Det Norske Veritas

Cost Effectivess Study of the use of Ante-mortem TSE Tests:

Final Report

Final Report for the Food Standards Agency
Report No: 22326940 - 4

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MANAGING RISK

Cost Effectiveness Study of the use of Ante-mortem TSE Tests
for

Food Standards Agency
Aviation House
125 Kingsway
London
WC2B 6NH

DET NORSKE VERITAS LTD.
Palace House
3 Cathedral Street
SE19DE London
Tel: +44 (0)20 7357 6080
Fax: +44 (0)20 7357 6048
Registered in England
Company No. 1503799

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Summary:

	Name and position	*Signature*
Prepared by:	Kate Huxtable, Consultant Dr Danny Matthews, TSE Specialist Philip Comer, Principal Consultant	
Verified by:	Philip Comer, Principal Consultant	
Approved by:	Karen Dodds, Head of Risk Management and Corporate Responsibility in the UK	
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Executive Summary

This report presents the results of a study carried out by Det Norske Veritas (DNV) for the Food Standards Agency (FSA) to assess the cost effectiveness of the introduction of ante-mortem tests for Transmissible Spongiform Encephalopathies (TSEs) in the United Kingdom. The study has focused on the implementation of ante-mortem tests for BSE in cattle at the current stage of the BSE epidemic, when prevalence levels are low.

The study has included a review of the state of the science relating to the development and approval of ante-mortem TSE tests. This included a review of the literature intended to inform the development of possible test scenarios, and consultation with scientists and companies known to be involved in the research and development of ante-mortem tests. This review led to the definition of a set of possible scenarios for the introduction of live animal tests for TSEs. These were discussed and reviewed at an Expert Workshop held in September 2009 and involving experts from a range of stakeholders, including regulators, the meat industry, veterinarians and people involved in test development. The outcome of the Expert Workshop was that the cost effectiveness assessment should focus on the introduction of a live test on healthy cattle carried out on farm before being sent for slaughter and involve either all cattle sent for slaughter or just adult cattle (over 48 months old at slaughter) as currently tested post-mortem. Testing of healthy cattle pre-slaughter at abattoirs was also considered, but it was felt that this would only be possible if the test provided an almost instant result and that this was unlikely to be achieved.

The cost effectiveness of the introduction of an ante-mortem TSE test has been evaluated by considering the range of costs that would be associated with implementing ante-mortem testing for the agreed scenarios compared with the benefit, measured as the estimated change in exposure to TSE infectivity resulting from the use of the test. The potential for TSE infectivity to enter the human food supply has been evaluated using a model based on the DNV SRM Controls Model that was developed for the FSA and used to estimate the impact of alternative supervision strategies for SRM Controls. The DNV SRM Controls Model has been previously reviewed by the Spongiform Encephalopathy Advisory Committee (SEAC) and the results for assessing alternative options for supervision of SRM controls accepted.

Study Conclusions:

1. The review of the state of the science has not identified any ante-mortem test for BSE that is currently ready for use or for approval. Taking into account the likely timescale for approval and modification of policy to enable the introduction of live animal tests, and the attitude of commercial companies to the challenges and risks involved, there appears to be little scope for their introduction in the foreseeable future.
2. Test specificity is likely to be critical to the acceptance of live animal testing, by both industry and consumers. The prospect of substantial numbers of false positive results that perpetuate fear of undetected or unconfirmed infection may undermine any attempts to introduce such tests.
3. Assuming that an ante-mortem test would be able to detect infectivity for a wider range of the incubation period than is currently possible with the present post-mortem tests, at least for the last 12 months of the incubation period, then it is estimated that the median exposure would reduce from 3 bovine oral ID_{50} units with the present post-mortem testing programme (range 0.2 to 52) to 0.02 bovine oral ID_{50} units (range <0.01 to 0.5) with the ante-mortem test applied only to animals older than 48 months slaughtered for food and a test with 100% sensitivity.

4. The estimated exposure for an ante-mortem test with 95% sensitivity is a median value of 0.3 (range 0.02 to 6) bovine oral ID_{50} units; this is still significantly less than the exposure with the present post-mortem test.
5. Testing the prime beef cattle in addition to those over 48 months of age at slaughter, does not result in any significant further reduction in exposure to TSE infectivity, as the untested prime beef animals contribute only 3% of the total exposure.
6. The cost of applying an ante-mortem test to over 48 month old cattle on farm prior to being sent for slaughter is estimated to be only slightly greater than that for the present post-mortem test; £6.9 million per year as opposed to £6.2 million. The higher costs of sampling on farm with the need for cattle handling and for a veterinarian to take the sample is partly off set by a reduction in MHS supervision costs.
7. The higher cost for ante-mortem testing is balanced by the additional reduction in TSE exposure, so that the cost effectiveness of the ante-mortem test applied to over 48 month animals is estimated to be 2.1 bovine oral ID_{50} units per £million spent as opposed to 0.9 for the post-mortem test. However, the significance of this increase in cost effectiveness needs to take into account the fact that the TSE exposure to the UK population from beef consumption is already at a very low level.
8. Applying an ante-mortem test to all cattle slaughtered increases the costs significantly with no additional benefit in terms of TSE exposure, resulting in a cost effectiveness value of 0.4.
9. With no data on the actual cost of an ante-mortem test it has been assumed that this would be the same as for the post-mortem test. If this cost were higher it would reduce the cost effectiveness of the ante-mortem test; however it has been shown that the ante-mortem test remains more cost effective provided that the cost of the test is no more than 3 times that of the post-mortem test.
10. The sampling costs for the ante-mortem test could be reduced by using a technical officer rather than a veterinarian to take the sample. It has been shown that this would increase the cost effectiveness only slightly from 2.1 to 2.4 Bovine oral ID50 units per million GBP.
11. If the introduction of a reliable ante-mortem test were to enable a change in the EU requirements for removing and disposing of SRM, this could result in a substantial cost saving, estimated to be £16 million. This estimated cost saving is greater than the cost of the test. However, discussions with the EU indicate that rapid ante-mortem tests will never be accepted as alternatives to the removal of SRM.
12. This study has demonstrated that although desirable, it is not necessary to have a live animal test that works at all stages of incubation. It has been shown that improving sensitivity from 3 to 12 months before clinical onset has a real impact, and potential benefits. This could help to define the minimum specification for a live animal test, i.e., 100% specificity and 95% sensitivity within 12 months of clinical onset, at least for the protection of the food chain.
13. This study has focussed on the application of TSE testing in cattle. Whilst there are grounds for greater optimism for the development of ante-mortem tests in small ruminants, there is currently little evidence of a test approach that could be used in a similar way as that investigated for bovines in this study.

Contents

Glossary

ABP	Animal By-Products
BB96	Born before 1996; cattle born before 1st August 1996 when the reinforced feed ban came into force remain excluded from the food chain.
BCMS	British Cattle Movement Service
BSE	Bovine Spongiform Encephalopathy
CNS	Central Nervous System
$CoID_{50}$	Cattle oral ID_{50} *The dose of BSE infectivity that would infect 50% of the cattle exposed by oral exposure*
CSF	Cerebro-Spinal Fluid
CWD	Chronic Wasting Disease
DRG	Dorsal Root Ganglia
EEG	Electroencephalogram
EFSA	European Food Safety Authority
ERAF	Erythroid associated factor
FBO	Food Business Operator
ID_{50}	Infectious Dose 50% *The dose of a pathogen that produces infections in 50% of the population*
LGC	Laboratory and analytical service provider (previously the Laboratory of the Government Chemist)
LFRA	Leatherhead Food Research Association
LRS	Lymphoreticular system
LVI	Local Veterinary Inspector
MHS	Meat Hygiene Service (now the Operations Group of the Food Standards Agency)
O48M	Over 48 Months
OIE	Office Internationale des Epizooties; the World Organisation for Animal Health
OTM	Over Thirty Months
OV	Official Veterinarian
PM	Post Mortem
PrP	Prion Protein
PrP^{BSE}	(PrP) Bovine Spongiform Encephalopathy
PrP^{res}	(PrP) protease resistant protein
PrP^{Sc}	(PrP) Scrapie
R4L	A meat carcase is classified for conformation (how well it is fleshed) and fat levels. Conformation is classed into one of five classes (E, U, R, O and P) and fat into 5 main classes from 1 (very lean) to 5 (very fat) with 3 and 4 split in High and Low.
RMOP	Required Method of Operation
SEAC	Spongiform Encephalopathy Advisory Committee
SRM	Specified Risk Material
TB	Tuberculosis
TSE	Transmissible Spongiform Encephalopathies
UTM	Under Thirty Months
vCJD	Variant Creutzfeldt-Jakob disease
VLA	Veterinary Laboratories Agency

1.0 Introduction

The Food Standards Agency's Strategic Plan for 2005-2010 commits the Agency to promote and aid the development of a sensitive, rapid live test for TSEs by 2010. This has been a key objective within the Agency's TSE research programme, and has been taken forward in co-ordination with other funding bodies. In 2007, a review of the TSE research programme concluded that this remains a priority, despite the continued reduction in the number of BSE cases in the UK, but that the Agency should consider carrying out a cost benefit analysis of the impact of an ante-mortem test for TSEs. As a result the Agency has contracted DNV to carry out this study to assess the cost effectiveness of the use of ante-mortem TSE tests.

During the course of the BSE epidemic, government departments and science councils invested substantial funds in the search for tests to confirm a diagnosis of prion disease in animals or humans prior to death. The existence of such tests at the outset would have simplified approaches to the eradication of BSE, as it would have enabled infected cattle to be detected and eliminated before they progressed to clinical onset, and ideally before dispersal or slaughter for human consumption. Infected and uninfected herds could have been identified, and traditional approaches involving herd certification, herd culling, and pre-movement testing would have enabled a more rapid truncation of the epidemic. In so doing it would have protected animal and human health, and prevented or reduced the scale of the crisis that arose following the announcement of a probable link between variant CJD in humans and BSE in cattle in March 1996.

1.1 Study Objectives and Approach

The overall objective of this study is to assess the cost effectiveness of the introduction of ante-mortem tests for BSE in Great Britain at the current stage of BSE epidemic, when prevalence levels are low. The study will focus on the implementation of ante-mortem tests for cattle, with applications to TSEs in other species considered qualitatively.

The detailed objectives as set out in the Scope of Work for the study were:

1. To review the types of ante-mortem TSE test that could be available, and Where, When and How they would be used.
2. To gather data and assess the costs associated with the implementation and use of ante-mortem tests for active herd and/or flock surveillance
3. To develop a risk model to assess the benefits of using ante-mortem tests in terms of the potential exposure of the population to the TSE infective agent.
4. To assess the potential impact on current TSE controls that could result from the successful use of an ante-mortem TSE test and the potential for cost savings.
5. To use the results to assess the cost benefits associated with the defined ante-mortem test scenarios, in terms of both cost savings (from changes to TSE controls) and in terms of human health risks (in terms of exposure to TSE infectivity).

There were 9 confirmed cases of BSE in Great Britain in 2009, none of which were clinically affected. There were 33 cases in 2008, of which only two were clinical suspects. This low prevalence sets the scene for this assessment.

The Agency has requested that the study should focus on the likely impact and costs of the introduction of ante-mortem tests, and probable benefits based on a set of proposed scenarios

for their use. A set of possible scenarios were developed by the study team and reviewed at an Expert Workshop held at Aviation House on the 10th September 2009. This is reported in Section 4.2. The cost effectiveness assessment (CEA) will determine the cost effectiveness of each of the agreed test scenarios. The approach for the CEA in this study is compatible with the Treasury "Green Book; Appraisal and Evaluation in Central Government". The main benefits considered from the implementation of ante-mortem TSE testing are those to human health risks (measured in terms of exposure to TSE infectivity), but any cost savings that might result (e.g., due to associated changes in SRM controls) have also been considered.

The impact of the ante-mortem testing scenarios on the potential for TSE infectivity to enter the human food supply has been evaluated using a model developed from the DNV SRM Controls Model that was developed for the FSA and used to estimate the impact of alternative supervision strategies for SRM Controls.

1.2 Acknowledgements

The project team acknowledges the help and support of the many individuals who willingly gave of their time and shared their knowledge and experience. It would not have been possible to complete this study without them. These individuals include those who participated in the Expert Workshop (listed in Appendix III), those from test developers who responded to our questionnaire, personnel from the Veterinary Laboratories Agency and the Neuropathogenesis Division of the Roslin Institute who provided information and support and personnel from the European Commission who provided input to the study.

2.0 Ante-Mortem Testing – The State of the Science

This section presents a summary of the review of the current state of the science relating to the development and approval of ante-mortem TSE tests that was produced as an Interim Report for the FSA in August 2009, and is given in full as Appendix I. This review is not intended to be a comprehensive study of the state of the science but to be sufficient to inform the consideration of test options in cattle and to enable a more realistic interpretation of the outcomes. Furthermore, knowledge of the specific issues raised by aspects of test development and approval could be critical to the design of any programme for the use of live-tests. The wider the age range of animals on which tests could be applied the greater the scope for adopting a flexible approach to testing, and the more effective they would be in enabling herds to be declared free of BSE. In other words, a test that is only effective in the three months before onset of clinical disease (as is the case for the present post-mortem tests), after an incubation period of circa five years, would be of value only in a much more targeted role than a test that worked from early in the incubation period.

A focused literature review was therefore carried out and supplemented by consultation with scientists and companies known to have been involved in research and development for live and post-mortem diagnostic test. Also key to the outcome was the likely criteria to be applied for the approval of live-animal tests, particularly in the European Union, but also world-wide. Both had the potential to determine whether or not the study outputs were to be realistic or purely theoretical.

2.1 Literature review[1]

A literature search was conducted in December 2008 (see Appendix I for search criteria), but subsequent relevant publications were also included in the review if appropriate.

The fact that broad reviews of the subject area generally outnumbered publications on the performance of specific live animal tests in domestic ruminants (other than lymphoid biopsies in sheep), is testament to the difficulties faced in this area. Few of the publications specifically addressed the diagnosis of BSE in cattle.

Key issues for consideration in the evaluation of test performance are:

- Whether or not the test can be performed on infected animals before the onset of clinical disease. Despite the potential value of a test that could have been applied to the 215,000 or so clinical suspects examined during the course of the epidemic, it is important to recognise that the majority of infected animals would have died or been killed before clinical onset, or without the recognition of clinical signs suggestive of BSE. (Ferguson & Donnelly, 2003)
- The application of such tests to clinically normal animals would enable the earlier detection of infected herds, the culling of infected animals, and the testing of animals before consumption.
- The pathogenesis of BSE is key to the performance of such tests in the pre-clinical stages. Reliance on the testing of CNS tissues limits their effectiveness in protecting human health, a key requirement if relaxation of other regulations is to be achieved, to the late stages of incubation. Arnold et al (2007) estimated that the window for detection using current post mortem tests on experimentally infected cattle extended to no more than a few months before onset of clinical disease (50% detected at 1.7 months before onset in the 1g dose group (95% bootstrap confidence interval 0.2:4.0), with detection falling close to zero at 3

[1] References for this section are given in the complete State of Science Report in Appendix I.

months before onset. This represented 97% of the incubation period. This is why the removal of SRM from the carcases of cattle intended for human consumption remains the primary protective measure. The current state of knowledge regarding the pathogenesis of BSE is briefly reviewed in the full scientific report at Appendix I.

2.2 Live test approaches

The description of a putative immunological test for scrapie as early as 1974 (Field & Shenton, 1974) offered hope for the differential diagnosis of neurological diseases of sheep. Unfortunately there is no evidence of any subsequent refinement or validation of the method in the literature. Avenues of investigation of sampling approaches and test methods for BSE were originally based on those already used for other diseases, including scrapie in sheep. With time, the combined benefits of scientific understanding and technological advances have given rise to claims of breakthroughs and hopes of success. Unfortunately, for prion diseases of both small ruminants and bovines, extensive reviews of the area in recent years consistently express disappointment at the lack of progress, with the exception of lymphoid biopsies in small ruminants and cervids (Brown, 2005; Brown et al, 2001; Gavier-Widen et al, 2005; Grassi, 2003; Grassi et al, 2008; Ingrosso et al, 2002; Lehto et al, 2006; Nunnally, 2002; Parveen et al, 2005; Soto, 2004).

While dependence on pathogenesis is recognised, the lack of analytical sensitivity of PrP detection methods is seen as a continuing obstacle to the development of tests even where infectivity appears to be present in blood (sheep, humans). In 2004, Soto estimated that immunodiagnostic methods were still 2-4 orders of magnitude away from being able to consistently detect PrP^{Sc} in blood. Despite subsequent enhancements to methodologies, the evaluation of tests for the screening of human blood, for use in blood transfusion, continues to face the same challenge.

