Method of administration / dose rate / duration of administration:

i. Treated (with the test product): subcutaneously into the neck region, 0.5 mL per animal per administration. Re-vaccination will take place on Study Days 0, 365, 720, 1080, 1440, 1880.

ii. Positive controls: Not applicable

iii. Negative controls: Not applicable

iv. Placebo treated controls: subcutaneously into the neck region, 0.5 mL per animal per administration. Re-treatment will take place on Study Days 0, 365, 720, 1080, 1440, 1880.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end on Study Day 1800 (approximately 5 years post-V1) for the majority of the study animals.

The ‘sub-population’ involved in post vaccination blood sampling will remain on the study until Study Day 2520 (approximately 7 years post-V1).

If an interim analysis is possible, then the above study end point will be different to these stated.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (e.g. animals that are to be sold on).

In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.

It is anticipated that at the end of the study period animals will undergo an industry standard SICCT test. If animals are shown as positive, animals will then undergo a blood IFN-γ test and PM. If both results from the blood IFN-γ test and PM show the animal as being bTB negative, the farm site will have no restriction/further testing. If results of the blood IFN-γ test or PM show the animal as being bTB positive the farm will be on ‘restrictions’ as per current policy and undergo repeat testing at 60 day intervals. This procedure is outwith that listed in section 5.5.

7 ASSESSMENT OF EFFICACY

The percentage reduction in the number of reactors will be compared between vaccine and control groups.

The number and severity of lesions observed at PM will be compared between vaccine and control groups.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and
protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 12 WPP6.2 – FIELD EFFICACY AND SAFETY OF BCG VACCINE, PROPOSED PROTOCOL

Field Efficacy and Safety of BCG Vaccine When Used in Neonatal Cattle to Reduce Infection with *Mycobacterium bovis* (bTB)

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1 TITLE
Field Efficacy and Safety of BCG Vaccine When Used in Neonatal Cattle to Reduce Infection with *Mycobacterium bovis* (bTB).

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
The primary objective is to generate data on the efficacy of the vaccine in neonatal animals which are tested to be bTB negative pre-vaccination. This study will focus on generating data on the effect at the individual animal level.

The secondary objectives for the study are listed below:

- To generate data on the reduction of lung lesions for all animals at post mortem.
- To generate data associated with injection site observations for animals of the minimum age intended for vaccination. This data has not been collected in any previous studies and is specifically required by the regulators.
- Generate blood samples from animals of a minimum age for vaccination or analysis via differentiating infected from vaccinated animals (DIVA). This objective is intended to support the proposed minimum age through assessment of DIVA in neonates.

5 STUDY DESIGN

5.1 General
A randomised, blinded, placebo-controlled, multicentre clinical field efficacy study to be carried out in different geographical regions within England and Wales.

5.2 Farms/Sites suitable for inclusion
Animals will be recruited from source farm sites which have tested negative for bTB for a minimum of 5 years. Animals from dairy and beef farms will be recruited in an approximately equal number. Thirty (30) days post-vaccination animals will be transferred to grazing Approved Finishing Units (AFUs) in high bTB risk areas. Up to 10 AFUs may be used in the study.

5.3 Animals inclusion
Animals of any gender, weight or breed are suitable for inclusion in the study.

Prior to vaccination animals which are scheduled to be a minimum of 15 days of age at the time of vaccination will be subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) testing to confirm their negative status.

The minimum number of cattle to be recruited onto the study is to be advised by the project statistician during the development of the final study protocol.

5.4 Study treatments and allocation
Animals will be recruited onto the study at a ratio of 1:1, IVP:CP. Animals will be randomly allocated to treatment.
5.5 Study schedule

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Data Collection</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Animal Details</td>
</tr>
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<td>Pre-Vaccination</td>
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<td></td>
</tr>
<tr>
<td>180</td>
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<td></td>
</tr>
<tr>
<td>360 (V2)</td>
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<td></td>
</tr>
<tr>
<td>540</td>
<td></td>
<td></td>
</tr>
<tr>
<td>720</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: a Performed daily. b Transfer to AFU. Flexibility (± X days) will be determined in the final study protocol. SICCT required even if DIVA validation performed prior to study start as study will generated data for DIVA use in neonates.

5.6 Treatment administration

Vaccination of IVP/CP will be on Study Day 0 and Study Day 360.

The IVP/CP will be administered at a dose rate of 0.5mL per animal per administration. This is the dose rate which has been shown to be safe and effective against the control of bTB in cattle, based on previous clinical studies.

The IVP/CP will be administered by subcutaneous injection into the neck region. The vaccine administration will typically be to the right side.

There is an assumed zero day withdrawal period for meat and offal.

5.7 Clinical examinations

Prior to each vaccination a veterinarian will undertake a clinical examination of each animal to ensure that the animal is suitable for inclusion or for continuation in the study. At the end of the study a final clinical examination will be performed to verify the animal has remained in good health throughout the study period.

The clinical examination will comprise an assessment of the general health status of the study animals and any abnormalities will be recorded.

5.8 Injection site observations

Injection site observations will be monitored for a period of 30 days post-vaccination or possibly longer if the injection site reaction persists. Observations should be completed and reactions resolved to normal prior to transfer to the AFU to help maintain blinding of AFU staff.

The following will be observed: erythema, heat and swelling.

5.9 Post mortem examination

A PM will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death.
- Animals which are despatched to slaughter as per standard farm practice (finished production) will undergo a routine PM.
- Animals which are positive to DIVA testing (i.e. considered to be bTB positive) will undergo a routine PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes, and in other organs.
Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

5.10 Samples and analysis

Blood samples will be collected as per the study schedule for blood IFN-γ DIVA testing to confirm the bTB status of each animal.

5.11 Adverse events

Adverse Events will be recorded throughout the study period for the collection of safety data.

5.12 Summary of trial details required for ATC

(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications: To be advised

Estimated duration of trial: 2 years.

Maximum no. of animals:

i. Treated (with the test product): number to be advised by project statistician
ii. Positive controls: not applicable
iii. Negative controls: not applicable
iv. Placebo treated controls: number to be advised by project statistician

Inclusion criteria: see 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: animals which are less than 15 days of age will not be included. Animals with a positive SICCT result prior to vaccination will not be included.

Description of safety monitoring: clinical examinations will be carried out on all animals prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be monitored for a period of 30 days post-vaccination.

Blood samples will be collected as per 5.5 Study schedule for DIVA testing.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortem examination is described in 5.9.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Injection site observations, 5.10 Samples and analysis.)

Method of administration / dose rate/ duration of administration:

i. Treated (with the test product): subcutaneously into the neck region, 0.5mL per animal per administration. Re-vaccination will take place on Study Days 365.
ii. Positive controls: Not applicable
iii. Negative controls: Not applicable
iv. Placebo treated controls: subcutaneously into the neck region, 0.5mL per animal per administration. Re-treatment will take place on Study Days 0 and 360.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will end approximately 24 months post vaccination. A decision to complete the study at Study Day 360 (prior to administration of V2) may be made depending upon the level of challenge within the AFU.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made. All animals will be sent for PM to generate lung lesion data.

Derogations are not applicable as the end point for this study design is PM. It should be noted that no other industry standard testing for bTB should be permitted during the study period.

7 ASSESSMENT OF EFFICACY

The percentage reduction in the number of reactors will be compared between vaccine and control groups.

The number and severity of lesions observed at PM will be compared between vaccine and control groups.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.

During study procedures handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.
APPENDIX 13 WPP6.3 – FIELD EFFICACY AND SAFETY OF BCG VACCINE, PROPOSED PROTOCOL

Field Efficacy and Safety of BCG Vaccine When Used in Cattle Herds to Reduce Infection with *Mycobacterium bovis* (bTB)

WPP 6, Design 3
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1 TITLE
Field Efficacy and Safety of BCG Vaccine When Used in Cattle Herds to Reduce Infection with *Mycobacterium bovis* (bTB).

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
The primary objective is to generate data at the farm level in support of a Marketing Authorisation (MA) application for BCG vaccine and provide data to confirm that vaccination has an effect on the incidence of bTB.

The secondary objective is to inform on transmission rate within farms.

5 STUDY DESIGN

5.1 General
A randomised, blinded, placebo-controlled, multicentre clinical field efficacy study to be carried out in different geographical regions within England and Wales. Farms will be recruited onto the study at a ratio of 1:1, IVP:CP with all animals on a single farm site either receiving the IVP or CP. Assessment will be made on a ‘per farm’ basis to determine the overall impact of incidence of bTB post vaccine use, as well as at the individual animal level (generation of efficacy and of some safety data required for an MA). This study design mirrors how the vaccine would be used in the field post grant of a MA.

5.2 Farms suitable for inclusion
Farm sites will be recruited from high risk bTB areas and will be ‘working’ dairy or beef farms. Farms will be ‘paired/matched’ with 100% vaccination with IVP on one farm and 100% vaccination with CP on the second farm. The paired farms will be matched as equally as possible based on standardised factors which may include herd type, size, bTB history and location.

Prior to vaccination all animals at the farm site will be subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) testing (see Section 5.5). All animals which are negative will be considered suitable for the study. All animals which are positive will be despatched to slaughter prior to the study start as per standard practice.

5.3 Animals suitable for inclusion
All animals on the farm at the time of Study Day 0 which are test negative for bTB will be suitable for vaccination. Further to this animals should be in overall good general health to be considered for the study.

Sample size is stated in the report issued by Cambridge University (see Appendix 3).

5.4 Study treatments and allocation
Farms will be recruited onto the study at a ratio of 1:1, IVP:CP and will be randomly allocated to treatment.
5.5 Study schedule

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Data Collection</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm Details</td>
<td>Animal Details</td>
</tr>
<tr>
<td>Pre-vaccination</td>
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</tr>
<tr>
<td>0 (V1)</td>
<td>❌</td>
<td>❌</td>
</tr>
<tr>
<td>180</td>
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<td>❌</td>
</tr>
<tr>
<td>360 (V2)</td>
<td>❌</td>
<td>❌</td>
</tr>
<tr>
<td>540</td>
<td>❌</td>
<td>❌</td>
</tr>
<tr>
<td>720</td>
<td>❌</td>
<td>❌</td>
</tr>
</tbody>
</table>

Notes: V1: Vaccination 1 (primary vaccination). V2: Re-vaccination. Flexibility (± X days) will be determined in the final study protocol.

At Study Day 720, farms could then undergo a repeat vaccination (i.e. year 3). This will be determined based on the bTB challenge rate at the farm site.

Alternatively blood samples may be collected at intervals post Study Day 720 (e.g. at approximately 6 monthly intervals) to monitor the farm for potential breakdowns.

Costings associated with this study design are based on the above study schedule and do not take into consideration additional samplings or vaccination.

It is a currently unclear whether the use of the SICCT pre-vaccination will affect the DIVA result from the sample collected on Study Day 0. It is anticipated that studies performed as part of WPP2 will inform whether the use of a pre-vaccination SICCT test is possible. If it is determined that SICCT impacts significantly the DIVA result, then SICCT will not be used prior to vaccination. In this instance the herd history will be the default for determining the suitability of the site for inclusion.

An additional consideration noted during the mathematical modelling would be to perform farm site vaccination every 6 months. For any animals brought or born onto the site (provided that such animals met the recruitment criteria). It is thought that this would increase the powering of the study. However, it should be noted that such a study design would increase costs and possibly complicate management at the site i.e. one animal could risk having two vaccinations within a 12 month period. Only annual vaccination has been described within this study design and its associated costings.

5.6 Treatment administration

Vaccination of IVP/CP will be on Study Day 0 (V1) and on Study Day 360 (V2).

The IVP/CP will be administered at a dose rate of 0.5mL per animal per administration. This is the dose rate which has been shown to be safe and effective for the control of bTB in cattle, based on previous clinical studies.

The IVP/CP will be administered by subcutaneous injection into the neck region. The vaccine administration will be to the right hand side on V1 and on the left hand side on V2.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.7 Clinical examinations

A veterinarian will undertake a clinical examination of each animal to ensure that the animal is suitable for inclusion (pre-V1) or continuation in the study (pre-V2). Clinical examinations will also be performed at the end of each study phase.

The clinical examination will include an examination of the general health status of the study animals, any abnormalities will be recorded.

5.8 Injection site observations

Injection site observations will not form part of the clinical trial as all animals on the same farm site will receive the same product and this would potentially generate biased data. It is therefore suggested that the injection site observations are performed as part of other studies e.g. WPP2.

---

SHORT TITLE: Feasibility study into testing and validating cattle BCG vaccine and DIVA

Final

Date 05 March 15

– Strictly Confidential –
5.9 Post mortem examination

A post mortem will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a PM in order to identify the cause of death.
- Animals which are despatched to slaughter as per standard farm practice (finished production) will undergo a routine PM.
- Animals which are positive to DIVA testing (i.e. considered to be bTB positive) will undergo a routine PM.

At PM the lungs and lymph nodes will be examined for signs of bTB infection. Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes, and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5cm to 1.0cm intervals.

Bacterial culture of swab samples from target organs/sites will be performed on the first 300 samples (or as many as are available) from animals with lesions at PM. Each time that a bacterial culture sample is taken from a suspect positive animal (with lesions) a comparable sample will be taken from a negative (without lesions) animal killed with PM on the same occasion. Tissues collected at PM for microscopic analysis will be fixed by immersion in 10% neutral buffered formalin. If present, microscopic granulomas will be classified. Samples for bacteriological examination will be collected into sealed, sterile containers using standardised techniques to minimise contamination risks.

5.10 Samples and analysis

Blood samples will be collected, as per the study schedule, for blood IFN-γ DIVA testing to confirm the bTB status of each animal.

5.11 Adverse events

Adverse Events will be recorded throughout the study period for the collection of safety data. Prior to farm recruitment onto the study, baseline data on the farms’ disease history will be collated and agreed with the farm sites’ own veterinarian e.g. background abortion rates. Adverse events will be reported as those outside of this baseline ‘normal’ for the farm site.

5.12 Summary of trial details required for ATC

(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)

**Nature and purpose of the test (objectives):** See section 4. Objectives.

**Pharmaceutical form:** Powder and solvent for suspension for injection.

**Target species:** Bos taurus

**Indications:** To be advised

**Estimated duration of trial:** 2 years.

**Maximum no. of animals:**
- i. Treated (with the test product): number as stated in Appendix 3 – modelling to support cattle BCG field trials
- ii. Positive controls: not applicable
- iii. Negative controls: not applicable
- iv. Placebo treated controls: number as stated in Appendix 3

**Inclusion criteria:** see 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.
Exclusion criteria: animals which are less than 15 days of age will not be included. Animals with a positive SICCT result prior to vaccination will not be included.

Description of safety monitoring: clinical examinations will be carried out on all animals prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Blood samples will be collected as per 5.5 Study schedule for DIVA testing.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post-mortem examination is described in 5.9.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Injection site observations, 5.10 Samples and analysis.)

Method of administration / dose rate/ duration of administration:

i. Treated (with the test product): subcutaneously into the neck region, 0.5mL per animal per administration. Re-vaccination will take place on Study Days 0 and 360.

ii. Positive controls: Not applicable

iii. Negative controls: Not applicable

iv. Placebo treated controls: subcutaneously into the neck region, 0.5mL per animal per administration. Re-treatment will take place on Study Days 0 and 360.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

At a minimum this will be Study Day 720.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (e.g. animals that are to be sold on).

In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.

It is anticipated that at the end of the study period animals will undergo an industry standard SICCT test. If animals are shown as positive, animals will then undergo a blood IFN-γ test and PM. If both results from the blood IFN-γ test and PM show the animal as being bTB negative, the farm site will have no restriction/further testing. If results of the blood IFN-γ test or PM show the animal as being bTB positive the farm will be on ‘restrictions’ as per current policy and undergo repeat testing at 60 day intervals. This procedure is outwith that listed in section 5.5.

7 ASSESSMENT OF EFFICACY

The study is designed to assess the indirect measures of efficacy.

The percentage reduction in the number of reactors will be compared between vaccine and control groups.

The number and severity of lesions observed at PM will be compared between vaccine and control groups.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.
From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
Field Safety of BCG Vaccine When Administered to Dairy Cattle (Generation of Milk Samples)

WPP 8, Design 1
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1 TITLE
Field Safety of BCG Vaccine When Administered to Dairy Cattle (Generation of Milk Samples).

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000). Milk sample analysis will be performed outside the scope of this study and will be performed according to GLP (see WPP9 and WPP10).

4 OBJECTIVES
The primary objective is to generate milk samples for analysis under a separate GLP study plan in respect of post-vaccination bacterial dissemination into milk.

The secondary objectives for the study are listed below:
- To generate data associated with injection site observations and temperature for this sub-population of animals i.e. lactating animals. This data has not been collected in any previous studies and is specifically requested by the regulators unless covered by data generated as part of WPP6.
- To generate pre- and post-vaccination farm site data on yield and milk composition (according to standard practice) to assess the safety of vaccine administration in dairy cattle in respect of the potential impact of vaccination on milk quality and quantity.

5 STUDY DESIGN
5.1 General
The field safety study will be a randomised, blinded, placebo controlled, multicentre trial carried out in different geographical regions within the United Kingdom.

5.2 Farms/Sites suitable for inclusion
Working dairy farms which are representative of standard farming practice for the region, located in low risk areas in order to ensure that data generated relates to the vaccine only. Only farms with a documented herd history of being bTB negative for the previous 10 years will be included in the study.

A minimum of two sites will be included in order to capture data from a range of breeds, and a range of milk compositions.

5.2.1 Farm Site Data
Pre-vaccination farm site data on milk yield and milk composition will be collected. This will include data for three months prior to study. The collection of this data will be via the farm site’s standard method of recording (e.g. National Milk Record).

5.3 Animals suitable for inclusion
Lactating cattle of any age and weight will be suitable for inclusion. Approximately 120 cows will be recruited on Study Day 0, 20 cows of each parity 0-5 (‘0’ = heifer). The total number of animals should include an approximately 5% overage to allow for drop out between V1 and V2 (re-vaccination, see 5.5).
Animals included should be at least 21 days post calving prior to vaccination on Study Day 0.

Prior to the study all cattle at the farm site will have been subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) routine industry testing to confirm the negative status of the farm site; this should have been performed within 12 months of the start of the study.

To positively confirm that the animals selected for study have remained bTB negative since the last testing at the farm site, animals will have an additional SICCT performed prior to each vaccination.

Animals will be scheduled for revaccination on Study Day 360 (V2). Animals which have remained in overall good general health and have calved prior to V2 will continue in the study and receive re-vaccination.

### 5.4 Study treatments and allocation

Animals will be recruited on to the study at a ratio of 1:1, IVP:CP. Animals will be assigned to treatment group using a Random Treatment Allocation Plan (RTAP), with parity as a blocking factor. All animals at a farm site will receive vaccination at a single time point.

Consideration should be given as to whether a placebo is used or whether animals allocated to the CP group are left unvaccinated as ‘in contact controls’. The benefits of using a placebo would be to collect the injection site observation data for lactating cattle within this WPP and not as part of WPP6. However, as previously mentioned, it is current understanding that the use of a placebo may require a project licence under the A(SP)A. Therefore it may be advisable, in the interest of saving time, to not use a placebo. The following sections include the use of a placebo as the “gold standard” and to generate additional safety data prior to conducting the pivotal field study.

### 5.5 Study schedule

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Notes: V1: Vaccination 1 (primary vaccination) V2: Re-vaccination. a. Blood Sample or SICCT (if study performed prior to DIVA validation, SICCT to be used. If performed post DIVA validation, blood sample collected). b. Pre-vaccination. Flexibility (± x days) will be determined in the final study protocol. h =hours.
5.6 Treatment administration
The first injection of IVP/CP will be administered on Study Day 0. Re-vaccination will then take place on Study Day 360.

The IVP/CP will be administered at a dose rate of 0.5mL per animal, per administration. This is the dose rate which has been shown to be safe and effective for the control of bTB in cattle, based on previous clinical studies.

The IVP/CP will be administered subcutaneously into the neck region. The vaccine administration will be to the right hand side on V1 and on the left hand side on V2.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.7 Clinical examinations
At the time of inclusion (Study Day -7) and prior to each vaccination a clinical examination will be performed by a veterinarian on all animals to ensure that the animal is suitable for inclusion or for continuation in the study. At the end of the study a final clinical examination will be performed to verify that the animal has remained in good health and can be signed off the study (returning to the commercial herd).

The clinical examination will include an examination of the general health status of the study animals by observing the main body systems (including an assessment of the animal’s suitability for milk sampling) and any abnormalities will be recorded.

5.7.1 Temperatures
Temperature (°C) to at least one decimal place will be measured in all study animals according to the schedule in Section 5.5. Temperature will be obtained using calibrated, certified thermometers. If temperatures are either abnormally low (<37.7°C) or abnormally high (≥ 39°C) these will be reported as adverse events (see 5.11).

5.8 Injection site observations
Injection site observations will be performed on all animals according to the schedule in Section 5.5.

Observations will include an assessment of erythema, heat and swelling.

5.9 Post mortem examination
A post mortem (PM) will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a full PM in order to identify the cause of death.
- Animals which are despatched to slaughter for welfare reasons will undergo a routine PM.

5.10 Samples and analysis

5.10.1 Blood samples
Blood samples will be collected as per the study schedule (Section 5.5) for blood IFN-γ DIVA testing. If the analytical method for blood IFN-γ DIVA test has not been validated prior to study, an industry standard SICCT test will be used to confirm that animals are negative prior to vaccination. If it is not possible to confirm the animal's negative status at the end of the study period, derogations will be required.

5.10.2 Milk dissemination
Milk samples will be collected aseptically according to the schedule in Section 5.5. Samples will be collected from each animal at each time point. Each sample will consist of a pooled sample representative of all udder quarters.
Milk samples will be allocated a cryptic alpha-numeric sample code used as the sole identifier in order to maintain blinding at the analytical site.

The procedure for milk sample collection and analysis for bacterial dissemination is covered in WPP9.

5.10.3 Milk Samples Composition

Two composite milk samples (primary and retained) will be taken from each cow at the time points specified in Section 5.5.

Samples will be collected from the milk jar/line or similar to obtain samples that are representative of the entire milking time point/all available quarters for the individual cow.

Primary samples will be sent to a locally designated milk quality laboratory for analysis. Retained samples will be stored frozen at the farm site/Contract Research Organisation until the analysis of the primary sample has been completed. Milk samples will be analysed for the following components: solids, fat, protein, lactose, Somatic Cell Counts.

5.11 Adverse events

Adverse events will be reported to the Monitor on an agreed timescale and to the Sponsor if considered serious.

Adverse event recording and reporting will continue until the end of the study.

5.12 Summary of trial details required for ATC

(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)


Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications: To be advised

Estimated duration of trial: 381 days

Maximum no. of animals:
  i. Treated (with the test product): 120 cows
  ii. Positive controls: not applicable
  iii. Negative controls: not applicable
  iv. Placebo treated controls: 120 cows

Inclusion criteria: lactating cattle of any age or weight and at least 21 days post-calving prior to vaccination. See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: Animals with a positive SICCT result prior to vaccination will be excluded.

Description of safety monitoring: clinical examinations will be carried out on all animals prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be performed as per 5.5 Study Schedule for all animals.

Blood samples will be collected as per 5.5 Study schedule for DIVA testing (if validated).

Milk samples will be collected as per 5.5 Study schedule, 5.10.2 Milk dissemination and 5.10.3 Milk samples composition.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post-mortem examination is described in 5.9.
Method of administration / dose rate / duration of administration:

i. Treated (with the test product): subcutaneously into the neck region, 0.5mL per animal per administration.

ii. Positive controls: Not applicable

iii. Negative controls: Not applicable

iv. Placebo treated controls: subcutaneously into the neck region, 0.5mL per animal per administration.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will continue until Study Day 381.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (e.g. animals that are to be sold on).

In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.

It is anticipated that at the end of the study period animals will undergo an industry standard SICCT test. If animals are shown as positive, animals will then undergo a blood IFN-γ test and PM. If both results from the blood IFN-γ test and PM show the animal as being bTB negative, the farm site will have no restriction/further testing. If results of the blood IFN-γ test or PM show the animal as being bTB positive the farm will be on ‘restrictions’ as per current policy and undergo repeat testing at 60 day intervals. This procedure is outwith that listed in section 5.5.

7 ASSESSMENT OF SAFETY

The results of the study will be compared between the IVP and CP vaccinated animals.

It has been suggested that the frequency of veterinarian visits relating to AEs and administration of concomitant medications can be compared between the groups to demonstrate any additional benefits of vaccination.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.
Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
Field Safety of BCG Vaccine When Administered to Cattle (Generation of Nasal Samples)

WPP 8, Design 2
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1 TITLE
Field safety of BCG Vaccine When Administered to Cattle (Generation of Nasal Samples).

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guidance on Good Clinical Practices (CVMP approved July 2000). Analysis of nasal swabs will be performed outside the scope of this study and will be performed according to GLP (see WPP9 and WPP10).

4 OBJECTIVES
The primary objective is to generate nasal swab samples for analysis under separate GLP study plans in respect of post-vaccination bacterial dissemination into nasal secretions.

The secondary objectives for the study are listed below:

- To generate data associated with injection site observations and temperature for this sub-population of animals. (This data has not been collected in any previous studies and is specifically requested by the regulators unless covered by data generated as part of WPP6.)

5 STUDY DESIGN

5.1 General
The field safety study will be a randomised, blinded, placebo controlled, multicentre trial carried out in different geographical regions within the United Kingdom.

5.2 Farms suitable for inclusion
Working farms (beef or dairy) which are representative of standard farming practice for the region, located in low risk areas in order to ensure that data generated relates to the vaccine only. Only farms with a herd history of being bTB negative for the previous 10 years will be included in the study.

A minimum of four sites will be included in order to capture data from a range of breeds.

5.3 Animals suitable for inclusion
Cattle of any breed and weight will be suitable for inclusion. Animals should be over the age of 6 months at the time of inclusion. Approximately 20 cows will be recruited per farm on Study Day 0 (therefore a minimum of 80 cows recruited). The total number of animals should include an approximate 5% overage to allow for drop out between V1 and V2 (re-vaccination, see Section 5.5).

Prior to the study all cattle at the farm site will have been subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) routine industry testing to confirm the negative status of the farm site; this should have been performed within 12 months of the start of the study.

To positively confirm that the animals selected for the study have remained bTB negative since the last testing at the farm site, animals will have an additional SICCT performed prior to each vaccination.

Animals will be scheduled for revaccination on Study Day 360 (V2).
5.4 Study treatments and allocation

Animals will be recruited onto the study at a ratio of 1:1 IVP:CP. Animals will be assigned to treatment group using a Random Treatment Allocation Plan (RTAP). All animals at a farm site will receive vaccination at a single time point.

Consideration should be given as to whether a placebo is used or whether animals allocated to the CP group are left unvaccinated as “in contact controls”. The benefits of using a placebo would be to collect the injection site observation data for lactating animals within this WPP and not as part of WPP6. However, as previously mentioned, it is current understanding that the use of a placebo may require a project licence under A(SP)A. Therefore it may be advisable, in the interest of saving time, to not use a placebo. The following sections include the use of a placebo as the “gold standard” and to generate additional safety data prior to conducting the pivotal field study.

5.5 Study schedule

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Notes: V1: Vaccination 1 (primary vaccination) V2: Re-vaccination. a. Blood Sample or SICCT (if study performed prior to DIVA validation, SICCT to be used. If performed post DIVA validation, blood sample collected). b. Pre-vaccination. Flexibility (± x days) will be determined in the final study protocol. h =hours.

5.6 Treatment administration

The first injection of IVP/CP will be administered on Study Day 0. Re-vaccination will then take place on Study Day 360.

The IVP/CP will be administered at a dose rate of 0.5mL per animal, per administration. This is the dose rate which has been shown to be safe and effective for the control of bTB in cattle, based on previous clinical studies.

The IVP/CP will be administered subcutaneously into the neck region. The vaccine administration will be to the right hand side on V1 and on the left hand side on V2.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.
5.7 Clinical examination
At the time point of inclusion (Study Day -7) and prior to each vaccination, a clinical examination will be performed by a veterinarian on all animals to ensure that they are suitable for inclusion or for continuation in the study. At the end of the study a final clinical examination will be performed to verify that the animal has remained in good health and can be signed off the study (returning to the commercial herd).

The clinical examination will include an assessment of the general health status of the study animals, any abnormalities will be recorded.

5.7.1 Temperatures
Temperature (°C) to at least one decimal place will be measured in all study animals according to the schedule in Section 5.5. Temperature will be obtained using calibrated, certified thermometers. If temperatures are either abnormally low (<37.7 °C) or abnormally high (≥39.9°C) these will be recorded as adverse events.

5.8 Injection site observations
Injection site observations will be performed on all animals according to the schedule in Section 5.5.

Observations will include an assessment of erythema, heat and swelling.

5.9 Post mortem examination
A post mortem (PM) will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a full PM in order to identify the cause of death.
- Animals which are despatched to slaughter for welfare reasons will undergo a routine PM.

5.10 Samples and analysis

5.10.1 Blood samples
Blood samples will be collected as per the study schedule (Section 5.5) for blood IFN-γ DIVA testing. If the analytical method for DIVA has not been validated prior to study, an industry standard SICCT test will be used to confirm that animals are negative prior to vaccination. If it is not possible to confirm the animal's negative status at the end of the study period, derogations will be required.

5.10.2 Nasal secretions, bacterial dissemination
Nasal swab samples will be collected aseptically according to the schedule in Section 5.5. Samples will be collected from each animal at each time point.

Nasal swabs will be allocated a cryptic alpha-numeric sample code used as the sole identifier in order to maintain blinding at the analytical site.

The procedure for obtaining nasal swabs and analysis for bacterial dissemination is covered in WPP10.

5.11 Adverse events
Adverse events will be reported to the Monitor on an agreed timescale and to the Sponsor if considered serious.

Adverse event recording and reporting will continue until the end of the study.

5.12 Summary of trial details required for ATC
(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’, VMD/L4/Authorisations/0321/C - #713143)

**Pharmaceutical form:** Powder and solvent for suspension for injection.

**Target species:** *Bos taurus*

**Indications:** To be advised

**Estimated duration of trial:** 381 days

**Maximum no. of animals:**
- i. Treated (with the test product): 40
- ii. Positive controls: not applicable
- iii. Negative controls: not applicable
- iv. Placebo treated controls: 40

**Inclusion criteria:** cattle of any breed or weight and at least 6 months old at the time of inclusion. See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

**Exclusion criteria:** Animals with a positive SICCT result prior to vaccination will be excluded.

**Description of safety monitoring:** clinical examinations will be carried out on all animals at the time of inclusion, prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be performed as per 5.5 Study Schedule for all animals.

Blood samples will be collected as per 5.5 Study schedule for DIVA testing (if validated).

Nasal swab samples will be collected as per 5.5 Study schedule and 5.10 Samples and analysis.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post-mortem examination is described in 5.9.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Injection site observations, 5.10 Samples and analysis.)

**Method of administration / dose rate / duration of administration:**
- i. Treated (with the test product): subcutaneously into the neck region, 0.5mL per animal per administration.
- ii. Positive controls: Not applicable
- iii. Negative controls: Not applicable
- iv. Placebo treated controls: subcutaneously into the neck region, 0.5mL per animal per administration.

**Disposal of unused product and empty containers:** to be advised.

**Disposal or fate of test food producing animals (not intended to enter the human food chain for food):** see 6.1 Fate of study animals.

### 6 STUDY END

The study will continue until Study Day 381.

#### 6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made. 

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (*e.g.* animals that are to be sold on).

In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.
It is anticipated that at the end of the study period animals will undergo an industry standard SICCT test. If animals are shown as positive, animals will then undergo a blood IFN-γ test and PM. If both results from the blood IFN-γ test and PM show the animal as being bTB negative, the farm site will have no restriction/further testing. If results of the blood IFN-γ test or PM show the animal as being bTB positive the farm will be on ‘restrictions’ as per current policy and undergo repeat testing at 60 day intervals. This procedure is outwith that listed in section 5.5.

7 ASSESSMENT OF SAFETY

The results of the study will be compared between the IVP and CP vaccinated animals.

It has been suggested that the frequency of veterinarian visits relating to AEs and administration of concomitant medications can be compared between the groups to demonstrate any additional benefits of vaccination.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 16 WPP8.3 - FIELD SAFETY OF BCG VACCINE, PROPOSED PROTOCOL

Project No.: PN1567

Field Safety of BCG Vaccine When Administered to Dairy Cattle
(Generation of Milk and Nasal Samples)

WPP 8, Design 3
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1 TITLE
Field safety of BCG Vaccine When Administered to Dairy Cattle. (Generation of Milk and Nasal Samples)

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
This study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guidance on Good Clinical Practices (CVMP approved July 2000). Analysis of milk samples and nasal swabs will be performed outside the scope of this study and will be performed according to GLP (see WPP9 and WPP10).

4 OBJECTIVES
The primary objective is to generate milk samples and nasal swabs for analysis under separate GLP study plans in respect of post-vaccination bacterial dissemination into milk and nasal secretions.

The secondary objectives for the study are listed below:

- To generate data associated with injection site observations and temperature for this sub-population of animals. (This data has not been collected in any previous studies and is specifically requested by the regulators unless covered by data generated as part of WPP6.)
- To generate pre and post-vaccination farm site data on yield and milk composition (according to standard practice) to assess the safety of vaccine administration in dairy cattle in respect of the potential impact of vaccination on milk quality and quantity.

5 STUDY DESIGN

5.1 General
The field safety study will be a randomised, blinded, placebo controlled, multicentre trial carried out in different geographical regions within the United Kingdom.

5.2 Farms/Sites suitable for inclusion
Working dairy farms which are representative of standard farming practice for the region, located in low risk areas in order to ensure that data generated relates to the vaccine only. Only farms with a herd history of being bTB negative for the previous 10 years will be included in the study.

5.2.1 Farm site data
Pre-vaccination farm site data on milk yield and milk composition will be collected. This will include data for three months prior to study. The collection of this data will be via the farm site’s standard method of recording (e.g. National Milk Record).

More than two sites will be included in order to capture data from a range of breeds and a range of milk compositions.

5.3 Animals suitable for inclusion
Lactating cattle of any age and weight will be suitable for inclusion. Approximately 120 cows will be recruited on Study Day 0, about 20 cows of each parity 0-5 (‘0’ = heifer). The total number of animals should include an approximately 5% overage to allow for drop out between V1 and V2.
Animals included should be at least 21 days post calving prior to vaccination on Study Day 0.

Prior to the study all cattle at the farm site will have been subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) routine industry testing to confirm the negative status of the farm site; this should have been performed within 12 months of the start of the study.

To positively confirm that the animals selected for the study have remained bTB negative since the last testing at the farm site, animals will have an additional SICCT performed prior to each vaccination.

Animals will be scheduled for revaccination on Study Day 360 (V2). Animals which have remained in overall good general health and have calved prior to V2 will continue in the study and receive re-vaccination.

5.4 Study treatments and allocation

Animals will be recruited onto the study at a ratio of 1:1 IVP:CP. Animals will be assigned to treatment group using a Random Treatment Allocation Plan (RTAP). All animals at a farm site will receive vaccination at a single time point.

Consideration should be given as to whether a placebo is used or whether animals allocated to the CP group are left unvaccinated as “in contact controls”. The benefits of using a placebo would be to collect the injection site observation data for lactating animals within this WPP and not as part of WPP6. However, as previously mentioned, it is current understanding that the use of a placebo may require a project licence under (A(SP)A). Therefore it may be advisable, in the interest of saving time, to not use a placebo. The following sections include the use of a placebo as the “gold standard” and to generate additional safety data prior to conducting the pivotal field study.
5.5 Study schedule

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Notes: V1: Vaccination 1 (primary vaccination) V2: Re-vaccination, a. Blood Sample or SICCT (if study performed prior to DIVA validation, SICCT to be used. If performed post DIVA validation, blood sample collected). b. Pre-vaccination. Flexibility (± x days) will be determined in the final study protocol. h = hours.

5.6 Treatment administration

The first injection of IVP/CP will be administered on Study Day 0. Re-vaccination will then take place on Study Day 360.

The IVP/CP will be administered at a dose rate of 0.5mL per animal, per administration. This is the dose rate which has been shown to be safe and effective for the control of bTB in cattle, based on previous clinical studies.

The IVP/CP will be administered subcutaneously into the neck region. The vaccine administration will be to the right hand side on V1 and on the left hand side on V2.

There is an assumed zero hour/day withdrawal period for milk, meat and offal.

5.7 Clinical examination

At the time point of inclusion (Study Day -7) and prior to each vaccination, a clinical examination will be performed by a veterinarian on all animals to ensure that the animal is suitable for inclusion or for continuation in the study. At the end of the study a final clinical examination will be performed to ensure the animal has remained in good health and can be signed off the study (returning to the commercial herd).

The clinical examination will include an examination of the general health status of the study animals, (including an assessment of the animal’s suitability for milk sampling); any abnormalities will be recorded.

5.7.1 Temperatures

Temperature (°C) to at least one decimal place will be measured in all study animals according to the schedule in Section 5.5. Temperature will be obtained using calibrated,
certified thermometers. If temperatures are either abnormally low (<37.7°C) or abnormally high (≥39.9°C) these will be recorded as adverse events.

5.8 Injection site observations

Injection site observations will be performed on all animals according to the schedule in Section 5.5.

Observations will include an assessment of erythema, heat and swelling.

5.9 Post mortem examination

A post mortem (PM) will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a full PM in order to identify the cause of death.
- Animals which are despatched to slaughter for welfare reasons will undergo a routine PM.

5.10 Samples and analysis

5.10.1 Blood samples

Blood samples will be collected as per the study schedule (Section 5.5) for blood IFN-γ DIVA testing. If the analytical method for DIVA has not been validated prior to study, an industry standard SICCT test will be used to confirm that animals are negative prior to vaccination. If it is not possible to confirm the animal's negative status at the end of the study period, derogations will be required.

5.10.2 Milk dissemination

Milk samples will be collected aseptically according to the schedule in Section 5.5. Samples will be collected from each animal at each time point. Each sample will consist of a pooled sample representative of all udder quarters.

Milk samples will be allocated a cryptic alpha-numeric sample code used as the sole identifier in order to maintain blinding at the analytical site.

The procedure for milk sample collection and analysis for bacterial dissemination is covered in WPP9.

5.10.3 Milk sample composition

Two composite milk samples (primary and retained) will be taken from each cow at the time points specified in Section 5.5

Samples will be collected from the milk jar/line or similar to obtain samples that are representative of the entire milking time point/all available quarters for the individual cow. Primary samples will be sent to a locally designated milk quality laboratory for analysis. Retained samples will be stored frozen at the farm site/Contract Research Organisation until the analysis of the primary sample has been completed. Milk samples will be analysed for the following components: solids, fat, protein, lactose, Somatic Cell Counts.

5.10.4 Nasal secretions dissemination

Nasal swab samples will be collected aseptically according to the schedule in Section 5.5. Samples will be collected from each animal at each time point.

Nasal swabs will be allocated a cryptic alpha-numeric sample code used as the sole identifier in order to maintain blinding at the analytical site.

The procedure for obtaining nasal swabs and analysis for bacterial dissemination is covered in WPP10.
5.11 Adverse events

Adverse events will be reported to the Monitor on an agreed timescale and to the Sponsor if considered serious.

Adverse event recording and reporting will continue until the end of the study.

5.12 Summary of trial details required for ATC

(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)

Nature and purpose of the test (objectives): See section 4, Objectives.

Pharmaceutical form: Powder and solvent for suspension for injection.

Target species: Bos taurus

Indications: To be advised

Estimated duration of trial: 381 days

Maximum no. of animals:

i. Treated (with the test product): 120 cows
ii. Positive controls: not applicable
iii. Negative controls: not applicable
iv. Placebo treated controls: 120 cows

Inclusion criteria: lactating cattle of any age or weight. See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: Animals with a positive SICCT result prior to vaccination will be excluded.

Description of safety monitoring: clinical examinations will be carried out on all animals at the time of inclusion, prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Injection site observations will include an assessment of erythema, heat and swelling and will be performed as per 5.5 Study schedule for all animals.

Blood samples will be collected as per 5.5 Study schedule for DIVA testing (if validated).

Milk samples will be collected as per 5.5 Study schedule, 5.10.2 Milk dissemination and 5.10.3 Milk sample composition.

Nasal swab samples will be collected as per 5.5 Study schedule and 5.10.4 Nasal secretions dissemination.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post-mortem examination is described in 5.9.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.8 Injection site observations, 5.10 Samples and analysis.)

Method of administration / dose rate/ duration of administration:

i. Treated (with the test product): subcutaneously into the neck region, 0.5mL per animal per administration.
ii. Positive controls: Not applicable
iii. Negative controls: Not applicable
iv. Placebo treated controls: subcutaneously into the neck region, 0.5mL per animal per administration.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.
6 STUDY END

The study will continue until Study Day 381.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

It should be ensured that details of the appropriate withdrawal period accompany any treated animals that are not intended to remain at the farm site at the end of the study (e.g. animals that are to be sold on).

In addition, any applicable derogation may be detailed on the animal passport (or similar). It should also be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.