2.2.1 Clinical signs and neurophysiological measurements

These approaches involve the full neurological examination of clinical suspects, and the comparison with healthy controls of measurements of heart rate variability, reduced frequency of rumination and changes in EEG. By definition clinical examination is insufficient for the detection of infected animals in the pre-clinical stages of infection. Supplementation by the measurement of neurophysiological parameters has not improved diagnostic sensitivity sufficiently to consider them further.

2.2.2 Blood tests

Blood is the most commonly used matrix for the testing of animals for the presence of infection with other pathogens or for the investigation of metabolic imbalances. Venous blood is accessible, and relatively large numbers of animals can be sampled in a working day with usual farm handling systems. Equivalent systems could be replicated at abattoirs if required. Published and unpublished claims of success with respect to BSE need to be interpreted with caution. Frequently they are based on small numbers of samples, usually unblinded, and even where appropriate numbers of blinded samples have been tested, the absence of appropriate negative controls means that a true evaluation of specificity has not been carried out.

Approaches include the detection of abnormal PrP in serum, the detection of surrogate markers that are claimed to be diagnostic, and the application of methods that measure non-specific changes with a view to establishing diagnostic fingerprints for BSE. While there remains some optimism that PrP detection has some value in small ruminants that are fully susceptible to infection with scrapie, and in humans infected with vCJD, facilitated by the recognised peripheral pathogenesis of disease in such circumstances, the lack of peripheral

involvement in BSE has hindered test development. Surrogate markers and non-specific approaches have also failed to demonstrate sufficient sensitivity and specificity for consideration.

2.2.3 Cerebrospinal fluid (CSF) based tests

Although used commonly in medicine to diagnose CJD and other neurological diseases of humans, the sampling of cattle presents far greater challenges than it does in humans, partly because it is difficult to control the conditions under which samples are collected on farm. Although feasible, the logistics, and inevitable limitations on the number of animals that could be safely sampled per day, would make this approach expensive as well as impractical other than in the most severe of economic circumstances. No study on markers detectable in CSF has demonstrated sufficient sensitivity and specificity to enable serious consideration in this study.

2.2.4 Urine and faecal tests

Urine represents another accessible test matrix, particularly in dairy cows, but early attempts to detect changes that were potentially diagnostic faced considerable challenges, not least of which was the diurnal variability in urine concentration in cattle. Most of the early research into urine based tests remains unpublished. Recent claims of success using bovine urine (Simon et al, 2008) remain to be substantiated on a sufficiently large number of BSE infected animals and appropriate controls. Meanwhile, even in laboratory rodents, where infectivity has been demonstrated in faeces, the testing of faeces cannot yet offer scope for the detection of infected animals throughout the incubation period.

2.2.5 Lymphoreticular biopsies

Despite the extensive body of evidence that lymphoreticular biopsies can be used for the screening of sheep exposed to scrapie (Espenes et al, 2006; Gonzalez et al, 2005, 2006, 2008, a, b; O'Rourke et al, 1998, 2000, 2002), or farmed and wild cervids infected with CWD (Keane et al, 2009; Spraker et al, 2009; Wild et al, 2002), there is no published evidence of any attempt to use lymphoid biopsies as a matrix for testing in bovines. Nevertheless, in the context of BSE, where testing of cattle must provide equivalent protection to the removal of SRM, none of the evidence from sheep and cervids suggests that satisfactory levels of sensitivity and specificity would have been achieved, even if bovine LRS had been found to be PrP^{res}–positive.

2.3 Consultations

2.3.1 Consultation with test-developers

A questionnaire was sent via the Veterinary Laboratories Agency (VLA) to 23 companies or institutes that had requested materials from the VLA TSE archive in the past for the purposes of test development or validation, with particular emphasis on live tests. Another company known to be involved in both research and continued test development, but which had not received materials from the VLA, was approached directly. In addition, some scientists funded by the FSA and Defra in recent years under the banner of "diagnostics" were sent an equivalent questionnaire, either via their funding body (FSA), or directly in the case of staff at the VLA and the Roslin Biocentre.

Four detailed questionnaires, and one background note, were received from scientists contacted via the FSA or directly. Two commercial companies, currently involved in marketing post-mortem tests for BSE and scrapie, responded with a completed questionnaire. Three

others confirmed their withdrawal from this field of development. Many others are known, on the basis of historical personal contact, to have re-directed their activities.

The following represents a summary of relevant detail provided in the six completed questionnaires. All six confirmed a desire to develop a test for use on blood, while two also expressed an interest in testing milk, and one also targeted CSF. Four approaches involved the detection of PrP^{Sc}, one used proteomics while the fifth targeted up-regulation of ERAF. The latter approach has since been abandoned because of the considerable variability in results from healthy large animals and humans which confounded discrimination between infected and healthy individuals.

Only three of the six responses indicated a desire or scope to test bovines; these three did not include the commercial companies. All six were interested in testing sheep both as an end in itself and as a model for test development. Only three groups had an immediate interest in developing a test for humans, while two felt there was scope for testing cervids (but subsequently abandoned by one).

It was suggested that a test for BSE in bovines could possibly be available within two to three years, but only if it was firstly demonstrated that test methodology that worked on sheep could actually detect PrP^{Sc} in bovine blood. The key challenge to test development was the continuing lack of understanding of pathogenesis.

The commercial companies confirmed that the absence of national and international policy for the future use of live tests was a factor that hindered test development. Because of the inability to define a future market, the costs of developing a test could not be compared with potential future income in order to offer confidence that there would be returns on investment.

In summary, none of the respondents indicated that a live test for BSE was likely to be available in the immediate future. Nevertheless, it is known that at least three commercial companies that were not amongst the respondents are still interested in marketing a test for CJD in humans, and can legitimately be considered to have a real interest in the testing of bovines. This is no longer a priority for them however, and will probably not be progressed if they fail to gain approval for the testing of human blood.

2.4 Consultation with the European Commission/EFSA

The European Commission was consulted with respect to its medium term expectations, in the context of the TSE RoadMap (EC, 2005), which acknowledges a need to de-regulate with time, and its open-call for test developers to submit tests for approval. The benchmark of 100% sensitivity and specificity established in the first evaluation of post-mortem tests conducted in 1999 remains a serious challenge to the approval of any live animal test.

The absence of a definitive gold standard to confirm the status of preclinical animals irrespective of stage of incubation remains a major obstacle to evaluating the true performance of ante-mortem tests. At present the EFSA guidelines for the evaluation of a live animal test are limited to a preliminary evaluation of sensitivity and specificity (EFSA, 2007b). How a formal evaluation of suitability for use on a wider scale will be conducted is partly dependent on the analyte to be measured, and whether appropriate test materials are available to enable such studies to be carried out. It is not however expected that rapid ante-mortem tests will ever be accepted as alternatives to the removal of SRM. It is also clear that tests will be expected to have diagnostic sensitivity not less than 100% (95% confidence limit not less than 98.5%)(EFSA 2007a).

The Commission cannot offer a forward plan for the integration of live animal testing within the EU programme of statutory testing.

Although it has no plans to extend the call for test evaluations any further than 2012, and cannot confirm its policy regarding the likely context in which such a test might be introduced, much will inevitably depend on timing, the prevalence of infection and costs of introduction at the time. If live animal tests are not available until such time as the EU testing programme has been reduced significantly, because of the low prevalence of BSE, the issue of equivalence with SRM removal will be less of an issue, and may enable a flexible and positive approach to application for herd certification if such a demand exists.

2.5 Consultation with the OIE

The OIE confirmed that although it could accommodate live animal tests within guidelines for international trade, and for diagnosis of BSE, it has not yet drafted protocols for the evaluation of live-animal tests, and has no plans to draft any at present. Consequently it has no proposals for the context in which it may accept the introduction of live tests, and will no doubt await evidence of successful development before taking this issue forward.

2.6 Small Ruminants

With respect to small ruminants it is clear that while there are grounds for greater optimism than with bovines, significant challenges remain. There is a need for clarity on the extent to which genotype confers resistance to infection with specific strains (absolute or relative) when exposed by natural routes. Within specific breed/genotype/scrapie strain combinations there is a need to confirm the performance of putative tests. Finally there will be difficulties in confirming the effectiveness of a test with respect to all strain/genotype combinations present within a national flock. Where there may only be a limited number of strains involved, there may be scope for live tests to be used in specific scenarios, such as flock/herd certification or culling of exposed animals. At the moment there is little evidence of a test approach that can be used for scenarios equivalent to those discussed for cattle in this study.

2.7 Conclusion

The review of the state of the science identified no test for BSE that is currently ready for use or for approval. Taking into account the likely timescale for approval and modification of policy to enable the introduction of live animal tests, and the attitude of commercial companies to the challenges and risks involved, there appears to be little scope for their use in the foreseeable future.

An issue that has also been highlighted by the review and the stakeholder consultation is that test specificity is likely to be critical to the acceptance of live animal testing, by both industry and consumers. While test sensitivity is already recognised as important when perception of risk is so important in determining the climate of national and international acceptability, the prospect of substantial numbers of false positive results that perpetuate fear of undetected or unconfirmed infection may undermine any attempts to introduce such tests. Realistically there is a need for not one but two tests that are capable of detecting infected animals at any stage of incubation, one for screening and one for confirmation. Failure to confirm the results of a primary positive result will critically undermine confidence in any screening programme. It will also be important to ensure that approaches to diagnosis in the screening and confirmatory tests are sufficiently different to ensure that they are not compromised for the same reason, in order to discriminate between false and true positives.

3.0 Slaughter and Processing of Cattle

The purpose of this section is to provide an outline description of typical slaughter and processing procedures in place in the United Kingdom, as a background for the definition of the Test Scenarios and to collate some of the information required for the assessment.

Abattoirs vary considerably in many respects, including design, layout, equipment, capacity, and range of species processed. The range of facilities means that there are differences in the way the slaughter process is carried out and managed and the way in which the relevant regulations are met and monitored by the Meat Hygiene Service (MHS). The role of the MHS is to ensure that the requirements of the regulations are met, whilst accommodating the differing work practices and layouts of individual plants. What is presented here is therefore a version of events which is considered to be representative, although there will be differences in the detailed procedures followed by different food business operators (FBOs).

3.1 Numbers of Cattle Slaughtered

Defra publish detailed statistics for cattle slaughtered in the United Kingdom. These give numbers slaughtered by month and year for a number of cattle types, with the main classes being Prime Beef and Adult cattle. Over the five years from 2004 through 2008 Prime Beef slaughter in the UK has reduced from 2.29 million head in 2004 to 2.03 million head in 2008. This decline seems to have levelled off with slaughter numbers for January through September 2009 being similar to those for the same period in 2008. It is proposed to use the average for the past 5 years as the basis for this study, i.e., 2.2 million head.

For Adult Beef the situation is more complex as no cattle over thirty months (OTM) old could be slaughtered for food from 1996 through to November 2005, when a system of post-mortem BSE testing for OTM cattle was introduced. Adult cattle slaughter has increased year by year from 375,000 in 2006 to 559,000 in 2008. For the 9 months to September, the numbers of Adult cattle were slightly less than for the same period in 2008, but this may be an artefact of the change in age for BSE testing from over thirty months to over 48 months at January 2009.

Data on the Active TSE surveillance programme[2] shows that 371,023 animals aged over 48 months at slaughter were tested for BSE in Great Britain from 1st January to 31st December 2009, plus an additional 77,833 in Northern Ireland. Together these give a total of 448,856 over 48 month old animals tested for BSE in the United Kingdom in the year. A value of 450,000 will be used to represent the number of over 48 month old animals slaughtered and tested post-mortem in the UK in 2009.

3.2 The Lairage

The farmer is primarily responsible for presenting animals that meet the required criteria for slaughter, namely that the animal must be:

- In good health and condition;
- Identifiable from accompanying passport and ear tag information.
- The animal should also be in the correct age range for the type of slaughter, e.g. Less than forty eight months of age for prime beef slaughter or born on or after 1st August 1996 for cull cow slaughter.

If an animal fails to meet these criteria, it may be refused entry to the abattoir or removed from the system with subsequent loss of income for the farmer. In Scotland the animal may be

[2] http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/bse/statistics/index.htm

returned to the farmer, but in England and Wales the animal must remain at the abattoir until the correct documentation has been supplied, and this must be done within 48 hours or the animal will be destroyed and regarded as SRM in its entirety.

With BSE testing only required for animals over 48 months of age at slaughter from 1st January 2009, many of the major slaughterhouses now slaughter up to 48 months old animals as "Clean Beef", and will not normally handle the older animals (Cull Cows). However, this means that they are required to get approval from the MHS for slaughter of animals older than 30 months, even though this group does not require BSE testing. Vertebral column removal is still required for OTM animals and has to be carried out in licensed cutting plants, but no longer requires 100% MHS supervision.

Most abattoirs handling older animals will also kill prime beef cattle. The first step on arrival of animals to the lairage is to check their identification and to separate the animals over 48 months old requiring a BSE test, In addition, any other animals that would require a BSE test (e.g. a casualty animal that is over 24 months) should also be identified, marked and segregated. It is the FBO's responsibility to ensure that animals entering the slaughter process are of the correct age, and have the appropriate documentation, although it would be usual for MHS personnel to observe and/or be involved with these age checks.

Whilst facilities vary between plants, it would be normal for the ear tags of each animal to be checked against the passport as the animals move though a race towards the stunning area. There should be facilities (e.g. a crush) to hold an animal if the tag is difficult to read (e.g. with a metal tag), and the ability to release any unwanted animal from the race. It would be normal for the sequential kill number to be applied at this stage.

At this time the Official Veterinarian (OV) completes an ante-mortem record form with the FBO or OV completing the first section and the OV noting any signs of BSE. A check of this type is likely to be conducted on a batch of animals, perhaps 25 at a time in a large facility. The OV would then note on the form that he/she was either satisfied with the inspection, or would remove suspect animals to an isolation pen, with the FBO being responsible for any animal handling activities.

3.3 Post-Mortem Identification Checks and SRM Removal

Slaughter

The animal is normally stunned using a captive bolt gun. The stunned animal then falls to the floor of the slaughter hall. Once stunned, the animal is shackled by one leg, hoisted and suspended above an area to contain the blood. The animal is then slaughtered by cutting the blood vessels in the throat. The blood is collected and can either go for Category 3 waste, if collected in a hygienic manner, or will be sent for rendering and subsequent disposal. For over 48 month old animals, the blood has to be retained in a secure tank and may only be released once a clear BSE test for the relevant batch has been received.

Identification Checks

Checks by MHS personnel on the identification of cattle are normally carried out post-mortem. Although done on all sites for all animals, there is nothing in the law to say that the MHS is responsible for PM identification checks. However, the SRM stamp effectively states that the individual MHS inspector is certifying that the meat is free of SRM and suitable for entry into the food chain, and a confirmation of the age and origin of the animal is considered central to any such assurance.

It is essential that a clear and consistent process is adopted by the abattoir in order that any difficulty identified with an animal can be traced to that carcass and all associated tissues before the meat is passed fit. The sequence of animals on the production line therefore needs to be carefully maintained, and this can be confirmed using MHS documentation and other mechanisms (e.g. identification tags).

Once the animal has been stunned and bled, the meat technician checks the ear tag and records the details. The passport may also be reconciled at this point, or this may be done elsewhere outside the slaughter hall.

At this stage the passport becomes the property of the MHS and the Inspector records the number of passports for the day. The passports are then placed in a sealed pouch and returned to the British Cattle Movement Service (BCMS). If an animal's passport is considered dubious, it is marked as not reconciled and returned accompanied by a form which describes the passport, apparent irregularities, and any action required. The entire carcass will then be retained together with any supporting evidence.

It is the role of Trading Standards Officers to follow up and investigate in cases of discrepancies or irregularities, and the farmer's responsibility to provide adequate information to identify the animal. The MHS responsibility is to ensure the animal does not enter the food chain in the event that there are inconsistencies. Although the MHS do not have direct authority to dispose of a carcass, the power to withhold health mark will lead to the carcass being held in the chill for an unlimited time, after which it may not be fit for consumption in any case.

3.4 Harvesting Head Meat and Tongue Removal

It is now common for head meat to be harvested in UK slaughterhouses. This is generally done on line to avoid the requirement for testing for the presence of contaminating CNS tissue. It is also not usual for the shot hole or *foramen magnum* to be sealed.

In some slaughterhouses, the hide is removed before the head using a hide puller. In this case the complete hide, including the skin of the head, will be removed. The head is removed using two knives to reduce the risk of contamination with spinal cord. The tongue is then removed at a point anterior to the tonsil. However, because tonsil is a disparate tissue, the incision is made at a point in the tongue significantly in front of the area where the majority of tonsil tissue is likely to extend so as to maximise the potential of excluding such tissue. Tongues are inspected by meat inspectors to ensure that they have been removed at the appropriate site. New guidance on tongue removal was issued in 2003 following an experimental result that there could be a low level of infectivity in tonsil tissue. It was considered unlikely that an incorrectly removed tongue would not be detected by MHS personnel. Head meat would not normally be harvested from the same area as the tonsils, so it is unlikely that head meat would be contaminated by tonsil tissue. However, head meat, once harvested, is not batch inspected for the presence of any erroneous tissue by MHS staff.

Once the head has been removed, ear tags can no longer be used to identify individual animals. FBOs have systems to identify animals once heads have been removed based on the 'kill order' on the production line and these will be verified and monitored by the MHS. As animals are on hooks on a continuous conveyor there is no opportunity for the order to change. There were no reports of failures in the carcass tracking systems.

3.5 Post-Mortem BSE Testing

Sampling of brain tissue for BSE testing has to carried out in a specifically designated location away from the main line, which is often, but not always, a separate room. The FBO is

responsible for taking the BSE test sample, and the operator taking the sample must have had the appropriate training. The sampling operation is supervised by the MHS. The Veterinary Laboratories Agency (VLA) has provided training for operators who are then able to train other operators at their plant. Defra keep a record of trained personnel. If the sample is not taken properly, so that the correct part of the brain stem is not available for analysis, the laboratory will declare the sample a “no-test”, with the result that the carcase will not be allowed into the food chain and have to be destroyed. This will be a major cost to the abattoir so that there is strong motivation to ensure that samples are correctly taken. The numbers of no tests have fallen steadily from a rate of about 0.1% in 2005 to about 0.02% by the end of 2007.

Before the sample is taken the operator will verify the animal identity, either by kill number or ear tag. The sample is taken and placed in a fresh pot, which is fitted with a tamper evident lid, and a printed label with bar code attached to identify the animal. Filled pots are placed in a tray and stored ready for dispatch to the laboratory at the end of the day.

3.6 Hide removal and evisceration

In some small abattoirs the carcass is flayed in a cradle to remove the hide before being lifted by a winch. In most large abattoirs the carcass will already be suspended and the hide removal will usually be assisted by a hide puller. The puller will do most of the work, but some assistance is usually needed from operators using knives. The carcass is then opened on a platform where the whole gut is removed, and enters the gut room via a chute. All guts, spleen and pluck (lungs, heart and liver for cattle) are inspected, although only the gut is now covered by the SRM controls. All items have to be maintained so as to be identifiable as coming from one animal, and if this is not done the entire batch may be condemned in the event of a test positive. There is therefore a major incentive for the FBO to maintain efficient tracking systems.

Guts are moved to the gut room. Everything beyond the abomasum (stomach) is considered to be SRM. The stomachs may be separated from the intestines and sold as pet food if harvested in an appropriate manner. The intestines from the duodenum to the rectum are classed as SRM and placed in the SRM bin for staining and disposal. Although the staining process is not witnessed on a continual basis, the MHS conduct regular checks of the area and process.

3.7 Spinal cord

The carcass is split using the splitting saw. In large abattoirs the saw will normally be operated from a hydraulic platform. Both band and reciprocating saws are used and will usually be fitted with a water cooling system. As the carcass is split in two the washings are collected and trapped in a 4mm drain trap. The material collected is specified solid waste and is treated as SRM. The operator then removes the spinal cord and cleans the canal using a soft water wash (i.e. low pressure). In some plants a vacuum head is used to finish the cleaning of the spinal canal. The spinal cord is placed in a SRM bin for staining and disposal. There is considered to be no obvious market for spinal cord in the event that it were not stained.

3.8 Final Inspection and Carcase Retention

Once the spinal cord and other SRMs have been removed the carcass is inspected by a MHS meat inspector who checks that the spinal cord has been removed. The examination is specifically looking for residues of spinal cord, as all other SRM tissues would be immediately

obvious if present. If spinal cord is found, the procedure is to gather evidence and recommend prosecution.

For animals that are subject to BSE testing the carcasses are routed to a chill room where they are retained under MHS supervision. When a rail or section is full, the MHS will apply a lock and seal that will be recorded. The carcases will only be released from retention once a clear set of BSE tests have been received from the laboratory, usually on the morning after slaughter. The health marks and TSE stamp are then applied at this time.

If there is a no-test then that carcase has to be identified and removed from the line before the remaining carcases can be released. If there is a BSE positive result then the one before and two after in the line must also be removed and destroyed to minimise the risk of any cross contamination. The carcase handling would be the responsibility of the FBO but would be done under MHS supervision.

3.9 Storage and Disposal of SRM

SRM bins are labelled with “Category 1 High Risk By-Product”. Material is transferred from the bins into a skip or similar large container which must also be labelled appropriately. Estimates of the quantity of SRM generated at the plant are recorded. This provides an estimate of weight of tissue which can then theoretically be reconciled to throughput. However, this can be a difficult process as there may also be condemned material, feet, paunch, etc., together with variations due to amount of water in the consignment. This problem is likely to increase in future with the decision to allow mixing of SRM with other categories of material, provided all are treated as SRM.

The responsibility for recording and maintaining a record of SRM weight lies with the FBO, although there are no regular, coordinated cross-checks to ensure what leaves the abattoir is what arrives at the disposal point. SRM material leaves the premises under triple documentation; FBO, haulier, and premises. The OV regularly audits the process of dispatch (potentially monthly inspections on full throughput, 3 monthly in low throughput), but does not inspect each consignment.

Once the SRM leaves the plant it ceases to be the responsibility of the MHS. The destination of the consignment should be known and come from an approved list of premises. It is Defra’s responsibility to check records at rendering plants.

3.10 On Farm Testing

One of the options considered for ante-mortem testing is to test the animals on farm before they are sent to market or the slaughterhouse. Farms will usually have appropriate handling facilities as they will be required to have tests for bovine TB carried out on a regular basis. The requirements for ante-mortem TSE testing are likely to be very similar to those for TB testing.

For a TB test the farmer is required to have suitable facilities available to enable the LVI (Local Veterinary Inspector) to check the identity of each animal to be tested, to prepare the animal (measure skin thickness and trim hair at test site) and then inject the tuberculin. For a TB test the LVI has to return after 72 hours to read the test.

It is assumed that the effort required for an on farm ante-mortem TSE test would be very similar to that for Day one of the TB test. The farmer will have to round up the animals to be tested, move them through a cattle handling system to a crush where the animal can be restrained for its identity to be checked and a sample taken, and then returned to its normal location.

4.0 Application of Ante-Mortem Tests

4.1 Scenarios for the use of in-vivo tests for TSEs

A number of possible scenarios for the introduction of live animal tests for TSEs, and specifically for BSE, were developed as a basis for the cost effectiveness assessment. They were based on past practise in the control of animal diseases, and no attempt was made to prioritise them prior to the Expert Workshop (held in London on the 10th September 2009). Prior to the Expert Workshop issues that were likely to arise as a result of each were summarised as a basis for the discussion. The full set of scenarios considered is listed below and presented in full in Appendix I. Following detailed discussion of each of the scenarios, two "Selected Options" were proposed for detailed assessment and further modelling.

The eight scenarios presented to the Expert Workshop for evaluation were:

1. The testing of clinically affected animals (BSE suspects)
2. The testing of BSE cohorts associated with index cases
3. The testing of healthy cattle, pre-slaughter, on farms
4. The testing of healthy cattle, pre-slaughter, at abattoirs
5. The testing of healthy cattle intended for export
6. The testing of healthy cattle for the purposes of herd certification
7. The testing of healthy cattle for the purposes of national accreditation
8. The testing of healthy cattle to detect resurgence of BSE

Costs associated with an ante-mortem test policy include those associated with travel and transportation to and from farms, time costs (for samplers) and testing costs. Infrastructure costs may also need to be taken into account if modification of facilities is required to enable cattle to be sampled, or testing to be carried out.

4.2 Workshop discussion of scenarios

A common position amongst experts at the workshop at the beginning of discussion was a belief that there were no clear-cut drivers for the introduction of a live animal test. No clear benefits were obvious, and there was relative contentment with current policy demands, in the expectation that with time regulatory burdens would decline in line with the prevalence of BSE.

Discussion of the proposed scenarios identified no additional plausible scenarios. It was recognised that several of the above scenarios could become feasible, and implemented within the constraints of EU and UK regulation at the time. Nevertheless, for the purposes of this exercise, it was clear that options three and four (the testing of pre-slaughter cattle on farm or at abattoirs) were the most appropriate for detailed modelling of risks and benefits. They impacted most directly on the FSA's areas of concern, directly influencing risk via the food chain. The table of scenarios in Appendix I identifies arguments for and against each of the scenarios as discussed at the meeting. It is however appropriate in the interest of clarity to expand on the issues that arose in relation to scenarios three and four.

4.2.1 General issues

Ideally the test used should be quick. A cow-side test that enables interpretation on the farm is preferable, although not essential. While alternative approaches, which involve the sending of

samples to a nearby laboratory, can be accommodated when sampling on farm, it cannot be assumed that sampling can be easily incorporated into working arrangements at abattoirs. Handling facilities may not be ideal for sampling, and welfare and health and safety issues may arise if sampling is attempted in circumstances that are not ideal.

The introduction of ante-mortem testing instead of post-mortem testing and/or SRM removal could be perceived as a retrograde step, with the potential to concern consumers about the reality of risk unless issues were clearly described in consultation prior to introduction. Furthermore, voluntary schemes were viewed as inappropriate, while compulsory testing would ensure that implementation was simpler and easier to monitor.

4.2.2 Testing on farm

The cost of sampling is likely to be significantly greater than the actual cost of testing the samples. Under the circumstances, the testing of single animals, or small groups, is likely to be disproportionate to the perceived benefit. If a test result remained valid for a period of months it should be possible to test larger groups of animals that are likely to be slaughtered within the following 6 or 12 months. The period of validity of a test result should be defined in any test approval, taking into account results on time-course samples from experimentally infected animals. If sensitivity is demonstrated to be 100%, there is little likelihood that the status of any individual test-negative animal would change over a short period.

One particular concern relating to testing on farm was the lack of evidence for benefit to the farmer, which may result in resistance to the implementation of such a policy. Abattoirs and possibly consumers were seen as the primary potential beneficiaries, and in the current low prevalence climate it was unlikely that farmers would benefit from higher prices for beef. Even if the farmer did not pay for the actual cost of the test (but who else would) they would have the additional work associated with carrying out the test.

4.2.3 Testing at the abattoir

If alternative arrangements are to be implemented at abattoirs they must represent clear benefits to management, who have put in place procedures for post-mortem testing that function relatively smoothly. These arrangements take into account the low probability of detecting positive animals using post-mortem PrP^{BSE} detection methods. Any suggestion that false-positive results will lead to the need to take remedial action on a significant number of occasions (such as unnecessary destruction of carcases and offals) will therefore potentially outweigh any benefits arising from the introduction of ante-mortem testing. False positive results would be less disruptive if the results were available before animals were slaughtered, thus eliminating consequential action with carcase components, especially if mixed with materials from other cattle. However, this would require an effectively instant result as abattoirs do not have sufficient lairage space to hold tested animals for any length of time prior to slaughter.

The likely cost of accreditation and quality assurance/proficiency testing could be a barrier to the establishment of testing facilities at abattoirs.

Because of the issues identified with ante-mortem testing at the abattoir, it was felt that this was unlikely to be a feasible option and so was not considered further.

4.3 Selection of sensitivity/specificity criteria for modelling

In the absence of any firm indications from the literature and company reviews of achievable sensitivities and specificities for live animal tests for BSE, it was necessary to consider hypothetical alternatives for modelling purposes. The upper limit for such sensitivities are

necessarily the best case target (100% sensitivity/specificity – 95% probability that sensitivity not less than 98.5% (EFSA 2007b). It is important to recognise the uncertainty surrounding the benchmark of 100% sensitivity and specificity achieved by current post-mortem tests. In the context of testing positive samples from clinically affected animals, the relatively small numbers of samples tested during evaluation (200) cannot guarantee that the result of the evaluation is absolute. Some uncertainty has to be accepted, and the testing of greater numbers of samples may have resulted in occasional false-negative results.

While this may still appear to represent an unachievable target for live animal tests, it is important to remember that none of the approved post-mortem tests would be capable of detecting infected animals in the early stages of incubation (Arnold et al, 2007). That is why the European Commission is correct to demand that the performance of a live animal test should be compared with risk reduction delivered by SRM removal rather than with the performance of the post-mortem tests. Indeed, it is conceivable that the detection of infected animals early in the incubation period with a live-animal test could reduce risk below that currently offered by a combination of age-related SRM rules and post-mortem testing, especially if such animals could be excluded from the food chain entirely.

Discussion at the expert workshop did however highlight the need for clear qualification of any EU approval of live animal tests. The ideal test should detect infected animals irrespective of age and/or stage of incubation. If this is not achievable, alternative approaches may still be feasible, perhaps with tests applicable to young (6-30 months) or older cattle.

The paucity of data on BSE test sensitivity and specificity, and consideration of examples from other disease scenarios are discussed more fully in Appendix I.

In considering what parameters should be modelled, it was however clear from the expert workshop that while test sensitivity was the most important in determining risk to consumers, test specificity was also critical in ensuring acceptability to both farming and slaughter industries. Test specificity of 99% could give rise to 1000 false positive results per 100,000 animals tested. If the sampling is done at abattoirs, and results are not available until after slaughter and dressing of carcases, this could imply 4-5,000 incidents that require consequential action if applied solely to mature cattle. Consequential action includes the destruction of tested carcases, plus one before and two after, and destruction of waste as SRM, at greater cost than if eligible for disposal as category 3 waste (assuming that introduction of testing had enabled SRM rules to be relaxed). This would cause significant disruption to abattoirs, and resistance to the implementation of such a policy.

A further complication to such scenarios, whether animals are tested on farm or at abattoirs, is whether or not false-positive results can be resolved quickly, securely and transparently. Any residual uncertainty or doubt would undermine confidence in the scientific basis of test interpretation, and potentially jeopardise consumer trust in the outcome. It could also impact on international acceptability of country or farm categorisation.

Consequently, in order to avoid total rejection of all resolved false-positive animals (inconclusives) from the food chain, it is important that approval of such tests incorporates a scientific rationale for the use of gold standard alternative approaches to identify the true infectious state of the animal. Such gold standards do not exist at present.

Under the circumstances, two sensitivity/specificity scenarios have been selected as the basis for the cost effectiveness study:

1. 100% sensitivity - 100% specificity, being the gold standard and preferred option based on post-mortem testing, and
2. 95% sensitivity – 100% specificity, as a more pragmatic, but not worst case, alternative.

The 100% sensitivity is modelled as a Beta distribution with zero false negatives in 200 samples (equivalent to the EFSA requirement of 95% probability that sensitivity not less than 98.5%); Beta(201,1). The 95% sensitivity is modelled as a Beta distribution with five false negatives in 100 samples; Beta(96,6).

Initially the 100% specificity was modelled as for 100% sensitivity, but as discussed in Section 5.4 this gives rise to an unacceptable level of false positive results. An alternative, optimistic, specificity was modelled assuming zero false positives in 100,000 samples (e.g. Beta (100001,1).

4.4 Selected Scenarios

In summary, the outcome of the Expert Workshop was that modelling should focus on the introduction of a live test on healthy cattle on farm before being sent for slaughter and involve either all cattle or just adult cattle as currently tested post-mortem. In each case the assessment would be done for the two sensitivity/specificity scenarios.

5.0 TSE Exposure Model

5.1 Model Overview

DNV's SRM Controls Model was developed in order to estimate the amount of BSE infective material, in terms of Bovine Oral ID_{50} units, that may enter the food chain for a defined set of SRM controls. The model was originally developed in 2004 as part of a project for the Food Standards Agency to assess the impact of alternative SRM control enforcement strategies and applied to prime beef slaughter. This was updated in 2006 to account for changes in the EC TSE regulations and again in 2008 to extend the model to cover slaughter of over thirty month (OTM) cattle and post-mortem BSE testing. This latter update also included input from an Expert Panel to determine some of the input parameters in the model.

The TSE exposure model used in this study has been developed from the last two versions of the SRM controls model so that it covers both prime beef and cull cow (OTM) slaughter. The main assumptions adopted in the previous versions of the SRM controls model have been incorporated in this version, with some changes to reflect the fact that from 2009 BSE testing is only required for animals aged over 48 months at slaughter rather than 30 months.

The model consists of a set of linked Event Trees in an Excel spreadsheet and is evaluated using @RISK[3], a commercial probabilistic risk assessment tool that works as an add-in to Excel. The model is run many times, and for each model run (or iteration) the combinations for each varying parameter are selected at random. These values are chosen from specific probability distributions that have been defined for that parameter. The number of model runs are high enough to ensure that all possible combinations of parameters have eventually been selected a sufficient number of times to allow an evaluation of the probability of occurrence of the corresponding risks. For the results reported here, the model has been run using Latin Hypercube sampling and 10,000 iterations. Latin Hypercube sampling is a stratified sampling technique that ensures the full range of the input distribution is sampled without the need for excessive iterations.