It is anticipated that at the end of the study period animals will undergo an industry standard SICCT test. If animals are shown as positive, animals will then undergo a blood IFN-γ test and PM. If both results from the blood IFN-γ test and PM show the animal as being bTB negative, the farm site will have no restriction/further testing. If results of the blood IFN-γ test or PM show the animal as being bTB positive the farm will be on ‘restrictions’ as per current policy and undergo repeat testing at 60 day intervals. This procedure is outwith that listed in section 5.5.

7 ASSESSMENT OF SAFETY

The results of the study will be compared between the IVP and CP vaccinated animals.

It has been suggested that the frequency of veterinarian visits relating to AEs and administration of concomitant medications can be compared between the groups to demonstrate any additional benefits of vaccination.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).

It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 17 WPP8.4 - FIELD SAFETY OF BCG VACCINE, PROPOSED PROTOCOL

Field Safety of BCG Vaccine When Administered to Breeding Bulls (Generation of Semen Samples)

WPP 8, Design 4
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1 TITLE
Field safety of BCG Vaccine When Administered to Breeding Bulls. (Generation of Semen Samples)

2 REGULATORY GUIDANCE
It is currently understood that the study will be performed under the authorisation of an ATC. It is, however, the experience of Triveritas that blood sample collections for purely experimental purposes, or use of a placebo, are unlikely to be considered procedures in the best interest of the individual animal and/or not recognised to be recognised veterinary practice and as such will not be allowed under an ATC. For such procedures, a project licence under the A(SP)A may be required. An additional degree of effort by the delivery contractor will be required running the two systems of trial clearance together but they are not mutually exclusive.

3 GOOD CLINICAL PRACTICE
At a minimum this study will be performed in compliance with the guidance of CVMP/VICH/595/98 VICH Topic GL9 Step 7 – Guideline on Good Clinical Practices (CVMP approved July 2000).

4 OBJECTIVES
The primary objective is to generate semen samples for analysis in respect of post-vaccination bacterial shedding and semen quality.

5 STUDY DESIGN
5.1 General
The safety study will be a randomised, blinded, placebo controlled trial.

5.2 Farms/Sites suitable for inclusion
The study will be carried out at a GLP or GCP compliant Contract Research Organisation (CRO) with experience in conducting target animal safety and reproductive toxicity studies.

5.3 Animals suitable for inclusion
Mature, male dairy or beef cattle will be suitable for inclusion. Ten IVP animals and ten CP animals will be recruited onto the study.
Prior to the start of the study, the animals will have been subject to bTB Single Intradermal Comparative Cervical Tuberculin (SICCT) routine industry testing to confirm negative status.
Animals will be scheduled for revaccination on Study Day 360 (V2).

5.3.1 Pre-study data collection
Prior to the start of the study, the prior reproductive history of each study animal will be recorded.

5.4 Study treatments and allocation
Animals will be recruited onto the study at a ratio of 1:1 IVP:CP. Animals will be randomly assigned to treatment group by means of a Random Treatment Allocation Plan (RTAP), with animals being blocked according to age and/or bodyweight. All study animals will receive vaccination at a single time point.
Consideration should be given as to whether a placebo is used or whether animals allocated to the CP group are left unvaccinated as “in contact controls”.

SHORT TITLE: Feasibility study into testing and validating cattle BCG vaccine and DIVA

Final
Date 05 March 15

– Strictly Confidential –
5.5 Study schedule

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Notes: V1: Vaccination 1 (primary vaccination) V2: Re-vaccination. a. Blood Sample or SICCT (if study performed prior to DIVA validation, SICCT to be used. If performed post DIVA validation, blood sample collected). b. Pre-vaccination. Flexibility (± x days) will be determined in the final study protocol. h = hours.

5.6 Treatment administration

The first injection of IVP/CP will be administered on Study Day 0. Re-vaccination will then take place on Study Day 360.

The IVP/CP will be administered at a dose rate of 0.5mL per animal, per administration. This is the dose rate which has been shown to be safe and effective for the control of bTB in cattle, based on previous clinical studies.

The IVP/CP will be administered subcutaneously into the neck region. The vaccine administration will be to the right hand side on V1 and on the left hand side on V2.

There is an assumed zero hour/day withdrawal period for meat and offal.

5.7 Clinical examinations

At the time point of inclusion, (Study Day -7) and prior to each vaccination, a clinical examination will be performed by a veterinarian on all animals to ensure that the animal is suitable for inclusion or for continuation in the study. At the end of the study, a final clinical examination will be performed to ensure that the animal has remained in good health and can be signed off the study.

The clinical examination will include an examination of the general health status of the study animals (including an assessment of the animal’s suitability for semen sampling); any abnormalities will be recorded.
5.8 Injection site observations
No injection site observations will be collected during this study, this data will be collected as part of WPP2 and WPP6.

5.9 Post mortem examination
A post mortem (PM) will be performed for the following reasons:

- Animals which die during the study, when the cause of death is unknown, will undergo a full PM in order to identify the cause of death.
- Animals which are despatched to slaughter for welfare reasons will undergo a routine PM.
- At the end of the study, all remaining animals will be despatched to slaughter and confirmation of bTB status obtained.

In addition to the standard PM procedures, in order to confirm that the animal is still bTB negative, the lungs and lymph nodes will be examined for signs of bTB infection.

Characteristic tuberculosis lesions occur most frequently in the lungs and the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, on serous membranes, and in other organs.

Scoring systems will be applied, with each lung lobe being examined separately and cross-sectioned at 0.5 to 1.0cm intervals.

5.10 Samples and analysis

5.10.1 Blood samples
Blood samples will be collected as per the study schedule (Section 5.5) for blood IFN-γ DIVA testing. If the analytical method for DIVA has not been validated prior to study, an industry standard SICCT test will be used to confirm that animals are negative prior to vaccination. If it is not possible to confirm the animal’s negative status at the end of the study period, derogations should be put in place.

5.10.2 Semen Samples
Semen samples will be aseptically collected according to the schedule in Section 5.5. Samples will be collected from each animal at each time point. The procedure for semen sampling will be as per the CRO’s Standard Operating Procedures (SOP’s).

Semen samples will be allocated a cryptic alpha-numeric sample code used as the sole identifier in order to maintain blinding at the analytical site.

Semen quality will be evaluated, including morphology (structure), motility (rate and percent of progressive forward movement), volume and concentration, along with analysis for bacterial dissemination (methodology to be developed).

5.11 Adverse events
Adverse events will be reported to the Monitor on an agreed timescale and to the Sponsor if considered serious.

Adverse event recording and reporting will continue until the end of the study.

5.12 Summary of trial details required for ATC
(taken from ‘Application for an animal test certificate (type A or B) using an immunological/biological product’; VMD/L4/Authorisations/0321/C - #713143)

Pharmaceutical form: Powder and solvent for suspension for injection.
Target species: Bos taurus
Indications: To be advised

Estimated duration of trial: 381 days

Maximum no. of animals:
i. Treated (with the test product): 10 male cattle
ii. Positive controls: not applicable
iii. Negative controls: not applicable
iv. Placebo treated controls: 10 male cattle

Inclusion criteria: mature, male cattle of any breed, over 6 months of age. See 5.2 Farms/Sites suitable for inclusion and 5.3 Animals suitable for inclusion.

Exclusion criteria: Animals with a positive SICCT result prior to vaccination will be excluded.

Description of safety monitoring: clinical examinations will be carried out on all animals at the time of inclusion, prior to vaccination and at the end of the study. The clinical examination will include an assessment of the general health status of the study animals by observing the main body systems.

Blood samples will be collected as per 5.5 Study schedule for DIVA testing (if validated).

Semen samples will be collected as per 5.5 Study schedule and 5.10.2 Semen samples.

Adverse events will be recorded throughout the study period for the collection of safety data.

Post mortem examination is described in 5.9.

(For further details see 5.5 Study schedule, 5.7 Clinical examinations, 5.10 Samples and analysis.)

Method of administration / dose rate/ duration of administration:
i. Treated (with the test product): subcutaneously into the neck region, 0.5mL per animal per administration.
ii. Positive controls: Not applicable
iii. Negative controls: Not applicable
iv. Placebo treated controls: subcutaneously into the neck region, 0.5mL per animal per administration.

Disposal of unused product and empty containers: to be advised.

Disposal or fate of test food producing animals (not intended to enter the human food chain for food): see 6.1 Fate of study animals.

6 STUDY END

The study will continue until Study Day 381.

6.1 Fate of study animals

Records of the fate of all study animals at the end of the study should be made.

In addition to this any applicable derogation will be detailed on the animal passport (or similar). Further to this it should be noted that no other industry standard testing for bTB should be permitted during the study period and this will also form part of the derogation.

7 ASSESSMENT OF SAFETY

The results of the study will be compared between the IVP and CP vaccinated animals.

8 OPERATOR SAFETY

From safety studies conducted to date, there is no evidence to suggest that the operator is at significant risk of bTB exposure either by the process of physical administration of the vaccine or by handling of vaccinated study animals.

From the data available there is no reason to suspect that bTB is shed through any biological output of vaccinated cattle (e.g. milk, saliva, faeces).
It is recommended that basic personal protective clothing is worn by the operators when working with cattle and handling of the vaccine. This may include steel toe-cap boots, overalls, and protective gloves. Additional user safety information directly relating to the vaccine is given in the SPC and should be supplied to all study participants prior to involvement in the study.

In addition, it should be ensured that the sites have safe handling facilities for use during study procedures. Handling of animals should be, whenever possible, kept to a minimum. When study procedures are scheduled, planning is paramount to avoid any unnecessary handling and stress to both the animals and handler. Raceways and crushes should be located and designed to allow handling/restraint of the animals in a way as to avoid injury to animals and handlers.

Farm sites, veterinarians acting in the role of Investigators and contract researchers involved in the study must ensure they have the correct liability insurance.
APPENDIX 18 WPP9- GLP STUDY PLAN: ANALYSIS OF MILK SAMPLES GATHERED IN THE FIELD FROM VACCINATED AND UNVACCINATED ANIMALS.

GLP STUDY PLAN OUTLINE: ANALYSIS OF MILK SAMPLES GATHERED IN THE FIELD FROM VACCINATED AND UNVACCINATED ANIMALS

WPP9
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1 TITLE
GLP Study Plan: Analysis of Milk Samples Gathered in the Field from Vaccinated and Unvaccinated Animals.

2 REGULATORY GUIDANCE
The current understanding is that the field study, from which these samples will be generated, will be performed under the authorisation of an Animal Test Certificate (ATC) and/or a project licence under the Animal (Scientific Procedures) Act 1986 (A(SP)A).

3 GOOD LABORATORY PRACTICE
Milk sample analysis will be carried out according to Good Laboratory Practice (GLP) in a facility that is within the UK GLP compliance programme.

4 OBJECTIVE
To analyse milk samples for the presence of BCG vaccine, in order to investigate post-vaccination bacterial dissemination into milk.

5 ANALYTICAL METHODS

5.1 Receipt of samples by the analytical laboratory
Samples will be despatched to the analytical laboratory with appropriate labelling and under controlled temperature conditions.

5.2 Culture of milk samples
A validated method for the culture of milk samples has been provided by APHA. As this method is the intellectual property of APHA, the full method is not given within this report.

Culture will be carried out under an approved Study Plan and according to the Laboratory’s Standard Operating Procedures (SOP’s).

5.3 Reporting of culture results
Results of analyses will be reported as a formal study report.

6 CONSIDERATIONS
It may be preferable for WPP9 and WPP10 (nasal swabs) to be conducted at the same facility, possibly under one Study Plan.

Selection of the facility should take into account the following: GLP status; capability to receive a large volume of samples from the field; capability to deal with potentially pathogenic samples.

Transfer validation of the test method should be performed where applicable.
APPENDIX 19 WPP10 – GLP STUDY PLAN: ANALYSIS OF NASAL SWABS GATHERED IN THE FIELD FROM VACCINATED AND UNVACCINATED ANIMALS

Project No.: PN1567

GLP OUTLINE STUDY PLAN: ANALYSIS OF NASAL SWABS GATHERED IN THE FIELD FROM VACCINATED AND UNVACCINATED ANIMALS

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1  TITLE
GLP Study Plan: Analysis of Nasal Swabs Gathered in the Field from Vaccinated and Unvaccinated Animals.

2  REGULATORY GUIDANCE
The current understanding is that the field study, from which these samples will be generated, will be performed under the authorisation of an Animal Test Certificate (ATC) and/or a project licence under the Animal (Scientific Procedures) Act 1986 (A(SP)A).

3  GOOD LABORATORY PRACTICE
Nasal swab analysis will be carried out according to Good Laboratory Practice (GLP) in a facility that is within the UK GLP compliance programme.

4  OBJECTIVE
To analyse nasal swabs for the presence of BCG vaccine, in order to investigate post-vaccination bacterial dissemination into nasal secretions.

5  ANALYTICAL METHODS
5.1 Receipt of samples by the analytical laboratory
Samples will be despatched to the analytical laboratory with appropriate labelling and under controlled temperature conditions.

5.2 Culture of nasal swabs
A validated method for the culture of saliva samples has been provided by APHA (formally AHVLA). This method will be adapted and validated for use in nasal swabs. Culture will be carried out under an approved Study Plan and according to the Laboratory’s Standard Operating Procedures (SOP’s).

5.3 Reporting of culture results
Results of analyses will be reported as a formal study report to an agreed timescale.

6  CONSIDERATIONS
It may be preferable for WPP9 (milk samples) and WPP10 to be conducted at the same facility, possibly under a single Study Plan.

Selection of the facility should take into account the following: GLP status; capability to receive a large volume of samples from the field; capability to deal with and potentially pathogenic samples.
APPENDIX 20 WPP11 FARMER PARTICIPATION IN BCG CATTLE VACCINATION TRIALS: KEY INFORMANT STUDY INTERIM REPORT

Defra SE3287

Farmer Participation in BCG Cattle Vaccination Trials: Key Informant Study Interim Report

School of Management and Business, Llanbadarn Centre, Aberystwyth University

Susan Fowler, Peter Midmore

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1. INTRODUCTION

The key informant study has the objectives of exploring practical issues of farmer participation in implementing any BCG vaccine trials and discussing other issues which might need to be taken into account in optimising the design. It is based on analysis of semi-structured interviews of representatives of the main stakeholders in the farming, veterinary, processing and retailing sectors. Participants were selected through network recruitment, and received a briefing document (Example given in Section 5 of this Appendix) outlining the evolving framework of trials design and the issues that these raise. Specific questions covered in the interview guide (Example given in Section 6 of this Appendix) related to attitudes to vaccination, willingness to participate, incentives, market and supply chain issues. Interviews were audio recorded, transcribed, and content-analysed.

Two waves of recruitment were scheduled, with the second wave recruited from suggestions of interviewees in the first wave. Initially seven key informants were identified for the first wave of interviews, although later was extended to include a further two participants. From assessment and comparison of suggestions made in the first wave, a further ten key informants for second wave interviews were identified and two further informants were provided. This report therefore provides a synthesis of the content of twenty-one interviews.

Informants were selected from the food supply chain and included: farmers, farmer representative organisations, levy bodies, supply chain including retailers, assurance bodies, veterinarians and an auctioneer. Most of the respondents were based in England, and three were based in Wales.

2. SUMMARY

Many respondents emphasized the need for a proactive communications campaign so that all stakeholders are engaged and fully informed, most importantly the farmers’ veterinarians, but also including retailers. The programme should be introduced by Defra/APHA and then farmers could be engaged through public meetings: smaller events would then be needed for potential participants, with individual farm meetings so that the details could be clarified. The participation of the farmers’ veterinarians was emphasized as representing a source of trusted information. The ideal facilitators for the larger meetings would be farmer groups or unions: it would be important for Defra or APHA to take a back seat at these meetings due to the cynicism felt by farmers towards these bodies.
It is deemed important that the entire farming family including stock-men/women should be involved because complete commitment would be needed. There would be an advantage in issuing contracts or having Memoranda of Understanding (MoUs) so that all engaged are very clear about what commitments are required.

Before agreeing to participate in trials farmers will need reassurance that their markets will not be affected; it may be helpful to have supply chain representatives at the initial meetings. In particular this suggests that safety trials need to be carried out concurrently with the DIVA validation so that adequate assurances regarding product safety can be given.

Financial incentives were not seen as vital but full compensation for any additional costs would be, particularly in areas of crisis (i.e. hot spot areas).

A consistent message from informants was the need for openness and honesty with all parties. A concern relating to informing consumers was that of highlighting the current situation that produce from reactor animals is in the food supply chain; however it was also recognised that this issue had risen previously without causing prolonged changes in consumer behaviour.

3. SPECIFIC QUESTIONS

Do farmers think vaccinating cattle is a good thing?

There was a range of opinions expressed, including that farmers didn’t have enough information to judge cattle vaccination. Responses ranged from: “the great white hope” to “conceptually it’s a very positive thing” but to “there’s a lot of cynicism, scepticism out there on farms … about the current approach.” Farmers would want to know more about it, that it is going to work and that it is safe. Farmers will need information including cost/benefit figures and would be more likely to take it up if recommended by their own vet. There needs to be careful explanation about what is achievable: “there’s a danger that they see it as a bit of silver bullet.”

Farmers will be very nervous of limitations on export and detriment to possible sale of culled cows, dairy cross calves etc. The market will pay less if it can get away with it so there is a need to address the market for vaccinates.

There a fear particularly in ‘clean’ areas where vaccination could be seen as bringing bTB into the area/farm and a risk of intimidation was mentioned. There is also some concern that vaccination could mask an underlying problem.

One farmer commented that if it might take them to bTB freedom, “get to the side you’ll be trampled”.

Would individual farmers be keen to participate in a trial?

Firstly farmers would need to be reassured there would be no impact on their markets: for products, vaccinates and progeny, even after the end of the trial, some stating that a guarantee would be required: “My cows are my livelihood. If they suddenly have no value and their product has no value I’m bankrupt”. “I’d want some sort of reassurance that if something went wrong there would be insurance liability that would be covering any unforeseen consequences”. In turn, retailers would require reassurance from the processors. To provide this reassurance to processors the BCG safety work needs to be completed before farm trials can commence.

The major fear for those without bTB would be of any change in status from participation, which will affect their willingness to engage. The status change could be either due to added restrictions from participating in the trial or from any new reactors: gamma interferon tests have a bad press, such that farmers may fear a change in bTB status and the attendant restrictions: "Now the questions I’d be asking are what's the risk and if the risk is either loss of animals or workload that suddenly increases it could be potential loss of value of all your stock if you then become TB restricted."

Farmers will also need a clear idea of the workload implications and expectations and how the trials will fit in with their current farming operations. Practical issues and cash flow need to be addressed. The support of the individual farm veterinarians is seen as vital. The DIVA validation will need full explanation so that farmers are reassured of the validity of any positives. Farmers will need time for consideration of the implications of participation.
It may also be a challenge to get engagement in non-commercial holdings, although, conversely, smallholders may be keen for their animals to be vaccinated even though their livestock may be less at risk.

There is mainly a communications challenge regarding messages and managing expectations. Normally vaccination is in response to screening, so this requires a different approach which would need explaining. There is also a real fear of introducing bTB onto the farm which will have to be addressed. Transparency would have to be handled carefully if farmers felt themselves vulnerable to activists.

There is hope that it could be possible to get insurance if stock are vaccinated.

In general participation is more likely “if there was an advantage to be gained from it; payment for the work or freedom from TB restrictions are advantages.” However, they would struggle to see the point if the wildlife reservoir were not also addressed.

**Would this differ in different bTB areas (high risk/edge/low risk)?**

Opinions vary considerably on this issue although there is agreement that willingness would vary across the country. There is general agreement that there would be interest in hotspot and edge areas but low risk areas may be more problematic. However, some comments suggest there may be interest in all areas: low risk, edge, endemic, and also where there is high-value stock. In general, perhaps the majority of farmers will only consider something that is proven to be of help, and others, possibly with greater knowledge and enthusiasm, may be willing to try anything to move the bTB world forward.

High risk area farmers are now cynical and reactive and some see significant scepticism in hotspot areas: the trials would need to help in the immediate term and not hinder farm operations. Others considered that farmers in hotspot areas would be keen for any potential solution: “farmers and veterinarians I’ve spoken to in the higher risk areas, they’re getting to the point where they’ve got nothing else to do.” If there is a likely to be a reduction in inconclusives, that might be seen as a positive reason to participate.

Some perceived more interest in edge and low-risk areas because farmers may be less reactive and more proactive (although with the caveat that many farmers would not engage until the disease was closer). Farmers in low risk areas (or clean but within annual testing areas) would be reluctant “…unless I knew a heck of a lot more about it”. Those unaffected but in a high risk area may also be reluctant: “more problematically I think is the issue of if they did come up with a TB reactor it would put them into a whole new ballgame which they’ve been nowhere near before.” There may be a need to use altruism to recruit low risk areas or clean farms within high risk areas “they may be the ones you’re going to have to work on a bit harder … for the good of the whole community rather than from their own individual…”

**Would the level of individual protection of 63% against bTB be a barrier?**

This message will be hard to get across, so good communications are vital. The extent of herd protective effect would need careful and full explanation: many respondents felt they, themselves, did not understand fully, and that some farmers would require a full understanding. This is a crucial role for the farm vet and there has to be an agreed, consistent message: one influential person (particularly a vet) could confound or detract from the overall message. This highlights the need for an explanation of how vaccination fits within the entire approach to bTB control (at farm, local or regional levels, as appropriate), and that this is only one aspect of the toolkit: “Needs explaining where this fits in overall picture.”

Farmers are unaware of the efficacy rates of other vaccines and just assume they will work: it may help to provide statistics on individual efficacy figures for other vaccines, e.g. leptospirosis. Farmers are more likely to conceptually think 63% is closer to ‘the flip of a coin’ rather than a 2/3 efficacy.

Farmers may recognise that the combination of 63% efficacy together with the use of placebos would reduce the likelihood that taking part in the trial will rid them of bTB.

It was suggested that participants may do better to think of themselves as being part of an experiment rather than a trial.
How would farmers react to the possibility of their cattle receiving a placebo or other blinding?

This was generally thought to be very challenging, because farmers do not like unknowns and they are “doers … not researchers” and it would also not be helping them to achieve bTB freedom in the near future, farmers will not see “the point of giving it a jab if it's not going to do the job.” It would need a significant education campaign: “it’s up to the people who are running the trial to explain that clearly what the implications are of that. Let’s face, it most of our farmers are not actually scientists.” It will be important to explain that this is necessary in scientific trials to achieve eventual licensing.

Some farmers would not understand the need and some would fear for their non-vaccinated stock: “one of the more sensitive areas I think, because you are potentially exposing an animal to a higher risk of TB than others”.

There are implications for requests for compensation: “Those ones that you've vaccinated are fine, those that you haven't are not, I’m blaming you, can I claim compensation off you?”

There could be an issue of accurate medicines recording which may need derogation, although the informant from an assurance body consulted did not anticipate a problem as long as processors could be confident that participation would not affect the farmers’ assurance status.

**Beef farmers may be more relaxed than dairy farmers:** “I’m talking about it from a dairy farmer’s position. A dairy farmer’s position might be very different from a beef farmer’s position, because … a beef… farmer's going to sell them on in 6 or 8, even 12 months’ time, he might have a different issue.”

Would incentives be necessary to achieve engagement, and if so, at what level?

There was no strong feeling that separate incentives would be needed, but financial compensation for farmer time, risk, extra labour and recording needs, etc., should be generous. This would be particularly important in areas of extreme stress, and to overcome scepticism. Farmers would want something done about wildlife vector.

One farmer stated that participation needs to be worthwhile for the farmer: “People are running a business. Full stop.” One farmer with large cattle numbers calculated it cost him £25 a head each time he tested in terms of stress, manpower, loss of weight gain.

Options may be to provide facilities such as handling equipment, isolation units, or helping to fence out the wildlife reservoir. Softer options could including supporting the farmer’s health planning during the regular visits, including, for example, taking blood samples for other simultaneous tests.

The potential of ‘earned recognition’¹ may be worth exploring so that farmers participating in the trial would be subject to fewer cross-compliance checks.

It is likely that any breakdowns suffered whilst participating in the trials would be seen as caused by participation therefore losses related to any adverse reactions, vet time etc. would need to be covered. There may need to be compensation over and above baseline values for breakdowns, or for stock carrying expensive embryos and agreed valuations for pedigree stock. Rate card payments would not be acceptable, particularly as they are likely to decrease in future with the potential bTB compensation rate changes: “if one of your vaccinated animals went down you’d have to offer to compensate at full value for that”. There was particular concern over realizing the value from dairy cross bull calves if positives were identified.

There were strong cautions against the damage that could be caused by changing policy or circumstances through trial. Also the role of Defra and APHA needs careful management. Initial publicity from Defra/ APHA would be important, but they should probably take a back seat at farmer meetings: “if it probably came straight from the word Defra it probably wouldn’t have that same level effect… or if it came straight from APHA, because a lot of these guys have been spending 20 years talking to government officials or APHA and they’ve come and gone and policy’s changed: vaccination, culling etc. … I suppose some of the scepticism could be understood” and “If it came straight from

¹ Earned recognition: where businesses that are able to demonstrate a history of good compliance with the legislation, or that are members of a private assurance scheme, would receive a lighter touch in terms of the number and type of official inspections.
Defra then it would be seen as, I think there would be an element of fear about it. What am I letting myself in for kind of thing?”

How should the protection offered by vaccination best be explained to farmers?

Use excellent presenters: presentation skills are seen as being more important than the speaker’s role. Having other people and organisations (such as feed merchants, buyers) at “pea and pies” meetings will be more likely to get farmers there. Farming families and workers should be invited to meetings to ensure real buy-in and understanding by all involved: this may also involve providing materials in different languages given the reliance on non-English temporary workers for milking.

There may be confusion between the 63% protective effect figure and the requirement for 80% coverage needed as promoted during the Bluetongue vaccination campaign. It may be useful to have efficacy figures for other vaccines (e.g. leptospirosis) to put the efficacy figure in context.

There was no single route identified as being best, but routes may need to be tailored to individual types of farmers. It was seen as important to keep feeding the information out. It is vital that it is a consistent message. Farmers will need cost benefit information. Vaccination needs presenting as one of several control measures, and as part of a 25 year eradication plan.

There is a need to trial communication methods and materials as it is vital to get it right: “if you go to meetings you want somebody who’s knowledgeable who puts it across at the level that you can understand.”

How do you think BCG vaccination trials will influence farmer attitudes to biosecurity?

Generally biosecurity is a problem anyway particularly on traditional farms with old buildings, and larger farms with multiple units. Although it should not affect other biosecurity principles because they are not just for bTB this is seen as a real risk link with over-expectations: “I don’t have to worry about putting in those badger fences…” (because my cattle are now being vaccinated.)

It might affect buying behaviour if farmers see vaccinates as clean, although participants will generally be the more engaged farmers and they will continue to prefer replacements from low-risk areas over vaccinates. Again, it is seen as all about communication and farmer education. As the BCG is an imperfect vaccine this may help the message, but it needs careful management as part of the package: “relax at your peril”. It might, conversely, raise awareness.

There is a continuing problem with farmers’ perceptions that buying livestock from a friend or relative (including as a favour in a dispersal sale) will be ‘safe’ due to the human relationship.

It appears that some farmers’ business plan depends on buying-in potentially infected stock: within the trial a protocol may be needed for participants that buy-in from high risk areas.

How would consumers react to meat and milk that comes from vaccinated animals?

It was generally thought that we do not know what the consumer view is, or that they do not have a view or particular interest in livestock treatments (as distinguished from diets, in which there is some interest) but it needs to be carefully managed because there is a risk of retailer positioning and because TB is an emotive issue. There is a need to have the Food Standards Agency (FSA) on board to reassure the public, as there is potential to raise consumer fears by those against vaccination for market, or other reasons. Specifically, Irish beef farmers have stated that they would attempt to gain market advantage by selling beef from unvaccinated animals, providing a suggestion of risk.

Some perceive a real risk of highlighting that there is current consumption of products from reactor animals. There were some concerns expressed relating to the use of live vaccines, so there is a clear need for safety information.

The reaction may however be positive because vaccination would reduce the need to cull badgers. The fact that most people and their children have the BCG vaccine was seen as a route for reassurance, also many consumers are aware of the protection from pasteurization.

It would be important that retailers are engaged and fully understand the issues, and therefore safety results need to be available to reassure the food supply chain. There is a risk that retailers could
initially be supportive of the trials but could change their view mid-way through trials, but producers need assurance for the lifetime of their animals and progeny. Thus it was seen as particularly important to have dairy processors on board.

Would farmers in low risk areas be more or less willing to purchase vaccinated cattle?

There was no clear answer to this inquiry. If livestock were ‘clean’ (i.e. from a four-year test area) and vaccinated then that could be seen as an additional security, but tested and ‘clean’ from a low risk area would be a first choice. There is concern that vaccinated animals could still be carriers: some in low risk areas may see vaccinated animals as a higher risk and would not touch them because of the risk that other farmers would blame them for bringing bTB into previously clean area, or due to fear of market implications. There would probably be resistance to buying vaccinated calves because: "why would you?"

There is a risk that some may buy vaccinates from high risk areas thinking they'll be clean: "It would make sense because they would be, they should be less risky." Also there was concern that, at this stage, while the efficacy of the vaccine is still low or unknown that a farmer wouldn't know with 100% certainty whether the animal in question has been successfully vaccinated against TB: “it's like a keg of dynamite almost, I'm not sure that, as a farmer, I would touch it, simply because of that, because the actual, the science in effect is still under trial”. One vet considered that ideally the trial could be designed so that animals only left for slaughter, or if they went to another farm, they should be specially traced.

The situation may change if risk-based trading gains traction: if buying from a vaccinated herd were to be seen as a plus in Defra's risk-based trading approach that would be an advantage.

It is vital that the DIVA test detects carriers: "it might vaccinate them but it could still be a carrier. That's the question I'd be asking anyway."

The trial, and subsequent markets, would be very vulnerable to any bad news stories.

What are the most common misconceptions of current bTB testing procedures, particularly skin test results?

Consistently animals seen as ‘false positives’ are the problem so myth busting is still needed: "And actually, yes, farmers are suspicious of it, all say it's useless". The problems of the perceptions of ‘false positives’ are made emotionally harder because farmers cannot be sure they are not buying in infection in the replacements. Pre-tested cattle turn up positive later.

However, many farmers do recognize it is the best we have got; others blame failures on poor testing procedures. There is no wide understanding that false positives are better than false negatives for general bTB control. There is still a need to change mind-sets: even of veterinarians who need to change their view of inconclusives when disease encroaches: that currently happens too late.

There is some farmer awareness (and use) of an interaction of the skin test with liver fluke infection, and farmers strategically test small groups for immediate sale rather than whole herds: "And I also think there’s a recognition that the skin test is a good herd test and not a particularly good individual test"

Those not experienced with bTB are less aware and need education.

Market impacts

For buyers within the industry it should not be an issue, but a communications strategy is seen as the key to avoiding problems due to the risk of food scares. Wider impacts (especially for milk, for cheeses and milk powders) depend on third country responses. The dairy supply chain would be very challenging if milk from vaccinates cannot go into exports, as it would be extremely difficult to separate milk from trial farms as there is frequent joint haulage. The cull cow export market is seen as vital, its loss would impact greatly on home market for steers and heifers.

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In livestock markets some expressed disquiet of the potential for a two-tier system with vaccinates having a different price, although the experience with Bluetongue vaccinates suggests the impact on price is impossible to predict. There was acknowledgement that a two-tier system may come about due to risk based trading anyway. Abattoirs could start including a box to be ticked for vaccinates: the beef market is very challenging at the moment with multiple causes contributing to reduced prices.

In contrast there was a suggestion that a legal ‘vaccinated herd’ status would help transparency and protect the market. It may be possible to devise a mechanism for selling of vaccinates (as with ‘orange markets’) with the help of auctioneers.

Beef finishers may not be very concerned, but there may be more of an issue for those with pedigree cattle, when vaccination may have a price implication.

Farmers will need to be reassured there are no implications for their buyers before they sign up for trials. Processors may decide they do not want to publically engage with this (due to risk of highlighting reactor produce) and may say they see it very much as a farmer issue. However, in the future, the assurance of a consistent supply following a successful trial would be a huge positive for retailers.

It is unlikely there would be impact on farm assurance and therefore trading status: “As long as the retailers and the processors are comfortable with what we’re doing we can certainly provide that statement I think” i.e. “this isn’t going to affect your farm assurance”.

Some specialist markets may be impacted (e.g. organic), and the unpasteurised cheese market may be threatened.

**Vaccine not being available after the trial?**

This is another issue where expectations need to be managed at the outset: generally, as long as the timescale was explained, there may be acceptance, but it was seen as very important that feedback followed the trial, as a long silence would add to cynicism.

It was clear that if farmers have been bTB free during the trial they would be very disappointed to lose the vaccine. It was suggested that Defra should plan now to enable vaccine to continue to be available, so that perhaps derogations could be arranged so that they could continue using the vaccine because: “If they’ve come to be convinced that’s why they’ve not got TB, they’ll pay holy Hell about it” although “people do participate in other field trials of other things and they wouldn’t necessarily see the benefit immediately”.

**Bureaucracy and testing/handling. Visitors to farms**

Dairy cattle are more used to being handled, and generally on-farm handling facilities are better than beef enterprises. There will be lower numbers involved with beef herds but they will be less compliant animals, and gathering from extensive systems will be a major problem and may require extra staff. Trial protocols will have to fit around the farming system since farmers will be unwilling to gather stock from distant fields during the summer. There needs to be clarity in commitments and expectations from the start, perhaps contracts, or at the least an MoU.

It was suggested that many farmers are taking things (the wildlife vector issue) into their own hands these days which may result in a reluctance to take part in the badger cull because the farmers’ own activities would be curtailed, so there may even be a badger increase in cull areas: these farmers would not want an increase in ‘authority’ visits.

If the visits were used as soft incentives to help with health planning that could help.

**Particular issues**

**Good communications vital:** it is absolutely essential to ensure the communications are right, to prevent rumour mills. It is important to be clear and up front with everyone including stating that there is no reason that participant will have any higher risk from participating.

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The biggest worry is false positives and false ‘outbreaks’ “but I think the loss of the TB free status would be more important than the actual economic loss of that one particular animal. “

Safety: “I would have more worry about the use of BGC vaccine than I would other vaccines, just because it’s a vaccine that we may need in the future and whether we’d be developing strains that become resistant to it and whether there’s any shedding.”

To achieve complete industry support farmer organisations need to be engaged and informed at all stages. If they are trusted, they are confident they can bring farmers along. Some Non-Government Organisations (NGOs) only care about badgers and may not raise objections (including food scare issues) because cattle vaccination could be seen as protecting badgers.

Control of vaccinates: “for safety’s sake, and for, I think it’d be a good thing that control is kept of these vaccinated animals” including young breeding animals - “you don’t want in ten years’, five years’ time, finding out there’s little mini outbreaks of TB in areas that have either been caused and, or been blamed on the vaccine trial. It doesn’t matter whether it’s likely to happen or not, if it gets blamed.”

Pasteurisation: one farmer who sells unpasteurised cheese would not participate in the trial if the trial required pasteurisation.

Separating milk out from trial participants would be very challenging and the respondent was not sure if it could be do-able.

Anything else we should consider? Other comments

Potential routes to farmers may be best achieved through their buyers for reassurance of markets. One good example of a successful communications campaign was regarding the Chernobyl meat coming onto the market. This took a huge effort and the Food Standards Agency (FSA) was crucial player, but it was very successful, with positive articles in the lay and trade press.

Other issues raised include:

- The whole thing is a tremendous challenge because of the fear of restrictions if the farm is not currently restricted,
- many farmers feel deeply wounded that they carry the burden of bTB and the wildlife vector is not addressed, this sometimes brings resentment and cynicism,
- the programme will be hard to sell if the wildlife reservoir is ignored,
- the issue of deer as carriers was raised where skin tests are no use,
- if the vaccine would be available for goats (from a respondent interested in goat milk sales),
- farmers are wary of the cost implications of administration of any new test,
- be wary of using APHA mailing list due to duplications,
- there need clear guidance for farmers, for example how to pre-movement test for sales off-holding during the trial, and what happens to casualties,
- negotiations should consider that bTB compensation may change during trials,
- perhaps there is a need for a new way of dealing with positives on trial farms,
- the number of foreign stockmen involved may require written materials to be provided in other languages,
- it is important that risk-based trading mediates impact on those in trial,
- beef farmers may engage more with health plans once EU Animal Health rules change to require annual veterinary visit,
- there needs to be a good explanation as to why the efficacy is at 63%,
- the gamma interferon name may put a few people off,
- this work emphasizes the urgent need for a health database, as on the continent,
• there is an issue of timing if vaccinations cannot be given at once (interaction between BVD and leptospirosis vaccine cited) and possible problems regarding scheduling e.g. for bluetongue vaccination, which would take priority in the farmers’ minds,

• a possible interaction between fluke worms and bTB skin test results was revealed,

• the issue of genetics and bTB susceptibility needs investigating, especially in dairy cattle,

• there is nightmare scenario which would be caused by reducing the sensitivity to get the DIVA test through and leaving more undisclosed infection.

• would there be any withdrawal periods?

4. CONCLUSION

Recruiting

The likely engagement of farmers with the bTB trials could be plotted as decreasing along an axis of distance from annual testing areas, particularly from disease hotspots. Perversely, however, farmers under extreme stress due to bTB restrictions for years may be the hardest to engage due to fatigue and cynicism.

A clear and well planned communications campaign is vital to achieve recruiting success. There is widespread institutional support for addressing the bTB problem and this should be utilized. With farmers the major obstacle to be overcome is fear, in the shorter and longer terms. The immediate fear that participating may bring bTB to the farm and the area, and the longer-term concern about any impact on the marketability of the produce: products, livestock and their progeny.

There were constructive suggestions as to how to best select participants. These included using those that have a working relationship with farmers such as veterinarians, farming unions and buyers. They will therefore be able to recommend responsible and engaged farmers.

Initial publicity will need to come from Defra and the APHA. Veterinary Practices and Farming Organisations should be fully briefed at an early stage, as farmers are likely to refer to them for advice if they hear about the programme. Farming unions and other trusted bodies would be ideal hosts for early public meetings during which they will need to adopt the farmers’ language and make messages clear and simple. Farm veterinarians should not be used for initial recruiting as they may appear to lose their objectivity, but they must be involved in individual farm discussions. Individual farm discussions should include farming families and herdsmen, and probably feed suppliers and buyers.

"I think that you’ll have some good meetings, but you want somebody who’s knowledgeable who puts it across well with some aids, clear and simple, as to what the trial is about, how it’s to work and why you want people and what you want them to sign up to with a bit of a hand-out and a phone number, email, all that stuff in it because again they’ll want to think about it, discuss it with each other etc., see what they think. I don’t think you’ll get vast rapid buy in. It isn’t a golden bullet. It’s a pain in the neck. It’s while there’s somebody else can do that what’s in it for me? And again if it’s a business and businessmen there’s no financial gain in it, in doing it. It’s just adding to my costs. That’s no good."

Trial design

In many farmers’ eyes, the bTB risk is related to the frequency of testing, for example four year testing areas are seen as “low risk”; this approach should be avoided in selecting participants as within the administrative testing areas there will be range of risk profiles. In Wales, for example, there are clearly hot spot, edge, and low risk areas within the country despite the fact the entire area went to annual testing in 2009.

In terms of trial design:

1. full safety information is needed at the outset so that the food supply chain is supportive; otherwise farmers are unlikely to engage,
2. safety assurances need to be robust, backed up with data and papers so that those that want to fully understand are given the information they need,

3. early and full engagement with processors and retailers is necessary so that farmers can be assured that their markets will not be disrupted,

4. a contract or an MoU covering expectations and requirements with compensation arrangements and rates, so that the farmer is reassured that risks are covered, will be needed,

5. this should include a range of issues and scenarios which need to have been thought through to achieve credibility,

6. it is important to engage and involve all the farm staff and family, and invitations to events should be addressed to the farming team, not just the farmer,

7. there may be some issues with timing of the vaccinations, relating to interactions with other vaccinations, and gaps which need to be left between vaccination programmes,

8. after the initial publicity, messages are best not seen as from APHA or Defra: trusted sources (farm veterinarians and/or farming organisations) should be used,

9. facilities and human resources for gathering stock may be limiting, particularly in extensive holdings.

Information and education have to be at the heart of the design. Key issues are that:

1. vaccination is only part of the strategy to reduce the incidence of bTB, it is not a solution in itself,

2. biosecurity has a continuing and important role to play, and it is just as important to maintain it during the trial,

3. expectations have to be managed both regarding the protective effect of the vaccine, but also need to understand the time-scale for any vaccine being commercially available.
Dear

Design of a field trial to test and validate the performance of cattle BCG vaccine and associated DIVA diagnostic test in England and Wales

Many thanks for agreeing to participate in our key informant interviews which will explore farmer involvement in the implementation of the vaccine trials and other issues which might need to be taken into account in optimising the design. I am writing to confirm formally our appointment, to provide you with details of the rationale of the proposed designs and a summary of the questions, and to give details about how we will use the interview material itself.

The issues and questions that we wish to explore are set out in the attached briefing note. This is not in itself necessarily comprehensive and only reflects an interim perspective on thinking on field trial design. Consequently we would welcome the opportunity to discuss any additional issues that you think are important, and also consider any significant changes to the approach which we may not have so far envisaged.