There are two kinds of data required for the model. The first concerns BSE infectivity for the various SRM tissues and the prevalence of BSE in the UK herd. The background and assumptions for BSE infectivity and prevalence are derived from the previous TSE risk assessment work carried out for the FSA and are set out in Appendix II. The second kind of data relates to the branch probabilities that concern SRM removal and the enforcement of SRM controls. In general there is little or no data to establish these probability values and it is necessary to rely on expert judgement. The values used here are generally the same as those used in the previous studies referenced above. For the OTM slaughter model these were established using a mini Delphi expert elicitation method in an Expert Workshop attended by representatives of the Meat Hygiene Service, food business operators, a consumer representative and representatives from both Defra and the FSA. The values for prime beef slaughter from the 2006 study were based on a more limited expert judgement and these have been updated with data from the OTM exercise with recognition of expected differences between prime beef and OTM slaughter facilities. The values used for the branch probabilities are given in Table 2.

For the purposes of the model, the slaughter process has been divided into a number of stages so that all the activities that can affect the possible risk of exposure to infectivity are included. It was found that the process can be represented in six stages for prime beef slaughter and nine

[3] Precision Tree and @RISK are Decision analysis and Risk analysis tools developed by the Palisade Corporation of 31 Decker Road, Newfield, NY 14867, USA. www.palisade-europe.com

stages for cull cow slaughter and each of these is modelled as an event tree. The event trees that make up the model are summarised in Table 1.

Table 1: List of Event Trees in TSE Exposure Model

No.	Event Tree	Prime Beef	Cull Cow
1	Cattle identification in lairage;	✓	✓
2	Ante-mortem inspection in lairage;	✓	✓
3	Post mortem inspection;	✓	✓
4	BSE testing;		✓
5	Harvesting the tongue;	✓	✓
6	Harvesting head meat;	✓	✓
7	Spinal cord removal and final inspection;	✓	✓
8	BSE test results and carcase dispatch		✓
9	OTM cutting plant		✓

Detailed descriptions of the event trees that make up the model are available in the two DNV reports to the Food Standards Agency: i) DNV Report 22115034 v3: Review of supervision of SRM Controls, March 2007, and ii) DNV Report 22125174 v3: Review of Supervision of SRM Controls in OTM Plant, September 2008.

Table 2: Branch Probability Values for TSE Exposure Model

Event Tree	Branch Description	Prime Beef Slaughter			Cull Cow Slaughter		
		Minimum	Best Estimate	Maximum	Minimum	Best Estimate	Maximum
1. Identification in lairage	Animal identification NOT checked in lairage	1:100,000	1:10,000	1:100	1:1,000,000	1:100,000	1:1000
	OTM animal NOT identified (if checked)	1:10,000	1:100	1:10			
	BB96 animal NOT identified (if checked)				1:100,000	1:1000	1:100
2. Ante-mortem inspection in lairage	Health NOT checked effectively	1:100,00	1:10,000	1:10	1:1,000,000	1:100,000	1:100
	High risk animal NOT identified	1:10,000	1:1000	1:100	1:100,000	1:10,000	1:1000
3. Post mortem inspection	Animal identification NOT checked Post Mortem	1:1,000,000	1:100,000	1:10,000	1:100,000	1:10,000	1:1000
	OTM animal NOT identified (dentition & ID check)	1:1,000,000	1:100,000	1:10,000			
	OTM animal identified but not removed	1:1,000,000	1:100,000	1:10,000			
	BB96 animal NOT identified				1:1,000,000	1:33,300	1:1000
4. BSE testing	Brain stem sample not taken				1:1,000,000	1:100,000	1:10,000
	Sample substituted				1:1,000,000	1:100,000	1:10,000
	Sample not correctly labelled				1:100,000	1:10,000	1:1000
5. Harvesting the tongue	Tongue not removed correctly	1:100,000	1:50,000	1:1000	1:100,000	1:50,000	1:1000
	Inspection fails to identify incorrectly trimmed tongue	1:100,000	1:2000	1:100	1:100,000	1:2000	1:100
6. Harvesting head meat	Head meat harvested in abattoir		0.75			0.75	
	Head not stained as SRM	1:1,000,000	1:100,000	1:10,000	1:1,000,000	1:100,000	1:10,000
7. Spinal cord removal and final inspection	Parts of spinal cord left on carcass	1:100,000	1:33,300	1:10,000	1:100,000	1:33,300	1:10,000
	Residual SRM not identified at final MHS inspection	1:1,000,000	1:100,000	1:10,000	1:1,000,000	1:100,000	1:10,000

Event Tree	Branch Description	Prime Beef Slaughter			Cull Cow Slaughter		
		Minimum	Best Estimate	Maximum	Minimum	Best Estimate	Maximum
8. BSE test results and carcase dispatch	One or more result is a "No Test"					2.8×10^{-4}	
	BSE Positive result received					7.4×10^{-6}	
	No Test carcase not identified and removed				1:1,000,000	1:333,000	1:100,000
	BSE positive and 1B2A not identified and removed				1:1,000,000	1:333,000	1:100,000
9. OTM cutting plant	OTM carcase not sent to approved cutting plant				1:1,000,000	1:333,000	1:100,000
	OTM meat sold bone-in (estimated as for UTM)					1:87	
	OTM carcase not identified in cutting plant so processed as UTM				1:1,000,000	1:100,000	1:100,000
	Proportion of cutting plant using traditional boning or excess DRG left on meat				1:1000	1:100	1:20
	VC not disposed as SRM				1:1,000,000	1:100,000	1:10,000
	VC used in human food				1:1,000,000	1:1,000,000	1:100,000

5.2 Exposure to Infectivity

The final stage in the model is to estimate the potential exposure to infectivity. The event tree model provides estimates of the likelihood that SRM tissues may get into the food chain (e.g. spinal cord left on carcass) and the probability that a carcase is derived from an animal born before August 1996 or a high risk animal. These are then combined with estimates of infectivity for each of the tissues and the likelihood that an animal is infected with BSE. The infectivity estimates are based on those used for the risk assessment carried out for the review of the Over Thirty Month rule (Comer and Huntly, 2004) updated by other studies, such as DNV reports on bovine tongue and beef-on-the-bone (DNV, 2003 & 2005) and any new scientific data. The new data includes estimates for the infectivity in peripheral nervous system tissues, following a number of recent reports of very low levels of infectivity found in some peripheral nervous system tissues using highly BSE sensitive mice (Buschmann & Groschup, 2005; Iwata et al, 2006).

The data used for the infectivity and prevalence estimates are presented in full in Appendix III, with the key results summarised below.

5.2.1 Infectivity of Bovine tissues

The infectivity profile used takes account of the most recent results from the attack rate experiments carried out by the VLA.

The infectivity titre in the brain of a clinically BSE infected bovine is assumed to follow the following distribution:

Log normal distribution with
Median (50 percentile): 5 cattle oral ID_{50}/gram
Higher 99 percentile: 100 cattle oral ID_{50} ($CoID_{50}$)/gram

It is assumed that infectivity increases over the incubation period with a 2 month doubling time.

The relative infectivity in different tissues are assumed to be as before, with the infectivity in DRG assumed to be as for brain and the infectivity in tonsil 5 orders of magnitude less. The values used are given in Table III.1.

5.2.2 Prevalence of BSE Infection

Estimates of the proportion of cattle that were slaughtered with BSE infection in 2009 have been provided by the VLA using the back-calculation model of Arnold and Wilesmith (2003) for three age groups:
1. Cattle born before 1 August 1996
2. Cattle born on or after 1 August 1996 that are over 30 months of age
3. Cattle under 30 months of age.

The estimates are given in Table III.2 for both before and after BSE testing where this is appropriate, and for three time periods: less than 3 months before onset, 4 to 6 months before onset and 7 to 12 months before onset. The last column in Table III.2 gives a weighted mean value, where the proportion with infection is weighted by the infection level for the incubation stage assuming a 2 month doubling time.

5.2.3 Exposure from Tongue

There is a possibility of exposure to infective material if tonsil tissue is left on the tongue. The potential level of infectivity in tonsil tissue is very low, and the model indicates that most tongues would be cut correctly. However, as noted by SEAC (2003) and also by EFSA (2008), even when cut correctly there may still be some lingual tonsil tissue remaining as there are large variations in the distribution of the tonsil tissue on the tongue (Wells et al, 2005). For this study, it is assumed that if the tongue is not cut correctly that 10% of the lingual tonsil tissue would remain on the tongue. This is the same as in the risk assessment presented to SEAC (DNV, 2003) and the previous version of the SRM controls model, and would be about 5g of tissue, which is likely to be a precautionary estimate. In this version of the model it is now also assumed that if cut correctly there may still be some lingual tonsil tissue present, but a factor of 10 less than on an incorrectly trimmed tongue.

5.2.4 Exposure from Spinal Cord

The inspection regime means that the likelihood of any spinal cord being left on a carcass is very low. If there is a failure it is assumed that 5% of the spinal cord (i.e. 10g) is left on the carcass. It is then assumed, as a worst case scenario, that this material would be consumed. In reality, as most meat is cut from the bone, it is likely that the major part of any contamination would in fact be disposed of and would not enter the human food supply.

There will also be some contamination on a carcase from spinal cord due to the fact that the carcase is split with a saw through the vertebral column so that some spinal cord tissue will be spread over the cut surface. In studies to measure the extent of this contamination Knight (2001) reported between 50 and 100 mg of CNS tissue on each cut surface. These values will be used as a uniform distribution. This contamination was included in the risk assessment for the OTM review where, in discussion with the FSA's Risk Assessment Group which acted as an expert review group for the study, it was decided to include a factor of 1% for the amount of this contamination that would be transferred to meat and so consumed (Comer and Huntly 2004). This recognised that the cut surfaces are mainly bone and that the majority of carcases are deboned before sale to the final consumer. This factor is also included in this assessment.

5.2.5 Exposure from Head Meat

As indicated in section 4.3, it was considered likely that there would be some level of contamination from CNS tissue on all heads. There is at present no data on the extent of possible contamination, although it is understood that there is now a study underway to determine this. EC rules require that a sample of the head meat harvested is tested for the presence of CNS tissue, unless the head meat is harvested with the head still on the hook or conveyor at the slaughterhouse. It is our understanding that at present all head meat is being harvested in this way, so that there has been no testing for CNS in GB.

In previous studies, the estimates from Cooper and Bird (2002) have been used. They studied slaughterhouse procedures and estimated the amount of CNS contamination per head as 1.28g (95% range 0.35 – 2.93). This study was based on the practices current at that time, which included transport of heads to specialist head boning operations, and would not reflect the current requirements and controls. This is therefore felt to be a high estimate for the current practice, and a distribution has been adopted with a median value a factor of 10 less and a 99 percentile of 1.28g. This is then applied to all heads from which the head meat is harvested.

5.2.6 Exposure from Dorsal Root Ganglia (DRG)

The exposure from dorsal root ganglia (DRG) has been estimated taking account of the data on alternative boning practices that was reported and used in DNV (2005). This showed that

with sheet boning only 0.5% of the DRG would be left with the meat, whilst with traditional boning about 7% of DRG may be left in the meat.

For OTM cattle all carcases have to be boned in approved cutting plant specifically authorised to remove bovine vertebral column and the vertebral column disposed of as SRM. There is no data on the proportion of approved cutting plant that use sheet boning, but at the Expert Workshop it was estimated that this would be the case for 99% or more. The proportion using traditional boning is taken to include the possibility of cutting plant failures such that more DRG is left on the meat. The chance that carcases may not be sent to an approved cutting plant is included in the model. In this situation meat may be sold on the bone (assumed to be the same frequency as for under 30 month animals) and it is assumed that the boning is done using the traditional method.

5.3 Exposure Model Results

The TSE Exposure Model has been run for both prime beef and cull cow slaughter in Great Britain for the conditions and regulations pertaining to 2009. The overall numbers of animals slaughtered and the estimated numbers of those born before August 1996 and those assumed to be high risk and sent to slaughter are shown in Table 3.

Table 3: Case Definition Parameters

	Prime Beef	Cull Cows	Total
Number of Cattle slaughtered (per year)	2,200,000	450,000	2,650,000
Proportion Prime Beef over 48 months old	0.010%		
Proportion Cull Cows born before August 96		0.03%	
Proportion of these high risk	0.10%	0.10%	
O48M animals to process (prime beef)	0.0		0.0
BB96 animals to process		1.53E-04	0.0
High risk animal slaughtered	41.9	0.86	42.8

The estimated quantities of BSE infectivity entering the human food supply due to failures in SRM controls and infectivity present in meat are presented in Table 4. The table shows the total infectivity from the animals slaughtered for each of the six scenarios. The values presented are the mean and median values from the probabilistic risk simulation plus the 2.5 and 97.5 percentiles of the distribution. The values represent the amount of BSE infectivity in terms of bovine oral ID_{50} units that is estimated to enter the food supply with the current annual slaughter of 2,200,000 prime beef animals and 450,000 cattle over 48 months old. The 2.5 and 97.5 percentiles represent the effective range of the results given the defined uncertainty in the input parameters. The results for each of the scenarios presented are discussed in the following sections.

The exposure has been presented in terms of bovine oral ID_{50} units in line with previous BSE exposure studies for the FSA and other published work. This is the amount of BSE infectivity that would infect 50% of the cattle exposed to it. It would be more satisfactory to express the exposure in terms of human exposure units or numbers of people who would be infected. However, there is still great uncertainty over the cattle to human species barrier and even more uncertainty over the potential for human infection. It is considered that it is best to express

results only in terms of bovine oral ID_{50} units, and use the available information on the cattle to human species barrier qualitatively to help interpret the results. In Appendix II, section II.6 it is shown that the exposure per expected vCJD case has been estimated to be about 4000 bovine oral ID_{50} units, calculated using a similar approach and assumptions as in this assessment.

Table 4: Estimated Infectivity into the Food Supply from Cattle Slaughtered in the UK

Bovine oral ID50/yr	Mean	Median	P2.5	P97.5
1. Base Case: current post mortem BSE tests	8.8	3.3	0.21	52
2. No BSE Testing	15	5.5	0.34	86
3. Ante-mortem testing on > 48m Cattle (100% sensitivity)	0.08	0.02	0.0004	0.5
4. Ante-mortem testing on >48m Cattle (95% sensitivity)	0.92	0.31	0.016	5.6
5. Ante-mortem testing on All Cattle (100% sensitivity)	0.08	0.02	0.0003	0.5
6. Ante-mortem testing on All Cattle (95% sensitivity)	0.92	0.31	0.016	5.6

5.3.1 Base Case: current post mortem BSE testing on animals older than 48 months

The first set of results represents the current situation in Great Britain in 2009 with post-mortem testing of cattle older than 48 months at slaughter. The model estimates a median exposure of 3 bovine oral ID_{50} units for the whole beef eating population of GB (range 0.2 to 52). This is almost all from animals over 48 months old, with the contribution from prime beef being negligible. This exposure is higher than that predicted in previous studies (DNV, 2007) due to changes in assumptions in the prevalence estimates. However, this does not change the overall conclusion from the previous study that taking account of the cattle to human species barrier (See appendix II.6) this represents an extremely low level of risk.

5.3.2 No BSE Testing

The second set of results are included to demonstrate the risk difference that would result if no post-mortem testing of older animals is carried out. It is estimated that the exposure to the population would increase from a median value to 3 bovine oral ID_{50} units to 5 bovine oral ID_{50} units (range 0.3 to 86). This shows that the exposure would increase by a factor of less than 2, with the overall exposure remaining very low. This is a result of the very low prevalence of BSE infection in UK cattle, even in the older animals.

5.3.3 Ante-mortem testing on animals older than 48 months

The next two sets of results (rows 3 and 4) are the estimated exposure assuming that animals over 48 months are tested before slaughter and not post-mortem with a) a test with 100% sensitivity and b) with only 95% sensitivity. Prime beef animals are not tested. The model estimates that the median exposure would reduce from 3 bovine oral ID_{50} units to 0.02 bovine oral ID_{50} units a reduction factor of 190. Whilst this is a significant reduction, it is a reduction from a low number to a very low number.

The reason that the exposure with an ante-mortem test is estimated to be so much less than that for a post-mortem test is that it is assumed, for the purposes of modelling, that the ante-mortem test would detect infectivity in an animal within the last 12 months of the incubation period, whilst the post-mortem test only has a significant impact on animals within the last month of incubation (Arnold, 2007). It might be expected that the residual infectivity with 100% sensitivity would be zero, but 100% sensitivity is modelled as a Beta distribution assuming zero false negatives in 200 samples (see Section 4.3) which allows for a low probability that some false negatives could occur. The adoption of a 12 month period of effectiveness for the test was a pragmatic attempt to assume that in routine use an ante-mortem test would perform better than current post-mortem tests, while accepting, given the state of the science, that extension into earlier stages of incubation was improbable.