We hope that you will consent for the interview to be audio recorded. This is of enormous benefit to us as researchers because note-taking becomes much less crucial and conversation can be natural and free flowing. However, we should reassure you that the recordings will remain entirely confidential and will be stored securely in compliance with data protection legislation. If we wish to directly quote from the recording in any publicly available report, we will seek your permission first and indicate the context in which the quotation is being made.

Please do let me know if we can be of assistance in any way prior to, during or after the interview.

Yours sincerely,

Peter Midmore
Professor of Economics
6. INTERVIEW GUIDE

INTERVIEW BRIEFING NOTES

Defra/WG funded project SE 3287 (Design of a field trial to test and validate the performance of cattle BCG vaccine and associated DIVA diagnostic test in England and Wales)

Our interview should take between 60 and 90 minutes, and will explore what might affect engagement of farmers in different types of area in a potential BCG cattle vaccine field trial and the validation of the diagnostic test differentiating vaccinated from infected animals. We are interested in your views, based on your personal expertise and knowledge of relevant stakeholder communities. We will treat everything you tell us with complete confidentiality and anything we wish to report will be anonymised. These briefing notes are designed to inform you of the purpose and content of our interviews, to shorten the time that they take and allow us to concentrate on essential issues. They are not a statement of policy or, at this stage, of proposals for the field trials themselves.

Context

The design of potential field trials of BCG vaccine for cattle is part of research into the development of new interventions to control bovine tuberculosis and achieving OTF status. Initial results suggest the vaccine is likely to provide individual protection to approximately 60% of vaccinated cattle. However, reduced transmission means that the degree of indirect protection ('herd immunity') could reduce the occurrence of disease.

Guidance from the European Food Safety Authority (EFSA) states that legislation could be changed to allow vaccination, provided that a number of necessary steps are taken, including successful field trials. Because BCG vaccination can cause cattle to test positive to the tuberculin skin test, an alternative diagnostic test is required to 'detect infected among vaccinated animals' (DIVA test). Options for a field trial are therefore being developed which include an evaluation of the current proposed DIVA test, as well as safety and efficacy double-blind randomised control trials of the vaccine that are required to secure a change in the law. Cattle vaccination alone will not eradicate bTB, but, used alongside existing TB control measures, it could, in future, help reduce the prevalence, incidence and spread of TB in the cattle population and could also reduce the severity of a herd breakdown.

Trial design

Different options for trial design are currently being evaluated, and the main purpose of these interviews is to understand the prospects for farmers to participate in them. Any trial is likely to proceed in three steps, with individual farmers involved perhaps in some, but not necessarily all of these:

- **DIVA test validation** has the main objective of demonstrating that the DIVA test will differentiate infected and vaccinated animals and its performance is at least as "good" as tuberculin skin testing. This requires generating samples from animals which are bTB negative pre-vaccination, vaccinated and then exposed to natural challenge perhaps in Approved Finishing Units. Sufficient samples of vaccinated, bTB negative cattle need to be compared with samples from vaccinated positives and unvaccinated negatives and positives.

- **Trials of the BCG vaccine** on working beef and dairy farms, including a proportion in high risk areas as well as some that have been negative for bTB for a minimum of 5 years; randomised blinded control trials may require some cattle to be vaccinated with a placebo (or blinded in other ways), and all will be revaccinated annually. They will subsequently be tested with the DIVA rather than the traditional tuberculin skin test.

- The trials will also involve the testing of a number of other food issues such as post-vaccine bacterial shedding.

Other work alongside the field trials will include modelling work to look at likely impacts of different levels of uptake of the vaccine and any changes in trading behaviour, and the design of a social study of the perceptions and behaviour of stakeholders towards adoption of the vaccine.
Questions for discussion

We would like to have your views and comments on the following questions:

- Do farmers think vaccinating cattle would be a good thing?
- Would individual farmers be keen to participate in a trial?
  - Would this differ in different bTB areas (high risk/edge/low risk)?
  - Would the level of individual protection of 63% against bTB be a barrier?
  - How would farmers react to the possibility of their cattle receiving a placebo, or the trials being blinded in other ways?
  - Would incentives be necessary to achieve engagement, and if so, at what level?
- How should the protection offered by vaccination best be explained to farmers?
- How do you think BCG vaccination trials will influence farmer attitudes to biosecurity?
- How would consumers react to meat and milk that comes from BCG vaccinated animals?
- Would farmers in low risk areas be more or less willing to purchase vaccinated cattle?
- What are the most common misconceptions of current bTB testing procedures, particularly skin test results?

Please could you also consider, in terms of their general and specific expertise, who else we could benefit from discussing these questions with (up to three individuals)?

School of Management and Business, Aberystwyth University, Ceredigion, SY23 3AL
Interviewers:
Susan Fowler, suf@aber.ac.uk, 01970 621834
Peter Midmore, pxm@aber.ac.uk, 01970 622251
Aberystwyth University Key informant checklist

Key informant ______________________________

- Do farmers think vaccinating cattle would be a good thing?
- Would individual farmers be keen to participate in a trial?
  - Would this differ in different bTB areas (high risk/edge/low risk)?
  - Would the level of individual protection of 63% against bTB be a barrier?
  - How would farmers react to the possibility of their cattle receiving a placebo or other blinding?
  - Would incentives be necessary to achieve engagement, and if so, at what level?
- How should the protection offered by vaccination best be explained to farmers?
- How do you think BCG vaccination trials will influence farmer attitudes to biosecurity?
- How would consumers react to meat and milk that comes from BCG vaccinated animals?
- Would farmers in low risk areas be more or less willing to purchase vaccinated cattle?
- What are the most common misconceptions of current bTB testing procedures, particularly skin test results?
- Market impacts?
- Vaccine not being available after the trial?
- Anything else we should consider?

Please could you also consider, in terms of their general and specific expertise, who we could benefit from discussing these questions with else (up to three individuals)?

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7. OPTIONS FOR SOCIO-ECONOMIC MONITORING AND EVALUATION OF THE FIELD TRIALS

Summary

Design of field trials to test and validate the performance of cattle BCG vaccine and the DIVA diagnostic test has been informed by socio-economic research through key informant interviews and Cost-Benefit Analysis. To ensure that any future application of such testing and validation contributes fully to the evidence base for policy development, options for further socioeconomic research are explored here. Two potential strands of inquiry are presented: a set of linked, sequential protocols for exploration of farmer attitudes and behaviour which result from use of vaccines and DIVA tests, with sufficient sampling (15-20 individual interviewees and 6 focus group discussions, replicated three times; and use of Farm Business Survey monitoring on farms participating in the field safety and efficacy trials to improve estimates of the economic impacts of vaccination. Three additional inquiries could update and improve upon estimates used in the design of the field trials for evaluation of future policy options: examination of the current public and private costs of bTB breakdowns; investigation of the psychological welfare impacts of bTB breakdowns; and improving understanding of the issues involved in use of Approved Finishing Units for validation of the DIVA test.

Introduction

This Appendix sets out the main issues to be addressed in terms of understanding, on the basis of field trials, how cattle vaccination could contribute to measures to control bTB. In particular it focuses on understanding farmer and vet attitudes towards vaccination, likelihood of its uptake outside of trial conditions, and analysing and interpreting the socio-economic impacts involved in field trials of BCG vaccination. It draws on the discussions in key informant interviews reported in Appendix 20, and is linked to the ex-ante Cost-Benefit Analysis in order to support a more accurate ex-post measurement of the efficiency and effectiveness of vaccination, based on monitoring of variations in farm costs, focusing on labour, and on the impacts on incidence of disease, and consequences for outputs, on field trial holdings. The issues which most concerned key informants in terms of recruitment of farms and vets into field trials included market risks, farm labour implications and health and safety, the extent to which vaccines provide protection from bTB, and the increased occurrence of "false positives". A major limitation of the Cost-Benefit Analysis was the uncertainty attached to cost-savings related to disease outbreaks, with larger cost-savings providing greater justification for vaccine deployment.

The Appendix addresses three main areas of concern. The first section discusses the collection of qualitative information to track the attitudes, concerns and practical issues that arise from participation in the vaccination field trials. The second section identifies the economic data that would be required from participating farms which would support a business case for inclusion of the option to vaccinate cattle as part of disease control efforts. The third identifies remaining evidence gaps which could be the subject of further research.

The objectives of socio-economic monitoring and evaluation are broader than those related to the life science component of the field trial, as they concern the overall context of attitudes and aspirations of all of the stakeholders. As a consequence, while there is no particular need for the socioeconomic research to map directly onto the field trials workpackages, it would be convenient and cost-efficient to involve the farms and their veterinary advisors recruited for the field safety and efficacy trials, since they alone would have first-hand experience of using the vaccine and, accordingly, of adapting their husbandry practices. Recruitment protocols developed for the field trials will ensure a mix of farm enterprises and sizes, and their location in annual testing areas provides a context in which vaccination is most likely to be used in future bTB control regimes. Because of the importance of the wider context, it should be made clear to the recruited participants that some engagement in socio-economic monitoring will also be required throughout the trial.

Conversely, the Approved Finishing Units (AFUs) involved in the blood IFN-γ DIVA test validation trials would not provide these characteristics and as such should not be recruited for socio-economic monitoring. However, advantage could be gained from the proposed design in which these trials are assumed to precede the main field safety and efficacy trials. Farm businesses and veterinary practices involved in the latter phase could be recruited in advance of the main trials' commencement, their perceptions and attitudes investigated and (in the case of farms) an assessment made of their

SHORT TITLE: Feasibility study into testing and validating cattle BCG vaccine and DIVA

Final

Date 05 March 15

– Strictly Confidential –
economic status, to provide a benchmark against which to track evolving perspectives and the economic consequences of introducing vaccination.

**Understanding the motivations and experiences of field trial participants and other stakeholders.**

The objective of qualitative socio-economic monitoring should be to understand participants’ attitudes toward the vaccine, the DIVA test(s) and other issues affecting uptake of vaccination in order to provide evidence to support decisions about whether vaccination becomes a significant element of future bTB control policy. Currently, evidence gaps which would be addressed by this element of the field trial are: how farmers might react in a context in which vaccines become available, in terms of biosecurity practice and cattle enterprise structure and management; how prominent concerns might best be allayed, such as higher probabilities of restrictions arising from relative DIVA sensitivity or market risks associated with vaccines and their products; and how veterinary practices with a high proportion of turnover derived from current testing regimes might be affected.

A wide range of methods can be used to explore perceptions and attitudes, ranging from structured questionnaires through to more discursive forms of inquiry. While standard questionnaire-based approaches are a cost-efficient means of collecting information in a way which can readily be analysed, they suffer from three crucial disadvantages in the current context: they are not particularly useful in providing insight into emotions or behaviour; they rely on having a clear understanding of the interplay between influences on individual decision-making, but this is not clear at the outset; and interpretations of questions may vary between individuals, particularly qualitative evaluation expressed in terms of a Likert scale. As a consequence, more discursive qualitative methods would be preferred for this small group of individuals. Since, additionally, some element of social desirability bias may be anticipated, a mix of complementary individual semi-structured interviews and focus group discussions is recommended. Because of the range of sensitive issues involved, some of the deeper issues involved may not be elicited in open discussions, although this has the obvious corollary that the researchers who conduct individual interviews should be experienced and empathetic. Individual interviews should in preference also be conducted prior to focus group discussions because some degree of experimentation to prompt unanticipated issues could help to develop the topic guide for the latter; interview approaches can also be modified and refined after each of the initial interviews. Focus groups, on the other hand, allow for interaction between participants which can stimulate ideas, challenge thinking and identify differences of view.

Moreover, the attitudes and perspectives of participants in both individual interviews and focus groups could contribute to the management and reporting of the field trials themselves. Prior to the trials, they could comment on the practical arrangements and have the opportunity to modify the trial protocols, in terms of practical implementation on farms. Within the trials, they could explore and resolve problems in administration. Afterwards they could have the opportunity to validate the results, and contribute to recommendations for policy change. A very prominent theme of the key informant interviews reported in Appendix 20 was the need for open and transparent communication. A relationship of trust between researchers and participants should also improve engagement and commitment of participants to the overall investigatory process. With systematic analysis of the resulting qualitative data, greater depth of understanding of the wider implications of vaccination would emerge to complement the central goal of regulatory approval.

The practical organisation of individual interviews and focus groups requires consideration of sampling, thematic content and protocol development, replication and repetition, data capture, and interpretation and reporting. If recruitment is based on those engaged in the WPP6.1 design, this would involve a high probability of being selected, and as a consequence the design of qualitative inquiry should consider minimising the additional burden on participants.

Considerations of validity and representativeness indicate that between 15-20 individual interviews generally need to be undertaken before ‘saturation’ (the likelihood that a representative range of perspectives and attitudes is fully reflected) is achieved. Correspondingly, focus groups normally involve between six to ten members. While some advantages exist in having overlapping (and consequently contrasting) participation in both individual interviews and focus groups, because of the additional burden that this might place on an already unusual level of commitment, it would be preferable to separate these obligations on participants and select for either one or the other activity.
For participating farmers, a minimum of three focus groups would provide reliable and accurate insights if memberships involve different degrees of homogeneity and heterogeneity (with respect to farm size, dairy and beef enterprises, and local disease prevalence); however, with respect to veterinary focus groups, since practitioners themselves will hold experience of working with a wide range of holdings, two focus groups should be sufficient, provided that they are held in contrasting locations.

Since considerable interest will focus on the evolution of attitudes and perspectives before, during and after introduction to the use of vaccines in a working farm context, qualitative monitoring could be conducted in three rounds: on recruitment, but prior to commencement of trials; within the duration of the trials themselves, when experience is both direct and ‘live’; and on conclusion, providing the opportunity to reflect on experiences (including feedback from the two previous rounds) and to contribute to a summative evaluation. Eriksson and Kovalainen (2008, p. 177) observe that focus group research “can be very empowering to the participants that they are treated as experts and allowed to work in close collaboration with the researcher when the focus group works well, it allows you to explore the topic as collective”.

As noted earlier, conducting individual interviews prior to the focus group discussions provides the opportunity to analyse evaluate and modify proposed topic guides. For that reason, detailed protocols are not being provided at this stage, although an outline of the issues that could be considered is provided in Table A.23.1.
### Table A.23.1 Qualitative Research Summaries

<table>
<thead>
<tr>
<th>Series</th>
<th>Timing (in relation to Safety and Efficiency Trials)</th>
<th>Participants</th>
<th>Discussion Themes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-trial semi-structured interviews</td>
<td>Month -5</td>
<td>15 trial farmers 5 veterinarians</td>
<td>1. Direct and indirect experience of impacts of bTB on farm business and farm family</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2. Understanding of and attitude towards vaccination</td>
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<td></td>
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<td></td>
<td>3. Concerns and aspirations regarding vaccination impacts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a. IVP-CP issues</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>b. Protection rate and biosecurity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c. The DIVA test and market risks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. Practical implications of adopting vaccination on farm</td>
</tr>
<tr>
<td>Pre-trial focus group discussions</td>
<td>Month -3</td>
<td>3 farmer groups (24) 2 vet groups (16) 1 stakeholder group (8)</td>
<td>1. Review understanding of and attitude towards vaccination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. Practical experience of adopting vaccination on farm</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3. Labour requirements of participation</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4. Impacts on future planning and enterprise mix.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5. Off-farm impacts, public and supply chain perceptions of vaccination</td>
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<td></td>
<td></td>
<td>6. Suggestions for modifications to field trial protocols to improve implementation</td>
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<tr>
<td>Intra-trial semi-structured interviews</td>
<td>Month +22</td>
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<td>1. Review understanding of and attitude towards vaccination</td>
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<tr>
<td>Intra-trial focus group discussions</td>
<td>Month +24</td>
<td>3 farmer groups (24) 2 vet groups (16) 1 stakeholder group (8)</td>
<td>2. Review scientific evidence arising from trials</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3. Current and future planned changes to structure and management of farm, in response to vaccination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. Suggestions for adoption and implementation of vaccines as component of bTB control strategy</td>
</tr>
<tr>
<td>Post-trial semi-structured interviews</td>
<td>Month +62 (+38 in 7-year trials)</td>
<td>15 trial farmers 5 veterinarians</td>
<td>1. Review experience of participating in trial</td>
</tr>
<tr>
<td>Post-trial focus group discussions</td>
<td>Month +64 (+40 in 7-year trials)</td>
<td>3 farmer groups (24) 2 vet groups (16) 1 stakeholder group (8)</td>
<td>2. Review scientific evidence arising from trials</td>
</tr>
<tr>
<td></td>
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<td>3. Current and future planned changes to structure and management of farm, in response to vaccination</td>
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<td></td>
<td></td>
<td></td>
<td>4. Suggestions for adoption and implementation of vaccines as component of bTB control strategy</td>
</tr>
</tbody>
</table>

Good practice requires providing full information to participants regarding the purpose and use of the material gathered; permission for audio (or video) recording and guarantee of anonymity; and member checks to validate the analysis, interpretation and reporting of excerpts used from transcripts.

This structured and systematic sequence of qualitative investigations which is based on (as far as possible) a balanced panel of participants, complemented with observations from the investigators managing the trials at farm level, forms an essential input into management of (and enhanced engagement with) the field trials. From a policy perspective, without this evidence base of attitudes and behaviour by the main stakeholders, the success of any future regime in which vaccination were approved for use and adopted on a wider scale would be put at risk.

**Understanding the cost and revenue implications of using BCG vaccine in bTB control**

The objective of financial monitoring of farms involved in the field trials should be to understand how the use of vaccination impacts on cost structures, overall business performance and mitigation of the impacts of any subsequent bTB breakdowns. The major uncertainty exposed by the ex-ante Cost-Benefit Analysis was the scale of avoided costs; alongside that, there is currently incomplete knowledge of numbers of animals requiring vaccination due its anticipated effects on transmission, which also has an effect on the economic performance of farms. Extrapolation of results from economic monitoring...
and vaccine efficiency, using farm optimisation models or ‘regional farm’ simulations, allows for different operational models to be investigated. A clearer understanding of both of these issues, based on whole-farm accounting information, would enable better evidence-based policy decisions to be made concerning conditions under which bTB vaccination may be used in future.

To improve on current estimates, observations of the performance of farms in the field trials need to be transformed into economic values, in the same way that plans for the trials themselves have been assessed. This introduces a number of choices in terms of what data should be collected, from whom, and at what frequency throughout the trial. Without a fully informed perspective, appropriate evidence for policy decisions would be unsatisfactory; nevertheless, a prominent additional consideration is that economic monitoring of trial farms places an additional burden on participants, which should be minimised as far as possible to reduce any possible barrier to involvement.

Options for the scope of financial monitoring include either partial budgeting or undertaking full farm income accounting.

- Partial budgeting could be cheap and simple to implement, using non-economist scientists or technicians involved in implementing other parts of the field trials. It would provide assessment of relevant net changes in costs and revenues, would be effective in assessing the direct economic implications of vaccination, and would allow early inferences to be made about the feasibility of its uptake. However, especially in the context of mixed farming systems, it would not consider consequential impacts on other enterprises. More generally, it neglects farms resource endowment constraints, which could be particularly important in the context of labour demand peaks, potential for diversification or off-farm work, or impacts on fixed costs.

- Full farm income accounting has advantages and disadvantages that mirror those of partial budgeting. It requires more resources and would take longer to collect information and provide interpretation. It does, however, provide a more coherent picture and, if combined with balance sheet information, could indicate effects of changing husbandry prompted by vaccination on fixed cost structures. However, there are further advantages and disadvantages that should be considered. Firstly, conducted under the aegis of the Farm Business Survey (FBS), experienced investigators would provide cost-efficiency and veracity in terms of the data provided; and analysis could include comparison with other non-trial farms monitored by the FBS, selected, for example, using propensity score matching. Secondly, however, participants in the FBS receive useful management accounting information which could and is suspected to have effects on their commercial operation; the so-called “observation effect”.

On balance the additional resources required to design, train investigators and accurately monitor partial cattle enterprise budgets would be substantial, in comparison to the scale and scope benefits offered by undertaking full farm accounts using the FBS. Consequently the latter is the recommended approach.

Alternative selection strategies include monitoring either a subsample, or all, of the 100 farms which would be involved in the efficacy and safety trials. While roughly 40% of these farms would be involved in the qualitative research discussed in the previous section, there are strong arguments for an overlap between this group and those selected for financially monitoring, since having focus group participants and individual interviewees undertake a full farm accounting exercise could provide a valuable triangulation perspective on both sets of evidence. Since these 100 farms form a relatively small group (compared to, for example, the 1,094 dairy or other grazing livestock holdings monitored overall by the FBS in England and Wales), for consistency and reliability of results, farm economic data should be collected from all participants.