5.3.4 Ante-mortem testing on all cattle

The final two sets of results (rows 5 and 6) give the estimated exposure assuming that all cull cows and prime beef animals intended for slaughter are tested pre-slaughter, again with 100% or 95% sensitivity. The results indicate that there would be no significant additional benefit from testing the 2,200,000 prime beef animals. The main reason for this is that the prevalence of BSE infection in slaughtered animals is very much less for prime beef animals than for those older than 48 months (See Table II.2 in Appendix II). Thus although testing the prime beef animals reduces their contribution to the exposure by a factor of 200, the untested prime beef animals contribute only 3% of the total exposure.

5.4 False Positive Results

Experience has shown that the incidence of false positive results with the post-mortem tests used has been very low indeed. This may not be the case with an ante-mortem test and will be difficult to demonstrate with the lack of a suitable confirmatory test. If the specificity of an ante-mortem test is defined in the same way as the sensitivity (95% probability that sensitivity not less than 98.5%) this would result in an estimated 2500 false positive results in the 450,000 over 48 month old cattle tested (range 60 to 9000). As discussed during the Expert Workshop, such a large number of false positive results would be a major problem for the industry and would be unlikely to be accepted.

As an alternative, a specificity of 100% has been modelled but with a 95% probability that the specificity is not less than 99.99%. This is modelled as a Beta distribution with 0 false positives in 100,000 samples (Beta(100001,1)). This results in an estimated 4 false positive results from the testing of 450,000 over 48 month old cattle (range 0 to17). If all cattle are tested the estimated number of false positive results is 26 (range 1 to 98). This alternative was selected following the discussion at the Expert Workshop as a value that was thought would be able to be tolerated by the industry. In effect, together with the impact of sensitivity, it sets potential target criteria for the acceptance of any ante-mortem test if the outcome of this analysis were to be considered acceptable.

6.0 Cost Effectiveness Assessment

6.1 Data for Cost Effectiveness Assessment

In order to carry out the cost effectiveness assessment cost data has been gathered for both the current post-mortem BSE test and the proposed on farm ante-mortem test. In addition, some information, such as carcase weights and values, is common to both. The data used and the source of the data are presented in the following sections and summarised in Table 7.

6.1.1 Common Data

6.1.1.1 Carcase weights

Data are required for weights of a carcase and weights of the various SRM and offals associated with it. There will be a range of carcase weights for animals slaughtered, depending for example on breed, whether the animal is a steer or heifer and prime beef or cull cow. It is not proposed to try to represent the full range here, as it would not have a major impact on the assessment. Data have been obtained from the study carried out by the Leatherhead Food Research Association (LFRA, 1997) that have been used in previous TSE risk assessment studies (DNV, 2008). The data is presented in Table 5**.** This is based on an animal with a weight before slaughter of 543kg and results in a carcase weight of 282kg.

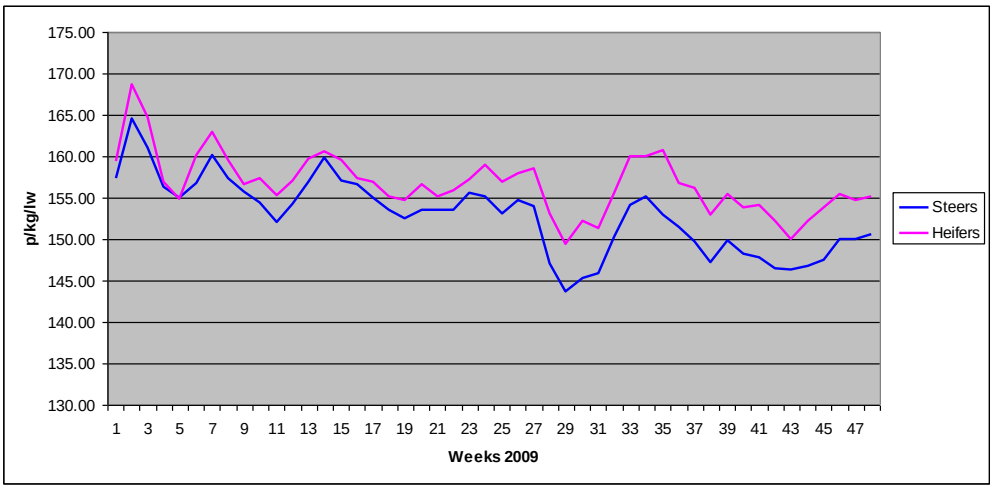
Monthly average carcase weights for prime cattle slaughtered in the UK in 2009, as published on the Defra website (see below), ranged from 337kg/head to 344kg/head over the year with an average value of 341kg/head. These weights will be used to adjust the values given in Table 5.

6.1.1.2 Carcase Values

The prices obtained for slaughtered cattle vary from year to year and over the course of the year. Data on the numbers of cattle killed, livestock values and carcase weights are all available on the Defra website in some detail, for both the current and past years (https://statistics.defra.gov.uk/esg/default.asp).

Prices for finished steers and heifers for the 48 weeks to 27th November 2009 are shown in Figure 1. This shows that prices for steers ranged from a high of £1.65/kg to a low of £1.44 with an average of £1.53, and for heifers from a high of £1.69/kg to a low of £1.49 with an average of £1.57.

Figure 1: Finished Cattle Values for England and Wales 2009



Information published by the Farmer's Weekly (http://www.fwi.co.uk/prices-trends/) give values for finished cattle as £2.85/kg dw (28 November 2009) for a carcass classified as R4L. A trend graph indicates that values over the year have been closely correlated with the finished cattle values as given in Figure 1 and this is assumed to be the case in this assessment.

Table 5: Carcase and Tissue Weights for Typical Bovine

Part of bovine		Weight (kg)	
Carcase (on bone)	Lean meat	170	
	Bone & waste	49	
	Fats	63	
	Total		**282**
Head & cheek meat			2
Fats			36
SRMs	Brain	0.5	
	Spinal cord	0.2	
	Dorsal root ganglia	0.03	
	Trigeminal ganglia	0.02	
	Lingual Tonsil	0.05	
	Distal ileum	0.8	
	Other intestine	14.2	
	Eyes	0.1	
	Other SRM	12.1	
	Total SRM		**28**
Offals	Kidneys	1	
	Heart	2	
	Liver	7	
	kirt	1	
	Tongue	2	
	Total offals		**13**
Stomachs			14
Hide			38
Blood			18
Other organs and waste			32
Gut Contents			80
Total Live weight			**543**

Data from LFRA (1997)

6.1.1.3 Animal by-product disposal

Charges for animal by-product disposal are given in the Meat Trades Journal, and indicate that disposal costs for SRM are typically about £35.00/tonne for larger contracts (over 50 tonne/week) rising to £50 or more (£56 in South West) for smaller quantities. This was confirmed in discussion with FBOs.

It is proposed to model this as a Beta Pert distribution with a best estimate of £35/tonne, minimum of £30 and maximum of £56.

There is no longer a market for blood, and this is often sent for disposal to facilities such as bio-gas plants. Costs of £45 to £50/tonne for disposal of blood were quoted.

6.1.1.4 Value of Offals

The value of offals was given as £8 to £9 per animal for a set of offals.

6.1.1.5 MHS costs

The Meat Hygiene Service (MHS) carries out official controls at approved meat premises throughout GB. The cost of these controls is charged directly to the FBO as set out in "A Guide for Food Business Operators to MHS Charges for Official Controls (The Charges Guide) September 2009." In addition, the MHS also carries out activities on behalf of government departments that are charged directly to them; this includes the supervision of the UTM SRM regulations, that are charged to the Food Standards Agency, supervision of the OTM SRM regulations and hygiene, BSE testing and disposal of animal by-products that are charged to Defra. Costs relating to cattle ID checks are shared between the RPA (75%), Defra (20%) and the FSA (5%). The MHS does not supervise all sample taking for BSE tests but checks a proportion of samples based on throughput and past compliance. Data for April 2009 to March 2010, the financial year following the change to testing over 48 month old animals, have been provided by the MHS and indicate that the costs relating to SRM hygiene and BSE test supervision for OTM animals average £5.83 per head. There is no data to break down the time spent on supervision of BSE testing, and it is thought that this would vary considerably from plant to plant. In discussion with MHS personnel it was estimated that costs relating to BSE testing could range from 30% to 50% of this total.

6.1.2 Post mortem testing

6.1.2.1 Post mortem testing costs

From the start of 2009 the cost of carrying out the BSE test on over 48 month old cattle has been paid directly by the FBO and not by government. This has opened up the market to competition and brought down prices. Discussions with representatives from meat industry bodies and some FBOs has indicated that the costs of tests now range from about £6.50 to £10.00 per test, with an average cost of £8.25. This cost includes the cost of materials, transport and analysis. LGC, who until the change in 2009 held the contract to supply BSE testing, were asked to provide a summary of test costs but declined to do so for commercial reasons. The price paid by the FBO for BSE testing excludes an EU co-financing of 5 Euros per test. It is assumed that this would also apply to ante-mortem tests and so is not taken into account.

In addition to the test cost the FBO bears the cost associated with taking the sample, and preparing samples for shipping to the laboratory. One FBO, West Devon Meats in Hatherleigh, estimated this cost to be about £1.00 per head.

6.1.2.2 Confirmatory Test

If a BSE positive result is found the sample is sent to the Veterinary Laboratories Agency (VLA) for testing to confirm the result. The cost of the confirmatory testing was reported by the VLA to be approximately £250 per animal.

6.1.3 Ante-Mortem Test

6.1.3.1 Cost of on-farm sampling

If the ante-mortem test is conducted on farm, there will be costs borne by the farmer for collecting the animals to be tested and moving them through the handling system for the veterinarian to take the necessary sample for the test in addition to the costs for the veterinarian. This will be a similar activity as required for testing cattle for bovine TB. Bennett

(2009) reports the results of a survey of 60 cattle farms on costs associated with pre-movement testing for bovine TB. It is reported that the average cost per head was £8.85, but with a range from £5 to over £30, with the variability being largely due to variations in the number of animals tested. For pre-movement TB testing the entire cost of the test is borne by the farmer. It is reported that the veterinary costs constituted 49% of this figure, and that 60% of the cost was associated with the injection part of the test. (A TB test requires the veterinarian to return to the farm on a second day to read the test). For this study it is assumed that the costs associated with ante-mortem TSE testing would be similar to those for the first (injection) stage of the bovine TB test, as summarised in Table 6 below; the split between the costs of the veterinarian and the costs to the farmer directly are shown for information only.

Table 6: Costs of Pre-movement Tests for Bovine TB

	AVERAGE	MIN	MAX
Cost of overall test (per head)	£8.85	£5	£30
Cost of first stage TB test (60%)	£5.31		
Costs of Veterinarian (49%)	£2.60		
Cost for farmer (51%)	£2.71		

Source: Bennett (2009)

The overall test cost is modelled as a Beta Pert distribution with a best estimate value of £8.85, a minimum value of £5 and a maximum of £30; with the 60% factor, as the cost of the ante-mortem test is assumed to be similar to only the first stage of the TB test, applied as a fixed value.

It may be possible that the ante-mortem sampling could be carried out by a Technical Officer rather than by a Veterinary Officer. The charge out rate for Animal Health personnel, which includes the cost of overheads etc., for a Veterinary Officer (VO) is £117.80 and for a Technical Officer (TO) is £60.79. Thus the cost of using a TO is 51.6% of the cost of a VO. However, this only applies to the 49% of the overall cost due to the veterinarian. Thus, using a TO would reduce the overall sampling cost by 23.7%. This will be considered as part of the sensitivity assessment.

6.1.3.2 Test cost

With no tests available, and therefore no data on test costs, it has been assumed that the costs associated with the test itself would be the same as that for post-mortem tests (see Section 6.1.2.1). As a variation to test the sensitivity to this assumption an alternative scenario in which the test costs are doubled will also be considered.

6.1.3.3 Confirmatory Test

It is assumed that there would have to be a confirmatory test available before an ante-mortem test could be used to confirm the results of any test positives. Following discussions with the VLA, who would be responsible for conducting the confirmatory test, it has been assumed that the cost of any confirmatory test would be the same as that for the post-mortem samples, i.e., £250 per animal.

Table 7: Summary of Data for Cost Effectiveness Assessment

Worksheet Input Data	Unit	Notes	Value	Average - Best Estimate	Min	Max
Weights						
Carcass (on bone)	kg	1	341	341	337	344
Carcass weight from LFRA	kg		282			
Carcass weight factor		2	1.21			
SRM	kg	3	33.8	28		
Blood	kg	3	21.8	18		
Offals	kg	3	15.7	13		
Other (head & cheek meat, fats, stomachs, hide, other organs & waste, gut contents)	kg	3	244.1	202		
Quantities						
Prime beef slaughtered	animals		2,200,000			
Over 48 month slaughtered	animals		450,000			
No of "no test" cases	No./year		90			
No of "positive" cases (within 3 months of onset)	No./year	8	3.00	2.50		
No of "positive" cases (within 12 months of onset)	No./year	8	9.00	9.10		
No of false positives, ante-mortem, cull cows	No./year	8	4.00	4.00		
No of false positives, ante-mortem, all cattle	No./year	8	26.0	26.0		
Blood per day/batch	tonnes		4.17			
No of animals slaughtered per day/size of batches			191.7	160.00	10	500
Cohort cull: number of cohorts and offspring killed per positive BSE case			6.97			
Values						
Finished cattle	£/kg lw	4	£1.56	£1.55	£1.47	£1.67
Carcass	£/kg dw	5	£2.95			
Carcass	£/head		£1,003.82			
Offals	£/head		£8.50	£8.50	£8.00	£9.00
ABP Category 3 waste value	£/tonne		£10.00	£10.00		
Non-pedigree bovine compensation values	£/head		£788.50	£788.50	£746.00	£831.00
Costs						
ABP disposal category 1 & 2 waste	£/tonne		£37.67	£35.00	£30.00	£56.00
Blood disposal	£/tonne		£45.00	£45.00	£40.00	£50.00
Test cost	£/head		£8.25	£8.25	£6.50	£10.00
Confirmatory test	£/head		£250.00			
Postmortem sampling	£/head		£1.00			
Cost of pre-movement test for bovine TB (eterinary plus farm costs)	£/head	6	£11.73	£8.85	£5	£30
Total sampling cost for ante-mortem test	£/head	7	7.04			
MHS Supervision of post mortem sampling	£/head		£2.33		£1.75	£2.92
Cost of cohort and offspring cull	£/head		£500			

Notes to Table 7

1 Monthly average carcase weights for prime cattle slaughtered in the UK in 2009
2 Factor to allow for difference between carcass weight in LFRA study and actual values
3 Values from the LFRA (1997) study adjusted by Carcass weight factor
4 Average price for finished steers and heifers in 2009 (Defra Statistics)
5 Liveweight based on values in Farmer's Weekly November 2009
6 Total on farm costs for pre-movement tests from Bennett (2009)
7 Cost for ante-mortem sampling assumed to be equivalent to part 1 of TB test (60% of total)
8 The number of positive cases or false positives are modelled as a Poission Distribution

6.2 Assumptions

6.2.1 Post-mortem Tests

In the event of a positive test result the positive carcase is removed plus the one before and two after on the kill sequence to reduce the chance of any cross contamination. These carcases are destroyed as SRM, as well as all the offals and blood associated with those carcases. The FBO reclaims the costs of any positive cases from government, including the values of the carcases and offals as well as the disposal costs.

Number of Test Positives

The results of ongoing active surveillance of BSE in the UK are published on the VLA website (http://www.defra.gov.uk/vla/science/sci_tse_stats_intro.htm). This data shows that there have been 8 BSE positive cases identified in GB in 2009, of which 7 were in fallen stock and one in an emergency slaughter animal. In addition there was one case identified by passive surveillance. In Northern Ireland there have been 3 positive cases, 2 in fallen stock and one in healthy slaughtered over 48 month cattle tested at fresh meat plants. In 2008 there were a total of 35 BSE positive cases identified (including 4 in NI) of which only 4 were in healthy slaughtered over 30 month cattle.

The prevalence estimates given in Appendix II, together with the total number of over 48 month old cattle slaughtered (450,000) indicate that the expected number of animals with BSE infection within 3 months of onset at slaughter for 2009 is 2.6 (9 within 12 months of onset). This predicted value is used in the assessment.

Cost of carcase disposal

The cost of disposing of the four carcasses as animal by-product category one waste is estimated from the carcase weights and the costs of ABP disposal as given above (Section 6.1.1.3).

Disposal of offal

Abattoirs have different systems for keeping track of offals and these have to be set down in the RMOP and agreed by the MHS. In some cases abattoirs will maintain identification of individual offal sets, but it is more common for these to be grouped in batches, particularly with the very low frequency of BSE positive cases. In the smaller abattoirs a batch may well be a day's production, whilst in the larger plants a days production would normally be divided into a number of batches. For this study a batch size of 160 head has been assumed as a best estimate, with a range from 10 to 500.

The disposal costs and loss of value for offal are then based on the costs associated with this batch size and not just the material from the four condemned carcases.

Disposal of blood

Blood is collected from the slaughter floor in a tank. The practices will again vary in different abattoirs, the requirement being that the FBO has to be able to identify which blood tank was used for any test positive animals slaughtered. It has been assumed that the same batch sizes as used for offals will apply.

No Tests

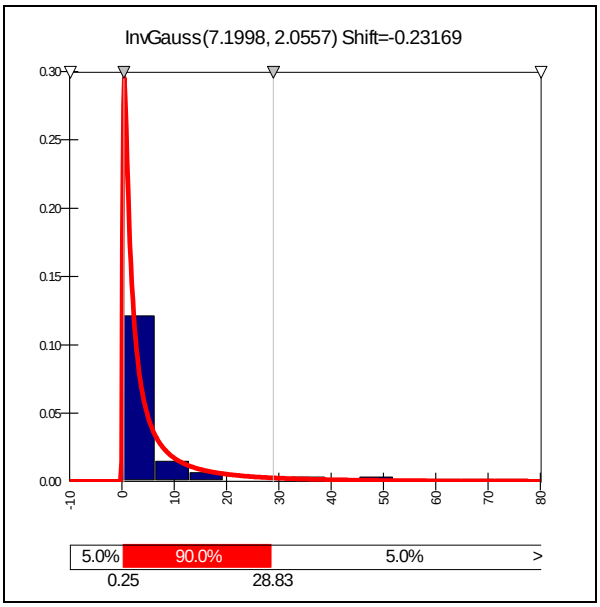
If the sample is not taken properly, so that the correct part of the brain stem is not available for analysis, the laboratory will declare the sample a "no-test", with the result that the carcase will not be allowed into the food chain and have to be destroyed. This will be a major cost to the abattoir so that there is strong motivation to ensure that samples are correctly taken. The

numbers of no tests have fallen steadily from a rate of about 0.1% in 2005 and levelling off at a rate of about 0.02% by the end of 2007. With a No-test it is only the single animal and related tissues that have to be removed and disposed of. A no-test rate of 0.02% is used in this assessment, which results in 90 no-tests in the 450,000 over 48 month old animals tested.

Cohort and Offspring Cull

Following confirmation of a BSE case the animal's cohorts and recent offspring (if a female BSE case) are identified, killed and disposed of. This results in additional costs to Government for the killing, disposal and compensation. The numbers of cohorts and offspring vary greatly. Data from Animal Health for the past three years (2007 to 2009) show that for a total of 116 BSE cases there have been 655 animals killed under the cohort and offspring provisions. These have ranged from zero to a maximum of 78 per case with a mean value of 7.0. The data have been fitted to a distribution as shown in Figure 2.

Figure 2: Distribution for Numbers of Cohorts and Offspring per BSE Case



Cohorts are killed either on farm by a veterinary officer or a knackerman, or transported live to a site and killed by a knackerman. Costs for the killing on farm have been estimated by Animal Health to be about:

- Consumables (drugs, needles, syringes) = £25.00 per animal
- Travel (40 miles at 40p per mile) = £16.00
- Time for VO, say 4 hours at £117.80 per hour for killing, paperwork etc for group of 5 animals = £97.24 per animal.

Total killing cost = £122.44 per head

In addition there would be costs for haulage, BSE sampling and disposal (incineration), which have been reported by the RPA to be:

- Collection/Haulage £218.17
- Sampling £10.08
- Incineration £147.12

Giving an average of £375.37 per cohort animal.

6.2.2 Ante-mortem testing

The following assumptions are made in the event of a positive test result from an ante-mortem test carried out on farm.

Number of Test Positives and False Positives

The predicted number of test positives for over 48 month old animals within 12 months of clinical onset is 9.4. The total estimate of all infected over 48 month animals is 46. The number within 12 months of onset will be used here. In addition to the test positives it is expected that there will be a number of false positive results associated with an ante-mortem test. This was discussed in Section 5.4 and the value of 4 proposed there has been assumed. The numbers of positives and false positives are modelled as Poisson distributions.

Cost of disposal of animal

The cost of disposal is for the single test positive animal (e.g., there would be no requirement for the one before and two after as in the abattoir).

Loss of value of animal

Defra publish a table of values for use as compensation payable for any animals compulsorily slaughtered for disease control purposes. The values from the table for November 2009 have been used here (http://www.defra.gov.uk/news/2009/091101.htm).

The table gives values for different ages, male and female and different sectors (e.g., beef, dairy, pedigree stock etc.). For this study the average value for male and female non pedigree beef animals over 20 months old, as given in the November 2009 table, have been used. This value is £788.50.

6.3 Data Analysis

The various costs presented in the previous sections are brought together to assess the costs associated with the current programme of post-mortem sampling and with the introduction of ante-mortem sampling for the scenarios summarised in Section 4.4. In both cases the costs are made up of two parts; firstly the actual costs associated with the sampling and testing, and secondly the costs associated with dealing with any positive cases including the costs associated with the cohort and offspring cull.

As for the TSE exposure model the cost effectiveness calculator has been evaluated using @risk using Latin Hypercube sampling and 10,000 iterations.

6.3.1 Post-mortem testing

The costs assessed for the current programme of post-mortem BSE testing on cattle older than 48 months at slaughter (cull cows) are summarised in Table 8. The results are given as the mean values from the simulation with the 2.5 percentile (P2.5) and 97.5 percentile (P97.5) values also being given for the total cost figures. The overall cost of post mortem sampling is estimated to be £6.2 million (range £5.4 to £6.9 million). The main part of this cost (67%) is the actual test costs. Other costs include the cost of MHS supervision of the sampling process (17%) and the cost to the FBO of taking the samples (9%). The cost associated with positive test results is estimated to be £33,000, 0.5% of the total cost, with £466,000 (7%) due to No-tests.

Table 8: Costs for Post-Mortem BSE Testing

Costs per year for post mortem testing	Mean	P2.5	P97.5
Post mortem testing			
MHS supervision	£1,049,400		
Sampling	£450,000		
Test costs (materials, shipping, analysis)	£4,162,496		
	£5,661,896	£5,013,030	£6,301,203
Post mortem "positive result"			
"Positive" result - 4 carcasses (1 before, 2 after) disposed of as animal by-product category 1	£51		
"Positive" result - value of 4 carcasses	£4,015		
Offals disposed of as animal by-product category 1	£113		
Value of offal	£1,629		
Disposal of other ABP as category 1 not 3	£2,231		
Blood disposal	£188		
Confirmatory test	£1,000		
Cost of cohort cull per positive test result	£3,484		
Total cost per positive test result	£12,712		
Total cost per year in UK	£33,143	£0	£102,843
Post mortem "no-test" result			
Total cost per positive test result	£5178		
Total cost per year in UK	£466,054		
Total cost of post-mortem test	**£6,161,093**	**£5,412,002**	**£6,904,551**

6.3.2 Ante-mortem testing

The estimated costs associated with the introduction of an ante-mortem test carried out on farm and testing only the over 48 month cattle are give in Table 9. This shows that the total cost for ante-mortem testing is predicted to be £6.9 million (range £5.2 to £9.6 million). The test costs for ante-mortem testing are assumed to be the same as for the current post-mortem test (as there is no data available), but the sampling costs are significantly greater due to the cost of the veterinarian required to take the sample and other on farm costs. The costs associated with test positive results are lower than for the post-mortem test as there is no longer the need to dispose of four carcases for every positive case and a complete batch of offals.

Provided the test specificity is very high (assumed to be one false positive in 100,000 samples) the costs associated with false positives are minor. Even if the rate of false positives were significantly greater the costs would still be a fairly small fraction of the total, but at the Expert Workshop it was felt significant numbers of false positives would result in a loss of confidence in the testing process.

Table 9: Costs for Ante-Mortem BSE Testing (over 48 month cattle)

Costs per year for ante-mortem testing - over 48 months	Mean	P2.5	P97.5
Ante-mortem testing			
Sampling	£3,167,986		
Test costs (materials, shipping, analysis)	£3,712,496		
	£6,880,482	£5,122,318	£9,525,300
Ante-mortem "positive" result			
Disposal of animal	£25		
Value of animal	£789		
Confirmatory test	£250		
Cost of cohort cull per positive test result	£3,484		
Total cost per positive test result	£4,547		
Total cost per year in UK (True positives)	£43,069	£6,671	£223,170
Total cost per year in UK (False Positives)	£18,287	£1,266	£95,133
Total cost of ante-mortem test	**£6,941,839**	**£5,179,513**	**£9,582,575**

The estimated costs associated with introducing an ante-mortem test for all cattle slaughtered for food in the UK is shown in Table 10. The total cost for testing all cattle slaughtered (2,650,000) is predicted to be £40.7 million (range £30.3 to £56.3 million), with most of this cost (99.9%) being the costs associated with sampling and testing. There are no additional costs for test positive cases over testing over 48 month old cattle as it is predicted that there would be no test positive cases found in the under 48 month old cattle. There are additional false positive cases, but again these are only a small contribution to costs.

Table 10: Costs for Ante-Mortem BSE Testing for All Cattle

Costs per year for ante-mortem testing - all cattle	Mean	P2.5	P97.5
Ante-mortem testing			
Sampling	£18,655,919		
Test costs (materials, shipping, analysis)	£21,862,477		
	£40,518,396	£30,164,764	£56,093,433
Ante-mortem "positive" result			
Disposal of animal	£25		
Value of animal	£789		
Confirmatory test	£250		
Cost of cohort cull per positive test result	£3,484		
Total cost per positive test result	£4,547		
Total cost per year in UK (True positives)	£43,069	£6,671	£223,170
Total cost per year in UK (False Positives)	£118,199	£24,543	£595,114
Total cost of ante-mortem test	**£40,679,665**	**£30,333,217**	**£56,251,173**

6.4 Cost Effectiveness Analysis

The cost effectiveness of the various test scenarios has been assessed by comparing the costs estimated with the predicted reduction in exposure to TSE infectivity. The results are shown in Table 11. The table presents the estimated cost associated with each scenario together with the assessed benefit in terms of the reduction in exposure to TSE infectivity as

predicted by the TSE Exposure Model. Results are given as the mean values for the simulation (10,000 iterations) together with the 2.5 and 97.5 percentile values. The cost effectiveness is the estimated reduction in bovine oral ID_{50} units per million pounds of cost.

The base case is given by the results for the present post-mortem testing of over 48 month old cattle, where the cost effectiveness is estimated to be 0.9 bovine oral ID_{50} units per million pounds spent (range 0.02 to 5.4).

For the ante-mortem test applied to the same group (with 100% sensitivity) the cost effectiveness is estimated to be 2.1 bovine oral ID_{50} units per million pounds spent (range 0.05 to 12.7). For 95% sensitivity the cost effectiveness is only slightly reduced to 2.0 bovine oral ID_{50} units per million pounds spent. These results indicate that the ante-mortem test applied to over 48 month cattle would be somewhat more cost effective than the present post-mortem test. (The difference is assessed to be statistically significant with a P value less than 0.0001). This is because an ante-mortem test is assumed to be able to detect infectivity in the last 12 months of the incubation period rather than only in the last month or two, and thus results in a greater reduction in exposure. However, the exposure for the base case is already very low. In both cases it is assumed that present SRM controls are in place.

Table 11: Cost Effectiveness Assessment

Cost Benefit Calculator	Mean	P2.5	P97.5
Current post mortem test (over 48 months)			
Cost	£6,161,093	£5,412,002	£6,904,551
Benefit (reduction in TSE exposure) bovine oral ID50 units	5.67	0.14	34.20
Cost effectiveness (Bovine oral ID50 units per million GBP)	0.92	0.02	5.44
Ante mortem blood test; on farm; over 48 months - cull cows; 100% sensitivity			
Cost	£6,941,839	£5,179,513	£9,582,575
Benefit (reduction in TSE exposure) bovine oral ID50 units	14.27	0.34	85.53
Cost effectiveness (Bovine oral ID50 units per million GBP)	2.11	0.05	12.66
Ante mortem blood test on farm; over 48 months - cull cows; 95% sensitivity			
Cost	£6,941,839	£5,179,513	£9,582,575
Benefit (reduction in TSE exposure) bovine oral ID50 units	13.44	0.33	81.58
Cost effectiveness (Bovine oral ID50 units per million GBP)	1.99	0.05	11.89
Ante mortem blood test, on farm; all cattle; 100% sensitivity			
Cost	£40,679,665	£30,333,217	£56,251,173
Benefit (reduction in TSE exposure) bovine oral ID50 units	14.27	0.35	85.54
Cost effectiveness (Bovine oral ID50 units per million GBP)	0.36	0.01	2.15
Ante mortem blood test, on farm; all cattle; 95% sensitivity			
Cost	£40,679,665	£30,333,217	£56,251,173
Benefit (reduction in TSE exposure) bovine oral ID50 units	13.44	0.33	81.59
Cost effectiveness (Bovine oral ID50 units per million GBP)	0.34	0.01	2.03

If the ante-mortem test is applied to all cattle then the cost effectiveness is estimated to be 0.36 bovine oral ID_{50} units per million pounds spent (range 0.01 to 2.2), significantly less than for the present post-mortem sampling activity.

As previously indicated, there is little information available on which to assess the likely cost of an ante-mortem test, if one was to be developed, and the cost has been assumed to be the same as for the current post-mortem test (overall cost including materials and transport). As a sensitivity case, the cost effectiveness has been assessed with the cost of the ante-mortem test being twice that of the present post-mortem tests. These results are shown in Table 12 a). This shows that by doubling the test cost the cost effectiveness for testing over 48 month old cattle reduces from 2.1 bovine oral ID_{50} units per million pounds spent to 1.4. Conversely, if the cost of the ante-mortem was less than that assumed it would increase. A plot of the cost effectiveness versus the test cost factor (1 = base case), is given in Figure 3. This shows that the cost effectiveness of the ante-mortem test is estimated to be the same as that for the current post-mortem test when the cost of the ante-mortem test is about 3.3 times that of the post-mortem test.

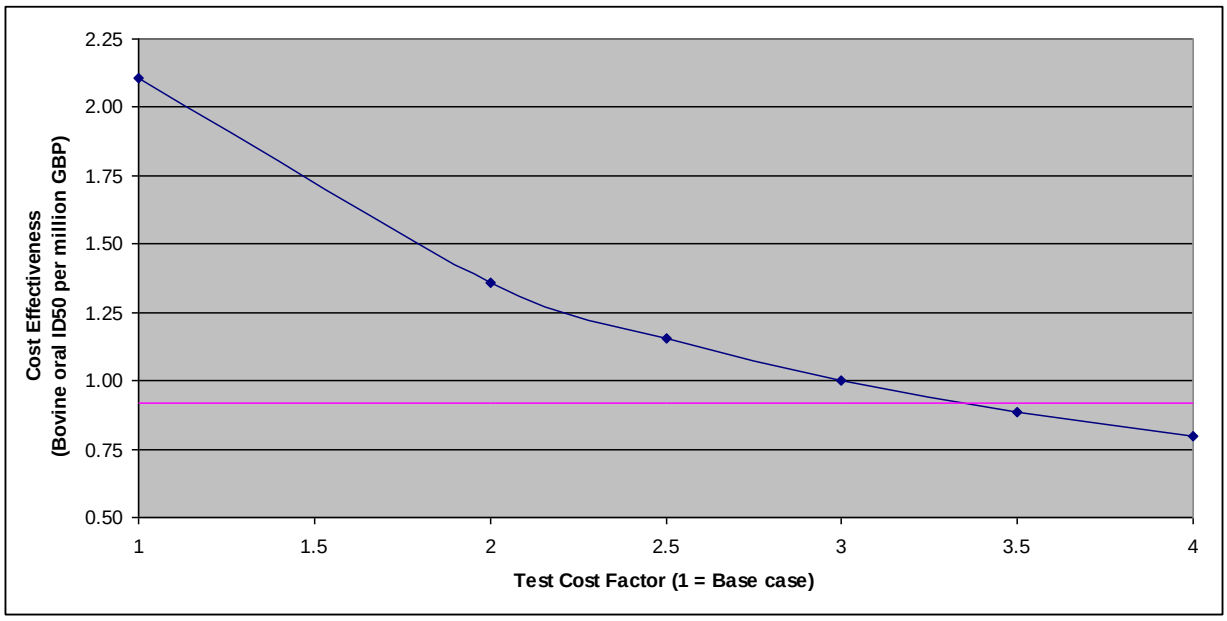
Table 12: Cost Effectiveness Assessment – Sensitivity Cases

a) Cost of Ante-mortem test twice that of Post-mortem test			
	Mean	**P2.5**	**P97.5**
Ante mortem blood test; on farm; over 48 months - cull cows; 100% sensitivity			
Cost	£10,654,335	£8,539,623	£13,471,286
Benefit (reduction in TSE exposure) bovine oral ID50 units	14.27	0.34	85.53
Cost effectiveness (Bovine oral ID50 units per million GBP)	1.36	0.03	8.25
Ante mortem blood test, on farm; all cattle; 100% sensitivity			
Cost	£62,542,142	£50,175,706	£79,179,825
Benefit (reduction in TSE exposure) bovine oral ID50 units	14.27	0.35	85.54
Cost effectiveness (Bovine oral ID50 units per million GBP)	0.23	0.01	1.40
b) Sampling carried out by Technical Officer rather than Veterinary Officer			
	Mean	**P2.5**	**P97.5**
Ante mortem blood test; on farm; over 48 months - cull cows; 100% sensitivity			
Cost	£6,190,587	£4,768,507	£8,245,569
Benefit (reduction in TSE exposure) bovine oral ID50 units	14.27	0.34	85.53
Cost effectiveness (Bovine oral ID50 units per million GBP)	2.35	0.06	14.13
Ante mortem blood test, on farm; all cattle; 100% sensitivity			
Cost	£36,255,631	£27,928,677	£48,374,871
Benefit (reduction in TSE exposure) bovine oral ID50 units	14.27	0.35	85.54
Cost effectiveness (Bovine oral ID50 units per million GBP)	0.40	0.01	2.41

In the base case the cost of taking the samples for the ante-mortem test have been assumed that the samples would be taken by a Veterinary Officer. Sampling costs could be reduced if this was able to be carried out by a Technical Officer as discussed in Section 6.1.3.1. The results of making this assumption are given in Table 12 b). This shows that using a Technical Officer for the ante-mortem sampling would increase the effectiveness only slightly from 2.1 Bovine oral ID50 units per million GBP to 2.4.