In terms of timing and repetition of data collection, the minimum required information set of farm accounts could be obtained from the same three points suggested for qualitative investigation; prior to, during, and after completion of, the field trials. In terms of impact assessment, a pre-trial baseline is essential to track the effect of vaccination on performance; the accounting information required could be collected ex-post for the financial year preceding study commencement.
However, the preferred recommendation is for continuous monitoring of farm accounts throughout the field trials period. Intermittent monitoring would involve additional set-up costs of data collection at each point in time, whereas continuity could allow trust to build between investigator and participant, and result in smoother data acquisition with enhanced validity. Perhaps more importantly, unanticipated changes in the external environment may affect interpretation of the results and a greater base of information would allow these to be more readily interpreted. Finally, in relative terms, the number of additional collections required would only be double that involved in intermittent monitoring (and less if the trials produced sufficient evidence on mid-term review to take the decision to end early). Alongside these other considerations, additional data collection effort is justified in terms of more reliable and better quality results.

In summary, we would suggest that full farm accounting information, under a specific contract with the FBS, involving all 100 farms participating in the field trials, be collected for 6 successive financial years, including the year before trials commence. This combination of evidence from field trial efficacy results, annual FBS accounts from each participating farm, and supplementary labour diaries to track additional requirements for unpaid family input, would provide the basis for understanding the benefits of vaccination. It would provide an empirical test of the Brooks-Pollock et al. (2014) modelling predictions. However, unlike the DIVA test performance validation study, selection of participating farms in the efficacy and safety trial would not be undertaken with the express intention of uncovering cases of bTB infection. Rather, the reverse would be anticipated to occur: although participants would be selected in areas of disease prevalence, the effect of vaccination would make breakdowns more unlikely, and where it did occur, the duration and severity of infection would likely be less. Thus to complement the qualitative and quantitative inquiries discussed so far, the final section addresses a few other hiatuses in the evidence base required for future policy decisions.

Identifying evidence gaps for further research

Three additional topics can be suggested to cover gaps in the evidence base not addressed in the two preceding sections. Two topics relate to the enhancement of Cost Benefit assessments of the desirability of using vaccination as an element of future bTB control policies; the other relates to proposals for conducting the DIVA test validation in AFUs.

- The sensitivity analysis conducted in Appendix 21 showed that the major uncertainty affecting the cost-benefit balance concerned benefits of avoided costs of bTB breakdowns; the source of the central estimate was the study by Bennett and Cooke (2006), which relied on data drawn from the years 1998-2000. Although the use of Farm Business Survey data appeared to be a promising means of identifying raised costs on participating farms receiving compensation for compulsorily slaughtered cattle, insufficient variability in the sample produced statistically insignificant estimates (for a discussion of the issues involved in deriving enterprise costs from whole farm accounting data, see Léon et al., 1999). Better evidence-based policy decisions could be implemented on the basis of a replication of the original Bennett and Cooke study.

- Central estimates, and the best-case scenario, of benefit-cost balances, based on actual monetary values, are negative; however, the sign would reverse if imputed values drawn from Bennett and Balcombe’s (2012) study of farmers’ willingness-to-pay for vaccine estimates are included. Again, this evidence is drawn from an empirical study concluded in 2008 (Defra, 2010), and the incidence and associated costs of bTB breakdowns may have changed considerably in the interim. There could also be concern that issues related to understanding of the efficacy of vaccination and its combination and interaction with other potential measures of disease control may affect willingness-to-pay estimates. Consequently, a broader assessment of willingness-to-pay for freedom from bTB could also contribute to better evidence-based policy decisions.

- Key informant interviews reported in Appendix 20 primarily focused on securing farmer engagement in field trials investigating efficacy and safety. Whilst respondents were informed of the intended role of AFUs in the DIVA validation trials, this did not feature prominently in the
concerns and issues that they raised. Of the 21 respondents, 5 discussed AFUs in passing; most stated that the validation trials would be unproblematic, although one raised concerns about the limited numbers currently registered for finishing animals from restricted sources, and whether adequate numbers exist for participation. Hence, prior to field trials commencing, there would be merit in undertaking qualitative interviews with a sample of AFU managers to explore the relevant subset of topics relating to participation which were used in the key informant interviews reported here.

References


APPENDIX 21 WPP12 COST-BENEFIT ANALYSIS OF FIELD TRIAL DESIGNS FOR CATTLE BCG VACCINE AND ASSOCIATED DIVA DIAGNOSTIC TEST IN ENGLAND AND WALES

Defra SE3287

School of Management and Business, Llanbadarn Centre, Aberystwyth University

Peter Midmore

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1. INTRODUCTION

The aim of this Appendix is to provide a financial assessment of the net present value, using the Treasury’s recommended discount rate, of the estimated costs and potential future benefits that accumulate from policy measures based on successful field trials. The field trials themselves consist of two major elements: validation of the diagnostic test which aims to differentiate infected from vaccinated animals (DIVA); and trials that satisfactorily demonstrate the safety and efficacy of the BCG vaccine for cattle such that regulatory approval for its use in the strategy to control bovine tuberculosis (bTB) can be obtained.

In outline, costs include professional fees for implementing, conducting, analysing and reporting the field trial designs themselves, which take account of costs of recruiting and providing information and training for participating farmers, veterinary practitioners’ involvement in administering vaccines and placebos (or similar), and payments to farmers to participate in the trials and to compensate them for any consequential losses which may arise. In net present value terms, these costs will vary according to the length of trial period and the numbers of animals and farms which are required. Once the trial is concluded, there will be additional costs associated with the DIVA test, which is likely to be more expensive than the current SICCT test. This will need to be used where animals which react to the SICCT test are known to be vaccinated, and also on farms experiencing a bTB breakdown. There would be a number of additional “false positives” (vaccinated animals which are not diseased but appear to be so); however, these increases would be offset by fewer bTB cases overall as a result of lower rates of disease. Both of these effects are addressed in the estimates discussed in following sections.
Benefits will arise from the adoption of vaccination which, despite current existing evidence of its partial protective effect, approximately 63%, is likely to have two major effects: it is expected to reduce within-herd transmission in the case of a breakdown, and consequently the average costs of compensation involved in each breakdown; it will also reduce between-herd transmission because of direct and indirect protective effects. Costs and benefits are assessed, in net present value constant (2014) price terms, over a 20 year time horizon.

As with all cost-benefit analyses, there are some very substantial uncertainties to consider. Predicted reductions in within-herd transmission and the rate of new breakdowns are derived from modelling studies. The total costs of breakdowns, both public and commercial, depend on overall market conditions which may affect the value of stock and of the opportunity cost of labour. Finally, there is also interdependence between costs and benefits, to the extent that earlier utilisation of vaccine following a shorter but possibly more expensive trial will bring forward anticipated benefits.

Some intangible factors can also be estimated, but not with any certainty. These include psycho-social costs of breakdowns on farmers and their businesses which, if reduced, would increase the value of benefits. Also on the positive side, reduction in the overall incidence of bTB would provide a more stable primary production base for the dairy and beef supply chains, which would improve their overall efficiency and profitability. Vaccination may be voluntary or compulsory (e.g. either in the whole of the UK, or just in annual testing areas); if voluntary, some farmers may choose not to use it. Intra-EU trade in vaccinated animals would probably not be permitted until substantial practical experience of vaccination and the DIVA test validation had been acquired; farmers may decide to opt not to vaccinate for export markets, or to vaccinate and forego export opportunities. However, note that in 2012, just fewer than 39,000 live cattle were exported from the UK, mainly to Spain and Ireland (House of Commons Library, 2013).

The rest of this Appendix is organised as follows. The next section summarises previous studies of costs and benefits which have influenced the approach taken here, and also identifies the major information sources utilised. Following sections describe the estimation of costs, benefits, and the sensitivity analyses conducted, and the paper concludes with a brief discussion and set of recommendations.

2. SOURCES

Cost-benefit analysis of animal health and disease remains relatively under-researched, despite clear indications of its importance (McInerney, 1996). With regard to bTB, an early example of cost-effectiveness analysis is provided by Barlow (1991) who compared different possum control strategies in a discounting framework. The foundations of more recent work are found in van Schaik et al. (1996), in which costs and benefits of paratuberculosis (Johne’s disease) vaccination are evaluated; the costs of vaccination were substantially outweighed from improved productivity benefits. Bernués et al. (1997) examine costs and benefits of control strategies for bTB in northern Spain, balancing production losses against control costs such as slaughter compensation, testing costs, additional labour requirements and policy administration. More sophisticated bioeconomic integration is provided by Horan and Wolf (2005), combining epidemiological modelling with cost-benefit analysis of bTB prevalence in wildlife, with benefits coming from hunter satisfaction.

The framework adopted in this paper closely follows recent Defra-funded work on badger culling and vaccination (Smith et al., 2007; Wilkinson et al., 2009), though parallel work (Biosecurity New Zealand, 2010) should be noted. Costs include additional policy effort required to produce the benefit, such as licensing and other administration, whereas benefits are conceived as saved expenditure and labour inputs brought about by a reduction in bTB incidence.

Secondary data used in this paper have been drawn from sources including official bTB statistics (Defra, 2014a); estimates of standard costs (Environment and Rural Affairs Committee, 2013; Defra, 2014b); and financial impacts of a bTB herd breakdown (Bennett and Cooke, 2006).

3. COSTS

Costs can be broadly divided into two groups; those associated with field validation of the DIVA test and the safety and efficacy field trials; and on-going additional test costs required subsequent to authorisation of vaccine use.
3.1. DIVA Validation

Six study designs have been suggested as part of the validation of the DIVA, with appropriate powering required to produce samples, respectively, of: vaccinated, test negative cattle which are confirmed bTB negative; vaccinated, test positive cattle which are confirmed bTB positive; and additional samples from unvaccinated bTB negative and positive cattle.

Undiscounted cost estimates for the six designs have been calculated by Triveritas. These do not include capital costs of animal handling equipment: some holdings will already have appropriate cattle crushes, whereas others would need to acquire them in order to participate in the trials. A major selection criterion to assess suitability for participation should be facilities and experience in close handling of cattle, in order to reduce health and safety risks.

Three alternative designs have been produced to generate blood samples from vaccinated cattle exposed to bTB (WPP2.1-2.3). They vary in terms of the proportions and sources of animals used as controls, and in consequence there are small differences in costs. The other three designs, respectively, generate blood samples from vaccinated cattle that remain bTB negative (WPP2.4); blood samples for DIVA testing from unvaccinated cattle (WPP2.5); and blood samples from vaccinated animals exposed to multiple SICCT tests (WPP2.6).

To arrive at a “present cost” estimate a number of assumptions need to be made. The validation study is projected to last around 16 months, from a pre-vaccination SICCT test to a post study clinical examination, blood sampling and enhanced post mortem examination for trial animals (all animals testing positive using the DIVA test, and a proportion of those testing negative). However, typically recruitment of farms into studies takes between 8-12 months; additionally, post-study data analysis, interpretation and reporting will typically take a further six months. Throughout this paper it has been assumed that animals are recruited into the study in equal increments over the initial 8 months, and transmitted in appropriate batch sizes to Approved Finishing Units (AFUs). This has the effect of bringing forward costs in time (it is much more likely that recruitment is bunched towards the end of the recruitment period); in discounting terms, this may slightly inflate present costs, especially if recruiting takes up to 12, rather than 8, months to complete. Thus, in designs WPP2.1-2.2, animals are recruited at a monthly rate of 3,750, the entire study takes 26 months to complete, and costs are allocated across three consecutive years. A further complication regarding WPP2.3 is that the animals vaccinated will occur according to the normal intake into the Approved Finishing Units, and so an additional period of up to a year will be required to recruit the required numbers. This extra lag in the process will extend the study across 36 months. It has also been assumed that the professional fees of the Contract Research Organisation are paid in equal monthly instalments.

Taking into account the investigator and labour costs (which include training, test procedures, and shipment costs), farm and Approved Finishing Unit costs (extra labour, animal handling, transport and maintaining animals longer than standard practice) and professional fees of the Contract Research Organisation, Table A21.3.1 provides total undiscounted and annual net present costs (using the Treasury recommended real discount rate of 3.5%) of the six DIVA validation designs.
Table A21.3.1 Cost Estimates for WPP2 DIVA Validation

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<th>£th 2014</th>
<th>WPP2.1</th>
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</tbody>
</table>

The total present cost of the recommended designs (WPP2.1 and WPP2.4) in 2014 prices, is £15.5 million.

3.2. Safety and Efficacy

Three trial designs have been produced to explore efficacy and field safety of the BCG vaccine. This is required to obtain a Marketing Authorisation, and to respond to EFSA concerns which if addressed, would facilitate the change in European Law necessary to allow trade in vaccinated animals. These designs, respectively, will produce the data necessary on efficacy and safety of the BCG vaccine in reducing bTB infection in cattle (WPP6.1); in bovine neonates (WPP6.2); and in cattle herds (WPP6.3).

The WPP6.1 trial design on safety and efficacy on individual animals would involve, on current projections and depending on the outcome of WPP2, 31,500 animals from herds in annual testing areas, studied over a five year period, with 50% of animals vaccinated and 50% controls; vaccinations will occur on commencement of the study and annually four times more, and with follow-up DIVA tests at six-monthly intervals. Animals which test DIVA positive during the study period will have a gamma blood test and enhanced post mortem examination. With a recruitment lead-in and post-trial analysis and reporting, the total time envisaged for the study is just over 6 years (73 months). However, an interim analysis will be conducted three years into the experiment, to assess whether sufficient bTB breakdowns have occurred, in this case with lead in time and reporting, the earliest that the trial could conclude would be just over four years (49 months).

The WPP6.2 trial design on neonates would involve 500 animals, vaccinated twice and tested five times for bTB status. This design is specifically to generate data relating to the reduction of lung lesions. This design, with recruitment lead-in and post-trial analysis and reporting, is envisaged to take 37 months, and could run concurrently with the first design, WPP6.1.

The third study design, WPP6.3, investigates any effects of the BCG vaccine on transmission at herd level and would involve paired whole farm treatments using either vaccine or Control treatment. The study would involve matching similar farms being recruited within groups of 2. One farm would go through vaccination while the second farm would go through acting as a control to its paired IVP farm. Based on the socio-economic findings, and as discussed in the Executive Summary of the main report we do not think such a matched pair whole farm design is feasible as part of initial UK field trials. A study design that would involve 240 farms was costed by Triveritas (WPP6.3) but would only have 30% statistical power, and a larger similar study using 1,000-1,500 matched herds (see Discussion in Main Report) would be required to achieve a more suitable 80-90% statistical power. Again, with lead-in and reporting allowances, the WPP6.3 design would last just over 3 years, in total 37 months. A design of this type could be implemented in the eighth year of the trials, if further evidence of efficacy were to be required by regulators. Table A21.3.2 sets out, on the same basis as Table A21.3.1, the discounted cost estimates.

---

4 This table, and those which follow, illustrate a minimum validation study period before field trials commence. In this instance, the selected DIVA validation study to continue beyond commencement of WPP6 due to the number of animals needed to generate sufficient (300) bTB positive samples. The implication is that if the efficacy of the vaccine is better than expected, then overall trial costs will also increase.
costs of trial designs for Work package 6.

### Table A21.3.2 Cost Estimates for WPP6 Safety and Efficacy Trials

<table>
<thead>
<tr>
<th>Net Present Values, £th 2014</th>
<th>WPP6.1</th>
<th>WPP6.1 (ended early)</th>
<th>WPP6.2</th>
<th>WPP6.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiscounted total</td>
<td>£33,456.4</td>
<td>£17,103.9</td>
<td>£1,633.9</td>
<td>£20,315.1</td>
</tr>
<tr>
<td>Discounted:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 4</td>
<td>£5,193.3</td>
<td>£5,214.0</td>
<td>£653.1</td>
<td></td>
</tr>
<tr>
<td>Year 5</td>
<td>£4,419.7</td>
<td>£4,463.1</td>
<td>£264.2</td>
<td></td>
</tr>
<tr>
<td>Year 6</td>
<td>£4,270.3</td>
<td>£4,659.2</td>
<td>£495.5</td>
<td></td>
</tr>
<tr>
<td>Year 7</td>
<td>£4,125.8</td>
<td>£72.8</td>
<td>£14.7</td>
<td></td>
</tr>
<tr>
<td>Year 8</td>
<td>£4,722.7</td>
<td></td>
<td></td>
<td>£6,308.6</td>
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<tr>
<td>Year 9</td>
<td>£4,026.6</td>
<td></td>
<td></td>
<td>£6,693.2</td>
</tr>
<tr>
<td>Year 10</td>
<td>£30.2</td>
<td></td>
<td></td>
<td>£2,518.7</td>
</tr>
<tr>
<td>Year 11</td>
<td></td>
<td></td>
<td></td>
<td>£30.1</td>
</tr>
<tr>
<td>Total</td>
<td>£26,788.6</td>
<td>£14,409.1</td>
<td>£1,427.5</td>
<td>£15,550.5</td>
</tr>
</tbody>
</table>

The total discounted present cost of the recommended designs (WPP6.1 and WPP6.2) in 2014 prices, is £28.2 million; potentially, with favourable interim results, this could be lower, at £15.8 million.

Four further trial designs address safety issues: these consider bacterial shedding through milk, animal respiration and semen. The numbers of animals involved are smaller and the durations are less than in the designs considered previously. The first (WPP8.1) involves sampling milk from vaccinated animals; the second (WPP8.2), nasal swabbing from vaccinated animals; the third (WPP8.3) uses combined sampling of milk and nasal swabs; and the final study (WPP8.4), which has costs estimated on the basis of a subcontract, involves sampling semen from vaccinated bulls. Numbers of animals involved are 240, 80, 240, and 20, respectively; each study, including recruitment and reporting, would last approximately 18 months. Arising from concerns expressed in the key stakeholder interviews, it is suggested in the Executive Summary that these safety trials could be carried out initially. Present cost estimates of these four designs appear in Table A221.3.3 (WPP8.1 and 8.2 plus WPP8.4 are the recommended designs, at a present discounted cost in 2014 prices of £1.3 million).

### Table A21.3.3 Cost Estimates for WPP8 Post-Vaccination Bacterial Dissemination

<table>
<thead>
<tr>
<th>£th 2014</th>
<th>WPP8.1</th>
<th>WPP8.2</th>
<th>WPP8.3</th>
<th>WPP8.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiscounted total</td>
<td>£522.7</td>
<td>£379.0</td>
<td>£583.9</td>
<td>£387.1</td>
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<tr>
<td>Discounted:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>£328.2</td>
<td>£271.1</td>
<td>£403.5</td>
<td>£244.2</td>
</tr>
<tr>
<td>Year 2</td>
<td>£187.9</td>
<td>£104.2</td>
<td>£174.3</td>
<td>£138.1</td>
</tr>
<tr>
<td>Total</td>
<td>£516.1</td>
<td>£375.3</td>
<td>£577.8</td>
<td>£382.3</td>
</tr>
</tbody>
</table>

#### 3.3. Post-Trial Vaccination and Testing Costs

Provided that the trials demonstrate that the vaccine is safe and effective, and that the DIVA test is shown to accurately and efficiently distinguish vaccinates from diseased animals, the regulatory framework will ultimately be modified to allow vaccination to be deployed more generally. The Commission view (published by Defra, 2013) is that EFSA’s recommendations, and marketing authorisation could be obtained 12-18 months after satisfactory trial results become available. A further period of experience would also be required (up to five years) during which vaccinated...
animals could not be traded between EU member states. However, as noted earlier, the numbers involved are relatively small, less than half of 1% of the total GB cattle population and it is likely that only a proportion of such animals would require vaccination. The annual average total export value of live cattle to other EU countries between 2011 and 2013 was less than half a million pounds.\(^5\)

Costs would be very substantial if all cattle in the UK were subject to compulsory annual vaccination, since subsequent assessments of bTB reactor status would involve use of the DIVA test. It is more likely that vaccination would be deployed only in the current annual testing and edge areas (Defra, 2007), even though in Wales this would involve vaccination in largely disease-free areas that are being annually tested as part of a robust eradication strategy. Current (2013) numbers of cattle in these areas in England are just under 3.5 million, of which 0.8 million are dairy breeding animals and 0.4 million are beef breeding animals. In Wales, total cattle number 1.1 million, of which 0.4 million each are dairy or beef breeding animals. However, of these, not all would require protection; breeding animals, young stock kept for breeding, store stock moving between holdings should be vaccinated, but veal calves and suckled beef animals finished on the holding of origin exist for too short a period to require it. Around 10% of the remaining male animals reared for slaughter are on holdings using a traditional suckler production system (EBLEX, 2009); consequently, assuming that the structure of the cattle sector remains similar to the present when vaccination is introduced, 4.4 million animals would be vaccinated annually.