The one potential benefit from introducing an ante-mortem test in addition to reducing potential exposure to TSE infectivity that has been identified in this study is the possibility that it could lead to changes to the present European Union requirements for separation and disposal of Specified Risk Material (SRM). However, discussions with the EU indicate that rapid ante-mortem tests will never be accepted as alternatives to the removal of SRM. The potential cost saving is estimated to be £16 million, resulting from a £12 million saving in MHS costs to government for SRM controls and a £4 million saving in the cost of disposing the SRM as Category 1 ABP.

Figure 3: Cost Effectiveness of Ante-mortem TSE test versus Test Cost (on farm for over 48 month old cattle, 100% sensitivity)



7.0 Conclusions

14. The review of the state of the science has not identified any ante-mortem test for BSE that is currently ready for use or for approval. Taking into account the likely timescale for approval and modification of policy to enable the introduction of live animal tests, and the attitude of commercial companies to the challenges and risks involved, there appears to be little scope for their introduction in the foreseeable future.

15. Test specificity is likely to be critical to the acceptance of live animal testing, by both industry and consumers. The prospect of substantial numbers of false positive results that perpetuate fear of undetected or unconfirmed infection may undermine any attempts to introduce such tests.

16. Assuming that an ante-mortem test would be able to detect infectivity for a wider range of the incubation period than is currently possible with the present post-mortem tests, at least for the last 12 months of the incubation period, then it is estimated that the median exposure would reduce from 3 bovine oral ID_{50} units with the present post-mortem testing programme (range 0.2 to 52) to 0.02 bovine oral ID_{50} units (range <0.01 to 0.5) with the ante-mortem test applied only to animals older than 48 months slaughtered for food and a test with 100% sensitivity.

17. The estimated exposure for an ante-mortem test with 95% sensitivity is a median value of 0.3 (range 0.02 to 6) bovine oral ID_{50} units; this is still significantly less than the exposure with the present post-mortem test.

18. Testing the prime beef cattle in addition to those over 48 months of age at slaughter, does not result in any significant further reduction in exposure to TSE infectivity, as the untested prime beef animals contribute only 3% of the total exposure.

19. The cost of applying an ante-mortem test to over 48 month old cattle on farm prior to being sent for slaughter is estimated to be only slightly greater than that for the present post-mortem test; £6.9 million per year as opposed to £6.2 million. The higher costs of sampling on farm with the need for cattle handling and for a veterinarian to take the sample is partly off set by a reduction in MHS supervision costs.

20. The higher cost for ante-mortem testing is balanced by the additional reduction in TSE exposure, so that the cost effectiveness of the ante-mortem test applied to over 48 month animals is estimated to be 2.1 bovine oral ID_{50} units per £million spent as opposed to 0.9 for the post-mortem test. However, the significance of this increase in cost effectiveness needs to take into account the fact that the TSE exposure to the UK population from beef consumption is already at a very low level.

21. Applying an ante-mortem test to all cattle slaughtered increases the costs significantly with no additional benefit in terms of TSE exposure, resulting in a cost effectiveness value of 0.4.

22. With no data on the actual cost of an ante-mortem test it has been assumed that this would be the same as for the post-mortem test. If this cost were higher it would reduce the cost effectiveness of the ante-mortem test; however it has been shown that the ante-mortem test remains more cost effective provided that the cost of the test is no more than 3 times that of the post-mortem test.

23. The sampling costs for the ante-mortem test could be reduced by using a technical officer rather than a veterinarian to take the sample. It has been shown that this would increase the cost effectiveness only slightly from 2.1 to 2.4 Bovine oral ID50 units per million GBP.

24. If the introduction of a reliable ante-mortem test were to enable a change in the EU requirements for removing and disposing of SRM, this could result in a substantial cost saving, estimated to be £16 million. This estimated cost saving is greater than the cost of the test. However, discussions with the EU indicate that rapid ante-mortem tests will never be accepted as alternatives to the removal of SRM.

25. This study has demonstrated that although desirable, it is not necessary to have a live animal test that works at all stages of incubation. It has been shown that improving sensitivity from 3 to 12 months before clinical onset has a real impact, and potential benefits. This could help to define the minimum specification for a live animal test, i.e., 100% specificity and 95% sensitivity within 12 months of clinical onset, at least for the protection of the food chain.

26. This study has focussed on the application of TSE testing in cattle. Whilst there are grounds for greater optimism for the development of ante-mortem tests in small ruminants, there is currently little evidence of a test approach that could be used in a similar way as that investigated for bovines in this study.

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Appendix I State of the Science

Table of Contents – Appendix I

I.1. Introduction

The Food Standards Agency's Strategic Plan for 2005-2010 commits the Agency to promote and aid the development of a sensitive, rapid live test for TSEs by 2010. This has been a key objective within the Agency's TSE research programme, and has been taken forward in co-ordination with other funding bodies. In 2007, a review of the TSE research programme concluded that this remains a priority, despite the continued reduction in the number of BSE cases in the UK, but that the Agency should consider carrying out a cost benefit analysis of the impact of an ante-mortem test for TSEs. As a result the Agency has contracted DNV to carry out this study to assess the cost effectiveness of the use of ante-mortem TSE tests. This Interim Report presents a summary of the current state of the science relating to the development and approval of ante-mortem TSE tests.

During the course of the BSE epidemic, government departments and science councils invested substantial funds in the search for tests to confirm a diagnosis of prion disease in animals or humans prior to death. The existence of such tests at the outset would have simplified approaches to the eradication of BSE, as it would have enabled infected cattle to be detected and eliminated before they progressed to clinical onset, and ideally before dispersal or slaughter for human consumption. Infected and uninfected herds could have been identified, and traditional approaches involving herd certification, herd culling, and pre-movement testing would have enabled a more rapid truncation of the epidemic. In so doing it would have protected animal and human health, and prevented or reduced the scale of the crisis that arose following the announcement of a probable link between variant CJD in humans and BSE in cattle in March 1996.

This project aims to assess the impact of the introduction of ante-mortem tests for BSE at the current stage of BSE epidemic, when prevalence levels are low. At the time of writing, only five confirmed cases have been detected in Great Britain in 2009, none of which were clinical suspects. There were 33 cases in 2008, of which only two were clinical suspects.

The Agency has requested that the study should focus on the likely impact and costs of the introduction of ante-mortem tests, and probable benefits based on a set of proposed scenarios for their use. This report will therefore limit the review of the scientific literature, and consultations, to that necessary to enable consideration of test options in cattle. Options for the

testing of small ruminants and cervids are discussed primarily as indicators of the breadth of approaches attempted in the past, so that the current situation *vis a vis* cattle can be considered in context. Although publications on the application of diagnostic approaches in humans were also reviewed, these were primarily limited to a review of abstracts, to ensure that all possible options for live animal tests in cattle had been considered.

I.2. Literature review

A literature search was conducted in December 2008, using the following search terms:

prion or prions or (spongiform encephalopathy) or scrapie or (chronic wasting disease) or (creutzfeldt* jakob disease)*
AND
(live and animal) or (blood test) or (blood tests) or (blood testing) or cerebrospinal or biopsies or (in vivo)
AND
Test or detect* or diagnose**

Appropriate later publications were also obtained and included in the review.

The fact that broad reviews of the subject area generally outnumbered publications on the performance of specific live animal tests in domestic ruminants (other than lymphoid biopsies in sheep), is testament to the difficulties faced in this area. Few of the publications specifically addressed the diagnosis of BSE in cattle.

Key issues for consideration in the evaluation of test performance are:

- Whether or not the test can be performed on infected animals before the onset of clinical disease. Despite the potential value of a test that could have been applied to the 215,000 of so clinical suspects examined during the course of the epidemic, it is important to recognise that the majority of infected animals would have died or been killed before clinical onset, or without the recognition of clinical signs suggestive of BSE. (Ferguson & Donnelly, 2003).
- The application of such tests to clinically normal animals would enable the earlier detection of infected herds, the culling of infected animals, and the testing of animals before consumption. Exposure of both cattle and consumers would therefore have been massively reduced.
- Key to the performance of such tests in the pre-clinical stages is the pathogenesis of BSE. Reliance on the testing of CNS tissues limits their effectiveness in protecting human health, a key requirement if relaxation of other regulations is to be achieved, to the late stages of incubation. Arnold et al (2007) estimated that the window for detection using current post mortem tests on experimentally infected cattle extended to no more than a few months before onset of clinical disease (50% detected at 1.7 months before onset in the 1g dose group (95% bootstrap confidence interval 0.2.:4.0), with detection falling close to zero at 3 months before onset. This represented 97% of the incubation period. This is why the removal of SRM from the carcases of cattle intended for human consumption remains the primary protective measure.

I.3. The pathogenesis of prion diseases in ruminants.

During the early years of statutory controls, human and animal health protection measures were based upon limited knowledge of the pathogenesis of scrapie in sheep and goats. Research to specifically investigate the pathogenesis of BSE in bovines has continued for almost 20 years, with each generation of studies using improved analytical tools, including transgenic mice and sensitive immunodiagnostics (SSC, 2001, 2002; WHO, 2006).

The study of **naturally infected**, terminally affected, cattle initially identified infectivity only in the brain, spinal cord and retina (Fraser & Foster, 1994). More recently the use of transgenic mice for tissue bioassay enabled the identification of a trace of infectivity in muscle although this may have arisen because it was not possible to exclude the possibility that the sample contained a small amount of peripheral nerve (Buschmann & Groschup, 2005). Direct inoculation of calves with tissue derived from clinically affected cattle produced equivocal results for the third eyelid (WHO, 2006).

The primary pathogenesis studies following **experimental challenge** identified infectivity only in brain, spinal cord, and ileum (Wells et al, 1994, 1996, 1998), with one equivocal result in bone marrow at or about the onset of clinical disease (Wells et al 1999, Sohn et al, 2009). Evidence of infectivity in tonsil at 10 months post exposure was obtained when some bioassays were repeated by inoculation into cattle rather than laboratory rodents (Wells et al 2005). The substitution of calves with transgenic mice as the assay model indicated that low levels of infectivity were detectable in palatine tonsil at multiple time points in the preclinical phase of incubation (Espinosa et al, 2007).

Incomplete studies in further pathogenesis studies have identified the present of abnormal PrP in autonomic nerves connecting the intestine to central nervous system before the CNS is positive (Hoffman et al, 2007). Additional studies using sensitive immunodiagnostics have demonstrated the involvement of peripheral nerves, both in naturally and experimentally infected cattle, but only at or after onset of clinical disease (Iwata et al, 2006; Masujin et al, 2007). The adrenal gland has also been shown to be infectious (Masujin et al, 2007).

With respect to timing of arrival of infectivity, and likely positivity when tested, the ileum has been shown to be infectious as early as six months post-exposure (Wells et al, 1994). On the other hand the brain and spinal cord remain negative until the later stages of incubation (Arnold et al, 2007). Studies in transgenic mice confirmed the presence of infectivity in CNS marginally before the tissues were positive using immunodiagnostic methods (Espinosa et al, 2007), and confirmed that tonsils remained infected during the course of incubation, but were significantly less infectious than the CNS. Despite hope that the detection of infectivity and PrP^{res} in ileal Peyer's Patches (PP) could lead to a target for the screening of cattle slaughtered for human consumption, and thereby offer an alternative to ante-mortem testing, the regression of PP during life, coupled with considerable variability in the frequency and intensity of immunostaining (Terry et al, 2003) highlight the unsuitability of the tissue for sampling. Irrespective of the sensitivity of the assay to be used, the degree of sampling error would be unacceptable, undermining the value of any negative results.

No infectivity has been detected in accessible fluids (blood, CSF, urine). With the exception of the trace of infectivity detected in palatine tonsil (Wells et al, 2005; Espinosa et al, 2007), peripheral lymphoid tissues have remained negative when examined either for infectivity or the presence of abnormal prion protein. Using transgenic mice that were demonstrated to be a more sensitive bioassay model than wild type mice or cattle, the lack of involvement of peripheral lymphoid tissue was further confirmed even at the terminal clinical phase in a naturally infected bovine (Buschmann & Groschup, 2005).

These results contrast with the recognised involvement of peripheral tissues in small ruminants, whether infected with scrapie or BSE (Dennis et al, 2009; Espenes et al, 2006); Gavier-Widen et al, 2005; González et al, 2005, 2006, 2008a,b; O'Rourke et al, 1998, 2000, 2002; Schreuder et al, 1996, 1998; and in cervids infected with CWD (Keane et al, 2009; Mathiason et al, 2006, 2009; Spraker et al, 2009; Wild et al, 2002). The successful experimental transmission of both scrapie and BSE by blood transfusion in sheep suggests that blood would prove to be a potential matrix for future testing of sheep (Houston et al, 2000; Hunter et al, 2002). Similar results have subsequently been obtained with CWD-infected white-tailed deer (Mathiason et al, 2009). The presence of infectivity and PrP^{Sc} in peripheral lymphoid tissue has led to the evaluation of biopsies of tonsil, third eyelid and rectoanal mucosa-associated lymphoid tissue (RAMALT) for the screening of small ruminants and cervids for evidence of infection. Despite the potential to identify infected animals in relatively early stages of incubation, the recognition that the involvement of peripheral tissues is dependent in part on genotype, but also potentially with strain of agent (e.g. atypical scrapie), highlights the challenges of using particular testing approaches other than in specific contexts (e.g. where the sheep genotype and infecting strain are known to be compatible with high sensitivity/specificity of tests).

In all instances, the evaluation of potential live animal tests is compromised by the lack of a reliable gold standard test for confirmation of disease status, irrespective of the stage of incubation (Ruth, 2003). While reliance is placed on the testing of CNS for confirmation of a test result, there will be continued reluctance to accept a negative result obtained with a live animal test in a relatively young animal. This is because the result cannot be verified by other means. The testing of lymphoid tissue in small ruminants and cervids has the potential to narrow the window of uncertainty in the early stages of infection, but in cattle it remains a very large window extending to almost the full natural lifespan. This is a serious challenge for any attempt to evaluate and introduce a live animal test for bovines. For other chronic infections of bovines, such as tuberculosis, the window of uncertainty is shorter, but still represents a serious challenge to confidence and acceptance of policy when animals that are slaughtered following a reaction to skin tests are slaughtered and necropsied without confirmation of infection by the isolation of *Mycobacterium tuberculosis* (de la Rua-Domenech et al, 2006).

I.4. Live test approaches

The description of a putative immunological test for scrapie as early as 1974 (Field & Shenton, 1974) offered hope for the differential diagnosis of neurological diseases of sheep. Unfortunately there is no evidence of any subsequent refinement or validation of the method in the literature. Avenues of investigation of sampling approaches and test methods for BSE were originally based on those already used for other diseases, including scrapie in sheep. With time, the combined benefits of scientific understanding and technological advances have given rise to claims of breakthroughs and hopes of success. Unfortunately, for prion diseases of both small ruminants and bovines extensive reviews of the area in recent years consistently express disappointment at the lack of progress, with the exception of lymphoid biopsies in small ruminants and cervids (Brown, 2005; Brown et al, 2001; Gavier-Widen et al, 2005; Grassi, 2003; Grassi et al, 2008; Ingrosso et al, 2002; Lehto et al, 2006; Nunnally, 2002; Parveen et al, 2005; Soto, 2004).