In the analysis of Section 3, current standard costs of vaccine and administration were estimated at £11.50, and the cost of the DIVA test at £30, plus a cost of £0.47 per sample transported by courier for laboratory analysis (on the basis of average batches of 50 samples). It has been suggested that vaccine sensitisation to the skin test “falls off so that after nine months only 10% of animals test positive as a result of the vaccination. Therefore, it may be that in a TB clear herd only 10% of animals will require the DIVA test” (Environment, Food and Rural Affairs Committee, 2013, p.14). However, in the absence of any experience in use of the DIVA as a serial test to confirm that a SICCT reaction is indeed vaccination-induced, this may prove to be optimistic; in the worst case, all vaccinates may need to be serially tested. The initial assumption used to estimate costs is that 40% of vaccinates require DIVA tests.

Moreover, the higher sensitivity of the DIVA test would increase detection of bTB positive animals earlier, leading initially to higher compensation costs. However, depending on its specificity, it could also falsely identify more cattle as positive, also leading to higher compensation costs (van Dijk, 2013, p. 4, suggests that the Y-interferon test would mostly likely result in 3 false-positive tests in an average size UK herd, compared to 2 resulting from the SICCT test). Consequently, the effects of reduced specificity of serial testing have been included, in terms of the average additional slaughter of one animal per herd tested using the DIVA.

However, testing would be several orders of magnitude greater than envisaged in the field trials, and considerable economies of scale might be envisaged. Advice from the AHVLA that suggests that at the estimated scale of testing, DIVA laboratory costs would be reduced to approximately £12: with an allowance of £3 added for drawing the samples (for which veterinary qualification would not be required) and transport, this would represent a 50% reduction in cost achieved from scale economies. This should be considered the lowest attainable level, since considerable learning costs would be experienced as DIVA testing is introduced more widely; the initial assumption used to estimate unit DIVA costs is mid-way between current and AHVLA forecast (before drawing and transport), in total £22.50 per test.

At current prices, with cattle populations in these areas similar to those of today, the initial annual cost of vaccinating these cattle would be £50.3 million, the cost of additional DIVA testing of vaccinates appearing positive at annual tests would be £10.0 million, additional short interval DIVA tests associated with breakdowns would cost £32.6 million annually, and higher levels of compulsory slaughter would cost £59.4 million.

Finally, fewer short interval tests can be anticipated as the number of breakdowns declines from reduced transmission of infection from vaccination; these numbers have been estimated on the same basis as that used to assess benefits, in Section 4. Table A21.3.4 provides net present costs in constant (2014) prices for vaccination and testing over the post-trial period of assessment, with the trials having different outcomes. The first variant assumes that the safety and efficacy trials can be completed after four years, where the interim analysis provides sufficient evidence for vaccination to be adopted as a control measure, with an extra year of lag anticipated for the regulatory approval process. The second variant is WPP6.1, completed after seven years.

<table>
<thead>
<tr>
<th>Table A21.3.4</th>
<th>Net Present Cost Estimates (£m 2014) for Post-Trial Vaccination and Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 year trials</td>
</tr>
<tr>
<td>Undiscounted total</td>
<td>£2,049.5</td>
</tr>
<tr>
<td>Discounted:</td>
<td></td>
</tr>
<tr>
<td>Year 8</td>
<td>£130.0</td>
</tr>
<tr>
<td>Year 9</td>
<td>£124.0</td>
</tr>
<tr>
<td>Year 10</td>
<td>£118.5</td>
</tr>
<tr>
<td>Year 11</td>
<td>£113.2</td>
</tr>
<tr>
<td>Year 12</td>
<td>£108.2</td>
</tr>
<tr>
<td>Year 13</td>
<td>£103.6</td>
</tr>
<tr>
<td>Year 14</td>
<td>£99.1</td>
</tr>
<tr>
<td>Year 15</td>
<td>£95.8</td>
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<tr>
<td>Year 16</td>
<td>£92.5</td>
</tr>
<tr>
<td>Year 17</td>
<td>£89.4</td>
</tr>
<tr>
<td>Year 18</td>
<td>£86.4</td>
</tr>
<tr>
<td>Year 19</td>
<td>£83.5</td>
</tr>
<tr>
<td>Year 20</td>
<td>£80.6</td>
</tr>
<tr>
<td>Total</td>
<td>£1,324.7</td>
</tr>
</tbody>
</table>

Clearly, post-trial costs are likely to be substantially greater than those of the trials themselves, even if unit costs of testing can be reduced substantially: the overall range of costs, in present value terms, is between £1.0 and £1.3 billion, as Table A21.3.5 shows. In relative terms, trial costs form 2.4% to 4.4% of overall estimated present costs.

<table>
<thead>
<tr>
<th>Table A21.3.5</th>
<th>Overall Comparative Costs (Net Present Values, £m 2014 prices)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 year trials</td>
</tr>
<tr>
<td>Trial Costs</td>
<td>£32.3</td>
</tr>
<tr>
<td>Post-Trial Vaccination and Testing Costs</td>
<td>£1,324.7</td>
</tr>
<tr>
<td>Total</td>
<td>£1,357.0</td>
</tr>
</tbody>
</table>

4. BENEFITS

The effects of vaccination are anticipated to help reduce bTB disease in cattle. However, predicting the epidemiological state of the disease and the likely impact of vaccination on herd breakdowns and infections rates a priori is hazardous in the extreme, for two reasons: first, vaccines cannot be used until the field trials are satisfactorily completed and the science case for use of BCG vaccine and DIVA test has been accepted, which will not happen before 2023, and could take until 2026; second, while it is hoped that both breakdowns that occur in vaccinated herds will be less severe in terms of numbers of reactors, and that fewer infected animals will lead to lower herd to herd transmission (or herd to environment to herd transmission), it is the field trials themselves that will produce the best predictive
evidence of such quantitative impacts. In the previous decade, substantial rates of increased infection were forecast, and although recent provisional statistics indicate declines in incidence (Defra, 2014a), this deviation from past trends cannot yet be viewed as established (Blake and Donnelly, 2014).

Some indication of the way in which the complex mix of interacting effects might be envisioned can be gleaned from a useful, recently available spatiotemporal dynamic stochastic modelling exercise (Brooks-Pollock et al., 2014; hereafter the “EBP model”). This combines within-farm and between-farm transmission (the former based on stochastic transmission, maintenance of environmental reservoirs of infection, and detection based on the sensitivity of the SICCT test; the latter on movements of individual cattle between farms). On the basis of this extensive framework, outcomes of various control measures based on hypothetical interventions between 2005 and 2010 are explored. These included reduced transmission (a simulation of the effect of vaccination) and improved testing (representing the likely DIVA impact). This provides a qualified basis for what might happen if BCG vaccination became widely used in the first six years of permitted vaccine use, although as each control measure is modelled separately it cannot be assumed that their impacts are additive. Hence the proportionate impact of vaccination, alone, without any effects of improved testing, has been used to estimate the benefits of vaccination in terms of reductions in bTB prevalence.

In this analysis of estimated benefits, the benchmark is taken as the average of number of herds under restriction and numbers of infected animals (either compulsorily slaughtered or detected at slaughterhouse) over the past three years (2011-2013). Then, estimates of the reduction in total animals compulsorily slaughtered and the total numbers of farms on movement restrictions in each year following the introduction of vaccination are made on the basis of the EBP model of reduced transmission, applying uniform decrements for six years to the benchmark level of 2011-2013. It is more likely that the effects would be cumulative, so this assumption will perhaps overstate the benefits when discounting is applied. Effects beyond six years are uncertain: more beneficial interactions could cumulate, or the impact could plateau. Since this is uncertain it has been assumed, *de minimus*, that the benefits in future years remain constant. Estimates are provided in Table A21.4.1.

| Table A21.4.1 Estimates of Post-Trial Vaccination Impact on Restricted Herds and bTB cases |
|-----------------------------------------------|-------------------------------------------------|
| **Compulsory Slaughter and Slaughterhouse confirmed cases** | **Herds that are not Officially bTB Free** |
| Benchmark | 35,156 | 8,817 |
| Post-trial Year 1 | 32,296 | 8,273 |
| Post-trial Year 2 | 29,437 | 7,729 |
| Post-trial Year 3 | 26,577 | 7,185 |
| Post-trial Year 4 | 23,718 | 6,641 |
| Post-trial Year 5 | 20,858 | 6,097 |

The financial costs of confirmed new bTB breakdowns were last estimated empirically by Bennett and Cooke (2006). These costs have been updated to 2014 using appropriate indicators and are shown in Table A21.4.2, below. These have been used as the basis of estimation of benefits of reduced infection and reduced transmission.

---

6 Using data from the Farm Business Survey, a regression of numbers of animals compulsorily slaughtered and control variables on total labour costs produced a positive, but statistically insignificant, coefficient for the additional labour costs. Therefore updated estimates of farm breakdown costs based on Bennett and Cook (2006) are the best available.
Table A21.4.2  Estimated Average Cost of a bTB Breakdown

<table>
<thead>
<tr>
<th></th>
<th>£ 2014</th>
<th>Cost to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Farmer</td>
</tr>
<tr>
<td>Slaughter (12.8 animals)</td>
<td>£4,080</td>
<td>£11,284</td>
</tr>
<tr>
<td>Movement restriction</td>
<td>£1,170</td>
<td>£0</td>
</tr>
<tr>
<td>Isolation</td>
<td>£480</td>
<td>£0</td>
</tr>
<tr>
<td>Testing (5.2 herd tests on 200)</td>
<td>£3,564</td>
<td>£8,352</td>
</tr>
<tr>
<td>Other tests (200 contiguous/traced tests)</td>
<td>£706</td>
<td>£1,655</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>£10,000</td>
<td>£21,291</td>
</tr>
</tbody>
</table>

These costs can be applied to the impacts projected in Table A21.4.1; however, for appropriate evaluation, a discount factor needs to be applied, and this depends, as in the previous section, on how quickly vaccination can be permitted to be widely adopted. Using the same procedure as adopted in Table A21.3.4, discounted values of the estimated benefit arising from successful field trials are provided in Table A21.4.3.

Table A21.4.3  Net Present Benefit Estimates (£m 2014) of Reduced Numbers, Duration and Severity of bTB Breakdowns

<table>
<thead>
<tr>
<th>£m 2014</th>
<th>7 year trials</th>
<th>10 year trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiscounted total</td>
<td>£665.3</td>
<td>£423.4</td>
</tr>
<tr>
<td>Discounted:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 9</td>
<td>£9.2</td>
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<tr>
<td>Year 10</td>
<td>£17.8</td>
<td>£8.3</td>
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<td>Year 11</td>
<td>£25.7</td>
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<tr>
<td>Year 12</td>
<td>£33.1</td>
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<td>Year 13</td>
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<td>Year 15</td>
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<td>Year 16</td>
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<td>Year 17</td>
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<td>Year 18</td>
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<tr>
<td>Year 19</td>
<td>£32.6</td>
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<tr>
<td>Year 20</td>
<td>£31.5</td>
<td>£31.5</td>
</tr>
<tr>
<td>Total</td>
<td>£370.6</td>
<td>£246.1</td>
</tr>
</tbody>
</table>

On this reasoning, costs outweigh benefits over the 20-year time horizon. Subtracting Present Benefits (Table A21.4.3) from Present Costs (Table A21.3.5), the balance of costs over benefits is £986 million in the early end scenario (with a Benefit-Cost ratio of 0.27); it is £768 million in the full term scenario (with a Benefit-Cost ratio of 0.24). However, this assessment has been based on highly restrictive assumptions, the variation of which will be explored in the following section.

5. SENSITIVITY ANALYSIS

The central estimates of costs and benefits derived in the two preceding sections may be varied to determine the importance of the main assumptions involved. In this section, the effects of modifying the major assumptions about the future are explored to assess the extent of deviation in the cost-benefit balance. However, as Boardman et al. (2011) note in their standard text on Cost-Benefit Analysis, the...
large number of uncertainties involved in most exercises make “the brute force approach of looking at all combinations of assumptions infeasible” (p. 177). The three more manageable approaches that they suggest are partial sensitivity analysis, in which the effects on the Benefit-Cost balance of varying one assumption, with all others held constant, are explored; worst- and best-case analysis, in which combinations of reasonable assumptions are explored to determine if the sign of the Benefit-Cost balance might be reversed; and Monte Carlo approaches, in which a distribution of net benefits is constructed by repeated random draws from ranges of key assumptions. The third approach is not explored here due to resource constraints; in successive sub-sections, four major groups of assumption are subjected to partial sensitivity analysis, followed by worst- and best case scenarios.

5.1. Post-Trial Testing and Vaccination Costs

Post-trial costs are based on current indications of vaccine prices, veterinary fees, and testing costs midway between current and AHVLA-based future estimates. Vaccination, administered (and possibly certified) by a veterinary professional would be compulsory for most animals (breeding stock and others moving between holdings) within annual testing and edge areas; the 40% of vaccinates testing positive, using the current SICCT test, would then be serially tested with the DIVA test.

The main sensitivities explored here concern DIVA testing costs (higher and lower than the central estimates); the rates of vaccinated cattle with positive SICCT reaction, requiring a further DIVA test; lower vaccination costs as a result of scale economies in production and purchasing; and different numbers of animals under intensive testing regimes where vaccination needs to be applied.

The first partial sensitivity regarding post-trial costs sets DIVA testing costs either at their current level (£30 per test), if anticipated advantages of testing on such a large scale are not realised, or at the level indicated as potentially achievable by the AHVLA (£15 per test). A more significant saving could be derived from development and validation of a DIVA skin test with costs at or near those of the current SICCT test, which would involve a 75% reduction. However, were the Cost-Benefit balance to rely on this development, the research and development costs associated with a skin DIVA test ought also to be included in the analysis and overall trial design and timetable would also be affected. Table A21.5.1 provides details.

<table>
<thead>
<tr>
<th>DIVA Test Cost</th>
<th>Cost</th>
<th>Benefit</th>
<th>Balance</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 year trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>£15.00</td>
<td>£1,257.4</td>
<td>£370.6</td>
<td>-£886.8</td>
<td>0.29</td>
</tr>
<tr>
<td>£22.50 (Central Estimate)</td>
<td>£1,357.0</td>
<td>£370.6</td>
<td>-£986.4</td>
<td>0.27</td>
</tr>
<tr>
<td>£30.00</td>
<td>£1,456.6</td>
<td>£370.6</td>
<td>-£1,086.0</td>
<td>0.25</td>
</tr>
<tr>
<td>10 year trials</td>
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<tr>
<td>£15.00</td>
<td>£939.6</td>
<td>£246.1</td>
<td>-£693.5</td>
<td>0.26</td>
</tr>
<tr>
<td>£22.50 (Central Estimate)</td>
<td>£1,013.6</td>
<td>£246.1</td>
<td>-£767.5</td>
<td>0.24</td>
</tr>
<tr>
<td>£30.00</td>
<td>£1,087.6</td>
<td>£246.1</td>
<td>-£841.5</td>
<td>0.23</td>
</tr>
</tbody>
</table>

There may also be some scope to reduce vaccine costs. The BCG vaccine is, of course widely used in the human population, with annual global production of 348 million doses; while “the production of vaccines is marked by high sunk and fixed costs, low marginal costs, and significant scale economies” (Moss, 2012, p.13; see also You et al., 2010), there is also a high degree of price-making power among pharmaceutical multinational conglomerates. Correspondingly, there would not be scope for any major scale economies in professional costs of administration; indeed, additional demands on professional veterinary services may raise prices. With a 75% reduction in vaccine cost but constant professional veterinary administration fees, the overall cost of vaccination would be 36% lower than that used in the central estimate. For symmetry, this
partial sensitivity analysis includes variations in vaccination costs 36% above and below that assumed in the central estimate, the latter to reflect potential inflation of professional fees.

Table A21.5.2  Sensitivity Test: Post-Trial Vaccination Costs

<table>
<thead>
<tr>
<th>Vaccine Cost</th>
<th>Cost</th>
<th>Benefit</th>
<th>Balance</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 year trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>£7.36</td>
<td>£1,188.8</td>
<td>£370.6</td>
<td>-£818.2</td>
<td>0.31</td>
</tr>
<tr>
<td>£11.50 (Central Estimate)</td>
<td>£1,357.0</td>
<td>£370.6</td>
<td>-£986.4</td>
<td>0.27</td>
</tr>
<tr>
<td>£15.64</td>
<td>£1,525.2</td>
<td>£370.6</td>
<td>-£1,154.6</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>10 year trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>£7.36</td>
<td>£891.2</td>
<td>£246.1</td>
<td>-£645.1</td>
<td>0.28</td>
</tr>
<tr>
<td>£11.50 (Central Estimate)</td>
<td>£1,013.6</td>
<td>£246.1</td>
<td>-£767.5</td>
<td>0.24</td>
</tr>
<tr>
<td>£15.64</td>
<td>£1,136.0</td>
<td>£246.1</td>
<td>-£889.9</td>
<td>0.22</td>
</tr>
</tbody>
</table>

With the scale of reductions in disease propagation envisaged in Section 4, there may also be scope to relax the annual testing regime in Edge areas once the benefits of increasing herd immunity materialise sufficiently. If, after five years, vaccination and DIVA tests in Edge areas cease to be compulsory, this implies 21% fewer animals than those assumed in the central estimate (the number of cattle in the Edge areas, and a corresponding proportionate reduction in Wales where, even at present, the case is being made for Anglesey to be declared OTF). However, an alternative could be that vaccination is less effective than anticipated in the central estimates, that five years after the trials, present Edge areas become high-risk areas, and a new Edge is established in neighbouring local authority areas (Bedfordshire, Cambridgeshire, Greater London, Greater Manchester, Hertford, Kent, Lincolnshire, Merseyside, South Yorkshire, Surrey, West Sussex), involving a 9% increase in vaccinated cattle. Table A21.5.3 indicates the effects of varying assumptions on levels of compulsory vaccination.

Table A21.5.3  Sensitivity Test: Numbers of Animals Requiring Vaccination and Testing

<table>
<thead>
<tr>
<th>Vaccinated Population</th>
<th>Cost</th>
<th>Benefit</th>
<th>Balance</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 year trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21% fewer vaccinates</td>
<td>£1,225.7</td>
<td>£370.6</td>
<td>-£855.1</td>
<td>0.30</td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,357.0</td>
<td>£370.6</td>
<td>-£986.4</td>
<td>0.27</td>
</tr>
<tr>
<td>9% more vaccinates</td>
<td>£1,413.3</td>
<td>£370.6</td>
<td>-£1,042.7</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>10 year trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21% fewer vaccinates</td>
<td>£935.8</td>
<td>£246.1</td>
<td>-£689.7</td>
<td>0.26</td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,013.6</td>
<td>£246.1</td>
<td>-£767.5</td>
<td>0.24</td>
</tr>
<tr>
<td>9% more vaccinates</td>
<td>£1,046.9</td>
<td>£246.1</td>
<td>-£800.8</td>
<td>0.24</td>
</tr>
</tbody>
</table>

5.2. Benefits: Level of Impacts and Cost Savings

The central projections of effects of vaccination on the level of herd breakdowns and number of individual cases of bTB are derived from the EBP dynamic stochastic simulations. The central estimates apply average proportionate reductions from the reduced transmission control measure to the benchmark cattle and holding population of average values in the three years 2011-2013. However, depending on the effect of other control measures, disease prevalence may be greater or less than the mean level. Because the projections stem from model-based statistical inference, the proportionate reductions implied by upper and lower 95% confidence limits around these mean levels obtained from model outputs could be applied to the benchmark population. This partial sensitivity analysis involves assuming either a higher or a lower response to
vaccination (represented by the upper and lower confidence limits, respectively) resulting in avoided costs of numbers of animals slaughtered either 30% higher or lower than assumed in the central estimate of the impact of avoided costs; correspondingly, reduction in herds under restriction could be 5% higher or lower. The effect of varying this assumption is shown in Table A21.5.4.

<table>
<thead>
<tr>
<th>Table A21.5.4</th>
<th>Sensitivity Test: Herd Breakdowns and Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost</td>
</tr>
<tr>
<td>7 year trials</td>
<td></td>
</tr>
<tr>
<td>Upper 95% Confidence Limit</td>
<td>£1,357.0</td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,357.0</td>
</tr>
<tr>
<td>Lower 95% Confidence Limit</td>
<td>£1,357.0</td>
</tr>
<tr>
<td>10 year trials</td>
<td></td>
</tr>
<tr>
<td>Upper 95% Confidence Limit</td>
<td>£1,013.6</td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,013.6</td>
</tr>
<tr>
<td>Lower 95% Confidence Limit</td>
<td>£1,013.6</td>
</tr>
</tbody>
</table>

The central estimates of Section 4 were based on the EBP model of reduced bTB transmission rates over 6 years, divided into uniform annual decrements of cases and herd breakdowns; these variables were then assumed to plateau for the remainder of the assessment horizon. However, it is possible that there could be a continuing decline. The next partial sensitivities make the assumption that disease incidence declines further, with the same annual decrements, for two and four more years. After four years the number of cases of bTB would have declined to just over 12,000 annually, and the number of affected holdings to 4,500 (decreases from benchmark of 35% and 51%, respectively).

<table>
<thead>
<tr>
<th>Table A21.5.5</th>
<th>Sensitivity Test: bTB Incidence Declining Further into Assessment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost</td>
</tr>
<tr>
<td>7 year trials</td>
<td></td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,357.0</td>
</tr>
<tr>
<td>8 Year Decline</td>
<td>£1,357.0</td>
</tr>
<tr>
<td>10 Year Decline</td>
<td>£1,357.0</td>
</tr>
<tr>
<td>10 year trials</td>
<td></td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,013.6</td>
</tr>
<tr>
<td>8 Year Decline</td>
<td>£1,013.6</td>
</tr>
<tr>
<td>10 Year Decline</td>
<td>£1,013.6</td>
</tr>
</tbody>
</table>

The other factor which affects benefits is the cost saving from fewer breakdowns. Bennett and Cooke (2006), on whose work this measure is based, stress the wide variation of costs across farms, with some experiencing negligible impacts while others endure extremely high levels. From a limited number of cases in the South West of England, Butler et al. (2010, p. 46) find an average cost to farmers of around £25,000, two and a half times higher than the estimates used as a base here. However, the reverse could also be true, especially if vaccination has most impact on smaller scale breakdowns and is less effective in dealing with incidence in larger herds. Table A21.5.6 sets out the impact on the central estimates of both double and half of cost savings on farms.
**5.3. Consequences of Delays**

The overall balance between costs and benefits is undertaken assuming that passage between any phases of the fieldwork proceeds more or less without interruption, and that the ability to use vaccination as a control measure is permitted within the "12-18 months" envisaged by the European Commissioner. In fact, there is likely to be delay in the decision-making process at each point, if not at others in the process. The consequences of delays affect both costs and benefits: costs will be incurred later in the time horizon used for analysis, and this will be discounted more heavily; the same applies to benefits, but as the flow of benefits will commence later, fewer on aggregate will accrue within a fixed time horizon.

Table A21.5.6 assesses the consequences of delays into the timetable in two ways. In the first variant, an extra year of delay is introduced between the end of a DIVA validation and commencement of the field trials; the second variant adds a further year of delay to the first, between the end of the field trials and widespread adoption of vaccination.

**Table A21.5.6 Sensitivity Test: Avoided Costs Farm of Breakdowns**

<table>
<thead>
<tr>
<th></th>
<th>Cost</th>
<th>Benefit</th>
<th>Balance</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 year trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% reduction in avoided farm costs</td>
<td>£1,357.00</td>
<td>£307.30</td>
<td>-£1,049.70</td>
<td>0.23</td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,357.00</td>
<td>£370.60</td>
<td>-£986.40</td>
<td>0.27</td>
</tr>
<tr>
<td>100% increase in avoided farm costs</td>
<td>£1,357.00</td>
<td>£497.10</td>
<td>-£859.90</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>10 year trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% reduction in avoided farm costs</td>
<td>£1,013.60</td>
<td>£204.10</td>
<td>-£809.50</td>
<td>0.20</td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,013.60</td>
<td>£246.10</td>
<td>-£767.50</td>
<td>0.24</td>
</tr>
<tr>
<td>100% increase in avoided farm costs</td>
<td>£1,013.60</td>
<td>£330.20</td>
<td>-£683.40</td>
<td>0.33</td>
</tr>
</tbody>
</table>

**Table A21.5.6 Sensitivity Test: Timetable Disruption**

<table>
<thead>
<tr>
<th></th>
<th>Cost</th>
<th>Benefit</th>
<th>Balance</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7 year trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,357.0</td>
<td>£370.6</td>
<td>-£986.4</td>
<td>0.27</td>
</tr>
<tr>
<td>1 year delay</td>
<td>£1,091.1</td>
<td>£286.2</td>
<td>-£804.9</td>
<td>0.26</td>
</tr>
<tr>
<td>2 years delay</td>
<td>£866.7</td>
<td>£207.4</td>
<td>-£659.3</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>10 year trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Estimate</td>
<td>£1,013.6</td>
<td>£246.1</td>
<td>-£767.5</td>
<td>0.24</td>
</tr>
<tr>
<td>1 year delay</td>
<td>£773.7</td>
<td>£207.4</td>
<td>-£566.3</td>
<td>0.27</td>
</tr>
<tr>
<td>2 years delay</td>
<td>£670.8</td>
<td>£170.0</td>
<td>-£500.8</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**5.4. Sensitivity Analyses: Best and Worst Cases**

Under reasonable assumptions, best and worst cases are examined to determine whether any combination of them might reverse the sign of net benefits. For pragmatic reasons, the paradoxical result of a slight delay in field trials which improves the cost-benefit balance is not included; correspondingly, the best case involves assuming that the trials would be ended early, enabling vaccines to be used more quickly, with correspondingly higher benefit-cost ratios, whereas the worst assumes that a five year efficacy trial is needed, which results in lower like-for-like benefit-cost ratios.

The main assumptions, variations, sources and consequences of the partial sensitivity analyses discussed in previous sub-sections and reported in Tables A21.5.1- A21.5.5 are set out in Table A21.5.7.
### Table A21.5.7  Summary of Partial Sensitivity Analyses

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Value</th>
<th>Source/justification</th>
<th>Sensitivity range</th>
<th>Effect on Benefit-Cost Ratio 7 year trial</th>
<th>Effect on Benefit-Cost Ratio 10 year trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIVA test cost</td>
<td>£22.50</td>
<td>AHVLA Advice</td>
<td>£15.00-£30.00</td>
<td>0.25-0.29</td>
<td>0.23-0.26</td>
</tr>
<tr>
<td>Vaccine cost</td>
<td>£11.50</td>
<td>Triveritas costing</td>
<td>£7.36-£15.64</td>
<td>0.24-0.31</td>
<td>0.22-0.28</td>
</tr>
<tr>
<td>Vaccinated population</td>
<td>4.5 million</td>
<td>June Survey of Agriculture and Horticulture local results</td>
<td>£3.6-4.9 million</td>
<td>0.26-0.30</td>
<td>0.24-0.26</td>
</tr>
<tr>
<td>Vaccination impact</td>
<td>14328 fewer cases 2720 fewer restricted herds 6 year extent</td>
<td>Brooks-Pollock <em>et al.</em>, 2014 (95% confidence limits of model)</td>
<td>15,638-13,325 fewer cases 2835-2660 fewer restricted herds 8-10 year extent</td>
<td>0.26-0.29</td>
<td>0.23-0.26</td>
</tr>
<tr>
<td>Avoided costs</td>
<td>£370.6-£246.1 million</td>
<td>Bennett and Cooke, 2006; Butler <em>et al.</em>, 2010</td>
<td>£307.3-£497.1 million £204.1-£330.2 million</td>
<td>0.23-0.37</td>
<td>0.20-0.33</td>
</tr>
</tbody>
</table>

Reduced testing and vaccination costs, greater incidence of disease to be tackled by vaccination with improvements occurring over a longer time span, and a higher level of cost saving associated with individual breakdowns, all improve benefit-cost ratios. Correspondingly, the worst-case scenarios involve increased testing and vaccination costs, a lower incidence of disease to be tackled by vaccination with impact restricted to six years, and lower levels of cost saving associated with individual breakdowns. The effects of these combinations of assumptions are set out in Table A21.5.8. Even the best-case scenario does not reverse the sign of the balance.

### Table A21.5.8  Sensitivity Test: Best and Worst Cases

<table>
<thead>
<tr>
<th>Net Present Values, £m 2014</th>
<th>Cost</th>
<th>Benefit</th>
<th>Balance</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best Case (7 year trials)</td>
<td>£972.0</td>
<td>£644.4</td>
<td>-£327.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Worst Case (10 year trials)</td>
<td>£1060.6</td>
<td>£181.2</td>
<td>-£879.4</td>
<td>0.17</td>
</tr>
</tbody>
</table>

### 6. CONCLUSION

Section 5 demonstrates that, even where substantial variations are made in the central estimate assumptions, estimates of monetary costs of the trials and additional post-trial vaccination and testing outweigh the projected benefits. If vaccination reduces the incidence of the disease, or the value of savings from reduced herd breakdowns is greater than assumed in the central estimates, or if the extent of testing is scaled back once vaccination has been established for a period of a few years, the extent to which costs exceed benefits in net present value terms only diminishes by up to 10 percentage points. Delays in completing the field trials or achieving the necessary regulatory approval, reduce costs, but reduce benefits more.

Significant improvement in the Cost-Benefit balance occurs only where assumptions concerning benefits are modified; costs exceed benefits by much smaller margins if vaccination makes a deeper and more sustained impact in terms of reducing incidence of bTB. A further implication is that the field
trials will only be financially worthwhile if some means of substantially reducing post-trial costs can be found. The main scope for cost reduction is in more focused and efficient use of vaccination that target the animals most likely to transmit the disease.

The analysis has been conducted on the assumption that the size and structure of the cattle sector remains broadly consistent with what exists today. However, the beef sector has been constrained by chronically poor margins (EBLEX, 2009) and consolidation is proceeding apace in the dairy sector. In view of this, cattle production may be reduced in volume in the future and concentrated into fewer, larger units. With a smaller overall cattle population, benefits would be reduced; however, some evidence (Brooks-Pollock and Keeling, 2009) suggests that persistence of infection is greater in larger farm units, which if so would increase the benefits (i.e., greater amounts of avoided costs).

In view of this analysis, the case for public support of vaccine field trials rests on the importance of intangible but nevertheless significant non-monetary benefits which can be obtained from reduction in the incidence of bTB. There are three main benefits that should be considered. First are the welfare and psychological costs to farms and farm families of the experience of a bTB breakdown (e.g. Skuce et al., 2011). Second, the improved efficiency of the beef and dairy supply chains which would result from reduced disruption of production volumes should be considered. Third, the compulsory slaughter of suspicious cattle is an animal welfare issue of significant public and political concern.

Some insight into the first issue can be derived, at least tangentially, from a Choice Experiment exercise relating to hypothetical availability of vaccines protecting against bTB (Bennett and Balcombe, 2012). Their main estimate of willingness to pay for a 90% effective vaccine is £35 per animal per single dose; these estimates were obtained from farm businesses in annually tested areas. Their rough estimate of the cost of the single dose was £14, so on their data for the average breakdown, it can be inferred that the value of avoided psychological costs is just over £2,000 annually. Scaled up over the annual testing and edge areas, the aggregate net present benefit could be between £510 and £720 million, which would reverse the Cost-Benefit balance in only a few of the partial sensitivity analyses. However, contingent valuation approaches can be challenged on a number of grounds. The three main issues of concern here are that respondents may not have full information regarding the likely costs of a breakdown, the effectiveness of the proposed BCG vaccine may be considerably lower than 90% (although still worthwhile) and so there may be overstatement of willingness-to-pay, and the perceived costs (negative benefits) in one farm context may not readily transfer to others.

The qualitative benefits of improved supply chain efficiency are well documented (for example, see Simons and Taylor, 2007) but alongside disruption caused by disease, issues of improved trust and sharing of enhanced value creation need to be considered in terms of the generation of enhanced value and its distribution along the chain itself. It may be that the latter factors are more significant than any volatility in primary production levels.

Avoided political and animal welfare costs have also been estimated using contingent valuation approaches (Bennett and Willis, 2008), with aggregated values of prevention of slaughter per animal of £3,298. In terms of additional benefits, these values are small, between £70 and £96 million in net present value terms, and if included would not reverse the negative sign of the cost-benefit balance.

Notwithstanding the need to avoid what has been termed confirmation bias (Oswald and Grosjean, 2004) in policy choice, the conclusion of this analysis is that substantial weight would need to be given to these intangible elements, for the field trials to achieve greater benefits than costs. This raises two further issues.

The first is that a significant proportion (just under a third) of the benefits of the trials flow to private farmers' businesses; these would be rather greater if the monetary estimates of intangibles alluded to above were taken into account. While no account is taken in Cost-Benefit Analysis of "who pays", there is certainly a feeling that farmers would be reluctant to compulsorily vaccinate their stock if they had to contribute to even a modest proportion of the costs (Defra, 2007, p. 31); if, however, vaccination were voluntary then a classical market failure problem would arise in terms of free-riding. A "new political economy" perspective (Swinnen, 2010) would suggest that the collective representation of land-based stakeholders (a relatively small group compared with society as a whole) would have strong incentives to argue for taxpayer support (representing a much larger group, with less direct interests, and considerably dispersed impact as the burden of support is spread very thinly).
The second issue is related, since bTB control policies, and research underpinning them, are the shared responsibility of the Westminster and devolved administrations (particularly in the latter case the Welsh Government). However, proportionately, testing and compensation costs are more significant in Welsh agriculture, not only because of the localised prevalence of the disease, but also because testing policies are more stringent. A distribution of costs and benefits on the basis of territory within Great Britain has not been attempted in this analysis, but should be noted as a potentially important consideration in the decision-making process.

7. REFERENCES


APPENDIX 22 WPP13 – PROVISIONAL COORDINATION PLAN

It is vital that there is a period of interaction between the contractors performing the design phase (SE3287) and the contractors who will be undertaking field trials. The design project includes a phase to allow handover/Knowledge Exchange and fine-tuning of protocol. This was designed so that there was no loss of continuity in the overall project to produce a BCG vaccine, validated DIVA test(s) and leading eventually to an improved bTB control/eradication programme for the UK. The Consortium very carefully considered the requirements for this vital process and made multiple proposals to facilitate the Knowledge Exchange in the most sustainable manner.

A detailed and timed programme for the Knowledge Exchange phase was produced as part of the original proposal for this study (Figure A22.1). However at present there is uncertainty around the timings of the tendering process for the field delivery phase therefore the specific dates given in the Gantt chart will not be achievable. The task names, duration, project plan linkages and resources shown on the Gantt chart are considered appropriate and will be taken forward with Defra and WG once the delivery phase tender has been completed.
**SHORT TITLE:** Feasibility study into testing and validating cattle BCG vaccine and DIVA

**Final Date:** 05 March 15

---

### Table

<table>
<thead>
<tr>
<th>Task Name</th>
<th>Duration</th>
<th>Start</th>
<th>Finish</th>
<th>Resource Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE3287 Report submission</td>
<td>0 days</td>
<td>Fri 29/03/14</td>
<td>Fri 29/03/14</td>
<td>TVT</td>
</tr>
<tr>
<td>SE3287 Report pass &amp; ALL OUTPUTS saved in secure database</td>
<td>1 day</td>
<td>Fri 29/03/14</td>
<td>Fri 29/03/14</td>
<td>TVT</td>
</tr>
<tr>
<td>Defra &amp; Welsh Govt get virtual database password to allow audit</td>
<td>1 day</td>
<td>Fri 29/03/14</td>
<td>Fri 29/03/14</td>
<td>DEF/W/GV</td>
</tr>
<tr>
<td>Defra &amp; Welsh Govt feedback on data room &amp; Trivistas upgrades if applicable</td>
<td>4 days</td>
<td>Mon 01/04/14</td>
<td>Thu 04/04/14</td>
<td>DEF/W/GV</td>
</tr>
<tr>
<td>SE3287 results presented by Trivistas at Defra IT meeting to Interested Parties</td>
<td>1 day</td>
<td>Wed 01/04/14</td>
<td>Wed 01/04/14</td>
<td>DEF/W/GV</td>
</tr>
<tr>
<td>Execution Phase tender solicitation &amp; Invitation to Tender process</td>
<td>0 days</td>
<td>Wed 01/04/14</td>
<td>Wed 01/04/14</td>
<td>DEF/W/GV</td>
</tr>
<tr>
<td>Defra &amp; Welsh Govt appoint contractor to undertake field trials</td>
<td>0 days</td>
<td>Wed 01/04/14</td>
<td>Wed 01/04/14</td>
<td>DEF/W/GV</td>
</tr>
<tr>
<td>Defra &amp; Welsh Govt advice Trivistas on the contractor for field trials (Field Trial Contractor)</td>
<td>1 day</td>
<td>Wed 01/04/14</td>
<td>Wed 01/04/14</td>
<td>DEF/W/GV</td>
</tr>
<tr>
<td>Trivistas provide password for Cloud virtual dataroom to Field Trial Contractor (FTC)</td>
<td>1 day</td>
<td>Thu 02/04/14</td>
<td>Thu 02/04/14</td>
<td>TVT</td>
</tr>
<tr>
<td>Trivistas provides access to VMD &amp; EFSA as required</td>
<td>1 day</td>
<td>Fri 03/04/14</td>
<td>Fri 03/04/14</td>
<td>TVT</td>
</tr>
<tr>
<td>New Contract review Virtual Data Room &amp; send any queries to Trivistas</td>
<td>20 days</td>
<td>Fri 03/04/14</td>
<td>Thu 02/05/14</td>
<td>FTC</td>
</tr>
<tr>
<td>Initial handover meeting (Trivistas &amp; New Contract) &amp; Defra &amp; Welsh Govt invited</td>
<td>0 days</td>
<td>Mon 05/04/15</td>
<td>Mon 05/04/15</td>
<td>TVT/FTC</td>
</tr>
<tr>
<td>Detailed handover Work Package A inputs &amp; outputs (Skype/Telecon or meeting)</td>
<td>1 day</td>
<td>Tue 06/04/15</td>
<td>Tue 06/04/15</td>
<td></td>
</tr>
<tr>
<td>1 Blood gamma interlaboratory validation</td>
<td>1 day</td>
<td>Tue 06/04/15</td>
<td>Tue 06/04/15</td>
<td>TVT/FTC</td>
</tr>
<tr>
<td>2 Protocol for field data capture, blood gamma-IF DIA</td>
<td>1 day</td>
<td>Tue 06/04/15</td>
<td>Tue 06/04/15</td>
<td>TVT/FTC</td>
</tr>
<tr>
<td>Detailed handover Work Package B inputs &amp; outputs (Skype/Telecon or meeting)</td>
<td>1 day</td>
<td>Wed 07/04/15</td>
<td>Wed 07/04/15</td>
<td></td>
</tr>
<tr>
<td>1 Protocol for BCG field efficacy and safety</td>
<td>1 day</td>
<td>Wed 07/04/15</td>
<td>Wed 07/04/15</td>
<td>TVT/FTC</td>
</tr>
<tr>
<td>2 Protocol for DIVA field efficacy and safety</td>
<td>1 day</td>
<td>Wed 07/04/15</td>
<td>Wed 07/04/15</td>
<td>TVT/FTC</td>
</tr>
<tr>
<td>Detailed handover Work Package C inputs &amp; outputs (Skype/Telecon or meeting)</td>
<td>1 day</td>
<td>Thu 08/04/15</td>
<td>Thu 08/04/15</td>
<td></td>
</tr>
<tr>
<td>1 Consideration of baseline sample collection &amp; QAP protocol</td>
<td>1 day</td>
<td>Thu 08/04/15</td>
<td>Thu 08/04/15</td>
<td>TVT/FTC, AH</td>
</tr>
<tr>
<td>2 Analytical protocol for milk samples</td>
<td>1 day</td>
<td>Thu 08/04/15</td>
<td>Thu 08/04/15</td>
<td>TVT/FTC, AH</td>
</tr>
<tr>
<td>3 Protocol for respiratory tract swabs</td>
<td>1 day</td>
<td>Thu 08/04/15</td>
<td>Thu 08/04/15</td>
<td>TVT/FTC, AH</td>
</tr>
<tr>
<td>Detailed handover Work Package D inputs &amp; outputs (Skype/Telecon or meeting)</td>
<td>1 day</td>
<td>Fri 09/04/15</td>
<td>Fri 09/04/15</td>
<td></td>
</tr>
<tr>
<td>1 Focus group &amp; individual interview structure design</td>
<td>1 day</td>
<td>Fri 09/04/15</td>
<td>Fri 09/04/15</td>
<td>TVT/FTC, VT</td>
</tr>
<tr>
<td>2 Cost benefit analysis for BCG vaccine development and use</td>
<td>1 day</td>
<td>Fri 09/04/15</td>
<td>Fri 09/04/15</td>
<td>TVT/FTC, VT</td>
</tr>
<tr>
<td>Detailed handover of Trivistas 'value-added outputs &amp; reports' (e.g. plc. services, supplier, Welsh language use, communication report, etc.)</td>
<td>1 day</td>
<td>Mon 12/04/15</td>
<td>Mon 12/04/15</td>
<td>TVT/FTC</td>
</tr>
<tr>
<td>Hardcopy deadline COMPLETE</td>
<td>0 days</td>
<td>Mon 12/04/15</td>
<td>Mon 12/04/15</td>
<td></td>
</tr>
<tr>
<td>Newly telecon &amp; Skype/telecon with New Contractor for quality assurance &amp; meeting, plus free discussion</td>
<td>4 days</td>
<td>Tue 13/04/15</td>
<td>Mon 01/05/15</td>
<td>TVT/FTC</td>
</tr>
</tbody>
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**Note:** Strictly Confidential