While dependence on pathogenesis is recognised, the lack of analytical sensitivity of PrP detection methods is seen as a continuing obstacle to the development of tests even where infectivity appears to be present in blood (sheep, humans). In 2004, Soto estimated that immunodiagnostic methods were still 2-4 orders of magnitude away from being able to consistently detect PrP^{Sc} in blood. Despite subsequent enhancements to methodologies, the

evaluation of tests for the screening of human blood, for use in blood transfusion, continues to face the same challenge.

I.4.1 Clinical signs and neurophysiological measurements

I.4.1.1 Clinical signs

By definition, clinical signs are of no value for the screening of a healthy population prior to slaughter. Even at the peak of the BSE epidemic in 1992, when veterinary diagnostic skills were honed through regular examination of clinical cases, approximately 15% of all clinical suspects that were slaughtered were BSE-negative. As prevalence has fallen the diagnostic rate has declined in line with those in other countries where the small number of cases presented has compromised recognition of early clinical signs. In 2008, a total of 32 clinical suspects were slaughtered in Great Britain, but only two were confirmed (93.8% negative).

Even when examinations have been conducted by clinicians with specialist expertise in conducting neurological examinations, sensitivities and specificities in relation to individual or grouped clinical signs have remained variable, and lower than would be required for use in large scale screening (Braun et al, 1998, 1999a, b, 2004; Konold et al, 2004, 2006; McElroy & Weavers, 2001; Saegermann et al, 2003, 2004, 2005; Scott & Henshaw, 1995; Wilesmith et al, 1992). The diagnostic value of examinations was also expected to decline to unacceptable levels in low prevalence scenarios (Braun et al, 1998). Furthermore, the range of diseases that present with clinical signs that overlap with those of BSE is considerable (Saegerman et al, 2003), suggesting the need for their inclusion in any evaluation of test specificity. The fact that such animals are now rarely presented as BSE suspects in Great Britain highlights the danger in relying on clinical signs alone to detect infected animals.

I.4.1.2 Heart rate variability

The recognition that clinically affected BSE cases were presenting with evidence of neurophysiological damage led to attempts to use them for diagnostic purposes. Heart rate was low in comparison with healthy controls despite the excitable nature of advanced clinical cases experienced early in the BSE epidemic, and responded when treated with atropine sulphate (Austin et al, 1996, 1997). As more sophisticated equipment became available, remote monitoring of data transmitted from cases confirmed not only the low heart rate, but also an underlying variability in heart rate, not dissimilar to that observed in respiratory sinus arrhythmia in humans under anaesthesia (Pomfrett et al, 2004). Similar results were obtained in scrapie-infected sheep (Glover et al, 2007)). While results on clinical cases of BSE proved promising, albeit without 100% sensitivity, attempts to translate such approaches into tests that could be applied at the preclinical stages proved difficult. While heart rate variability could separate exposed and unexposed populations, the approach could not be relied upon for the identification of individual infected animals. Furthermore, the absence of a gold standard for confirmation of the infectious state of an animal meant that resolution of status proved difficult.

I.4.1.3 Rumination measurements

Early investigation of the clinical signs of BSE led to the recognition that while appetite generally remained healthy, BSE-affected animals suffered from impaired rumination, resulting in an inability to fully digest ingested feed and consequential weight loss (Austin & Simmons, 1993). Attempts to devise remote sensing devices that could measure the degree of rumination suppression proved impossible at the time and were not pursued. No attempt was made to investigate rumination patterns in preclinical animals.

I 4.1.4 EEG

Limited attempts were made to examine clinically affected cattle by the recording of electroencephalograms (EEGs) (Scott et al, 1988, 1989). Although changes in EEG patterns were recorded in affected cattle, this line of research was not pursued to the point of establishing diagnostic sensitivity. EEGs could not be recorded easily on farm, and it was generally necessary to transfer such animals to a specialist clinic. No attempt was made to record EEGs in preclinical animals.

I.4.2 Blood tests

Blood is the most commonly used matrix for the testing of animals for the presence of infection or for the investigation of metabolic imbalances. Venous blood is accessible, and relatively large numbers of animals can be sampled in a working day with usual farm handling systems. Equivalent systems could be replicated at abattoirs if required. Published and unpublished claims of success need to be interpreted with caution. Frequently they are based on small numbers of samples, usually unblinded, and even where appropriate numbers of blinded samples have been tested the absence of appropriate negative controls means that a true evaluation of specificity has not been carried out.

I.4.2.1 PrP detection

It cannot be assumed that tests developed for use on brain (or lymphoid) tissue can be used directly on blood in any species. Consequently no attempt has been made to review test methodologies per se, unless already applied to blood, at least in laboratory models.

Research into the use of blood as a matrix for testing has been based primarily on the use of laboratory rodent models, which demonstrated that both infectivity and PrP^{res} could be detected (Castilla et al, 2005; Cervenáková et al, 2003; Chang et al, 2007; Gregori et al, 2008; Grosset et al, 2005; Saa et al, 2006; Tcherkasskaya et al, 2005; Tsukui et al, 2007) . Given the need to reduce the risk of human to human transmission via blood transfusion, as has been demonstrated experimentally in sheep, and considered likely to have happened in humans following epidemiological investigations (Hewitt et al, 2006; Llewelyn et al, 2004; Peden et al, 2004), initial attempts to find a blood test for BSE were overtaken by the need for a test for vCJD.

Nevertheless, there is no doubt that even in species where infectivity can be detected in blood, such as rodent models or sheep, the amounts of infectivity present is very small, as demonstrated by bioassay. Consequently, the analytical sensitivity of test formats currently available appears to be insufficient to consistently detect samples from positive sources (Brown, 2005; Brown et al, 2001; Soto, 2004). The application of methods claimed to be highly sensitive to sheep blood has failed to generate consistent results, and particularly to cope with variability in peripheral circulating infectivity arising from host genotype and stage of incubation (Carmona et al, 2004; Everest et al, 2007; Jackman et al, 2006; Thorne et al, 2008;). The evaluation of tests for use on human blood is still in progress in the United Kingdom, and uses spiked blood, as well as blood from scrapie and BSE-infected sheep for the purposes of initial evaluation.

The Protein Misfolding Cyclic Amplification Assay (PMCA) (Castilla et al, 2005; Jones et al, 2007; Orrú et al, 2009; Saborio et al, 2001; Soto et al, 2002, 2005) or equivalent approaches (QuIC assay) (Atarashi et al, 2007, 2008), appear to be approaching levels of sensitivity that could offer opportunities for the testing of blood, but current timescales for completion of testing in the laboratory [circa 48-72 hours minimum] present real challenges for conversion into high throughput tests, and would be unacceptable if testing was conducted at abattoirs. The need to use PrP^{C} derived from the host species as substrate for this test limited the scope for further development, but, more recently, success has been achieved with the use of recombinant PrP^{C} and with substitution of sonication with shaking (Orrú et al, 2009). Both offer potential for

improvements in test turn-round time, and for conversion into commercial platforms. Indeed, there may also be scope for developing universal substrates to overcome the apparent dependence on the use of homologous tissue, especially as hamster rPrP appeared capable of supporting conversion of both human and sheep PrP^{res} in test material (Orrú et al, 2009). Although the PMCA/QuIC methodologies have recently been shown to be able to generate infectivity/PrP^{res} de novo, suggesting a risk of false positive results, the extreme test conditions used for such demonstrations suggest that it may be possible to optimise formats to minimise the risk of such incidents. Other approaches that attempt to increase analytical sensitivity by combining different methods of amplification (amplifying either the analyte, as in PMCA, or the capture/detection antibodies when bound to antigen) show promise, but have yet to progress beyond the experimental stage (Barletta et al, 2005; Bellon et al, 2003; Bieschke et al, 2000; Birkmann et al, 2006; Chang et al, 2009; Dabaghian et al, 2006; Dietrich et al, 2005; Fuji et al, 2007; Trieschmann et al, 2005). All such amplifications carry the risk of false positive results if care is not taken to eliminate all residues of PrP^{C} before amplification.

There has been little success in using bovine blood. This may simply reflect the absence of a consistent haematogenous phase to BSE pathogenesis. The rare claims of success in discriminating small numbers of clinically affected animals from unexposed control populations have not progressed to the publication of robust data on large numbers of animals, including appropriate negative controls, or the development of a commercial test. Recent success in demonstrating the effectiveness of PMCA on sheep blood, especially from sheep carrying the VRQ allele (Thorne et al, 2008), has yet to be followed up in bovines where genotype effects are less likely.

The identification of molecules that bind specifically to abnormal PrP, and not to PrP^{C}, have yet to lead to the development of live animal tests. One currently approved post-mortem test (IDEXX HerdChek*BSE AntigenTest) utilises the proprietary ligand “seprion” developed by Microsens Biotechnologies, but attempts to use the same approach for live tests remain incomplete. Plasminogen was another molecule mooted to be capable of binding to disease-specific PrP(Fischer et al, 2000), but has more recently been demonstrated to bind to PrP^{C} also (Cuccioloni et al, 2005; Kornblatt et al, 2004; Negred et al, 2007) .

I.4.2.2 Surrogate markers

Surrogate markers have been evaluated most frequently in humans, where in a clinical scenario with patients suffering from neurological diseases the results can be combined with a range of additional parameters measured at relative leisure, and repeatedly. Sensitivity and specificity have rarely approached 100%. For the testing of livestock, opportunities to apply multiple tests simultaneously will be rare, and the failure of any such test to demonstrate effectiveness in clinical cases of BSE, let alone in preclinical animals, rules them out for the purposes of this study.

Markers that have been evaluated in laboratory models, humans and in bovines (see reviews for background) include 14-3-3 protein, neurone specific enolase (NSE), fatty acid binding protein, γ-interferon, prostaglandin E2, laminin receptor protein, C-reactive protein, cystatin C, blood metabolites, and erythroid associated factor (ERAF/EDRF) (Brown et al, 2007; Clinton et al, 2006; Maury et al, 2001; Miele et al, 2001). None have proved satisfactory in bovines.

Research is still ongoing into the potential diagnostic value of circulating nucleic acids (CNAs)(Beck et al, 2009; Brenig et al, 2003; Schütz et al, 2005), where there is evidence of differences between clinically affected animals, and their cohorts of origin, and other healthy control populations. Evaluation of performance in experimentally infected animals, both cervids and bovines, shows promise, but early results in cattle suggested that results were only diagnostic in cattle shortly before clinical onset (Gordon et al, 2009).

One further hypothetical diagnostic marker has failed to gain appropriate peer review since first proposed in 1996 (Bergmann & Preddie, 2008; Ruth, 2003). The “prionin”, a small β-sheet derived protein supposedly expressed from within the prion protein gene, is claimed to be present in all infected animals. Exposure to externally derived prionin also supposedly results in the production of antibodies to the molecule. Diagnostic approaches can involve the detection of either prionin or antibody. Unfortunately, the absence of gold standard materials of known status for evaluation purposes means that claims of positivity arising at testing of supposedly unaffected animals cannot be verified. Unfortunately, the absence of external validation of such results has not prevented its marketing in the EU for non-statutory purposes (Ruth, 2003).

I.4.2.3 Non-specific approaches

This traditional approach to diagnosis in farm animals, measuring changes in normal physiological parameters, has also failed to deliver sufficient sensitivity and specificity with BSE even in clinically affected cattle, let alone in preclinical stages. While some methods, such as Fourier-Transform Infrared Spectroscopy (FT-IR) simply measure the spectrum of differences between test samples from cases and controls, without initially attempting to measure what those differences represent, they have delivered reported sensitivities and specificities of 85–96% and 90-92% respectively with clinical cases (Carmona et al, 2005; Lasch et al, 2003; Martin et al, 2004; Schmitt et al, 2002). Translation into preclinical stages proved far more problematic. Only one such method did progress to formal evaluation by EFSA on behalf of the European Commission, and achieved sensitivity and specificity using two approaches (variations of test parameters) of 88.2-88.7% (95% confidence range 74.4-95.8%) and 63.5 – 78.4 (range 58.1-82.3%) respectively (EFSA, 2006). It was not approved for wider use or further evaluation at that stage.

While such approaches focus on the general, others look at the minute detail of changes to genetic regulation of normal physiological processes – looking for up or down regulation of thousands of genes. Such approaches have from time to time identified potential diagnostic candidates, especially in rodent models, but have failed to translate into large animals, whether naturally or experimentally infected [e.g., EDRF/ERAF] (Brown et al, 2007; Clinton et al, 2006; Maury et al, 2001; Miele et al, 2001). Research continues in this area, using materials collected during time-course studies in experimentally infected cattle. Expression studies have the potential to elucidate whether specific genes, other than *PRNP*, are directly involved in the pathogenesis of BSE. If so, their up or down-regulation offers opportunities for the development of diagnostic tests, or alternatively use as gold standards for the confirmation of disease status when evaluating other tests at the preclinical stages (Khania et al, 2009;Tang et al, 2009).

I.4.3 Cerebrospinal fluid (CSF) based tests

Although used commonly in medicine to diagnose CJD and other neurological diseases of humans, the sampling of cattle presents far greater challenges than it does in humans, partly because it is difficult to control the conditions under which samples would be collected on farm. Although feasible, the logistics, and inevitable limitations on the number of animals that could be safely sampled per day, would make this approach expensive as well as impractical other than in the most severe of economic circumstances.

Evaluations of the diagnostic value of 14-3-3 protein (Dawson et al, 1997; Hsich et al, 1006; Lee & Harrington, 1997; Yutzy et al, 2007), apolipoprotein E (Dawson et al, 1997; Jones et al, 1996), S100b (Green et al, 1999; Shimada et al, 2005) and Tau have all proved disappointing even in the clinical phase, even for CJD (Pennington et al, 2009), and, with the exception of S100B, have not been evaluated in preclinical animals. Results for time-course analyses for S100B have not been published. An earlier broader approach to differential diagnosis, through

the examination of CSF protein and cellular components, offered some scope for the elimination of animals affected by other diseases, at the clinical stages, but could not specifically identify BSE cases (Scott et al, 1989; Scott & Trenti, 1994). Preliminary data on the evaluation of α-synuclein in humans as a diagnostic marker appears not yet to have been followed up in animals (Mollenhauer et al, 2006).

I.4.4 Urine and faecal tests

Urine represents another accessible test matrix, particularly in dairy cows, but early attempts to detect changes that were potentially diagnostic faced considerable challenges, not least of which was the diurnal variability in urine concentration in cattle. Most of the early research into urine based tests remains unpublished.

More recently, claims that PrP^{res} could be detected in hamster (and bovine) (Shaked et al, 2001) urine led to challenges that such results may have arisen from non-specific detection of immunoglobulins (Serban et al, 2004), or bacterial proteins (Furukawa et al, 2004), but the detection of infectivity in hamster urine suggests that the approach is not without value. The low levels of infectivity even in the hamster model does however suggest that sampling error is likely to diminish test sensitivity to the point where testing cannot be applied with confidence (Gregori et al, 2008). Care will also be needed to ensure that test approaches can discriminate between PrP^{C} and PrP^{Sc} in urine (Andrievskaia et al, 2008).

Early results of Canadian research into variability in surrogate markers suggest that the presence of clusterin may be indicative of infection, but given the small number of animals tested, and the lack of sufficient and appropriate negative controls means that proof of specificity is required before its scope for large screen testing can be thoroughly evaluated (Simon et al, 2008*).* Clusterin and other markers detected in the study were claimed to be capable of detecting infected animals as early as eight months post exposure. The authors stressed the need for further validation, highlighted by the need to exclude results from one BSE-confirmed animal from the analyses, on the grounds of non-conformity.

Confirmation that infectivity can also be detected in faeces in hamster models argues in favour of consideration of faeces as a potential test matrix (De Motes et al, 2008; Safar et al, 2008). It is however a challenging matrix to deal with, although methods for the extraction of PrP^{res} from faeces have been developed. It seems clear however that despite the success of the research in hamsters within the first few days following infection, demonstration of the detection of PrP^{res} or infectivity in later stages of incubation remained a challenge. As this represents the majority of the life of an infected ruminant, it will be necessary to both demonstrate the continued excretion of infectivity throughout the incubation period, and to significantly improve the sensitivity of analytical approaches, before such an approach could be considered. The absence of evidence for horizontal transmission of BSE in cattle suggests that faecal excretion is not significant, and that faeces are unlikely to represent an effective test medium.

I.4.5 Lymphoreticular biopsies

Despite the extensive body of evidence that lymphoreticular biopsies can be used for the screening of sheep exposed to scrapie (Espenes et al, 2006; Gonzalez et al, 2005, 2006, 2008, a, b; O'Rourke et al, 1998, 2000, 2002), or farmed and wild cervids infected with CWD (Keane et al, 2009; Spraker et al, 2009; Wild et al, 2002), there is no published evidence of any attempt to use lymphoid biopsies as a matrix for testing in bovines. Nevertheless, in the context of BSE, where testing of cattle must provide equivalent protection to the removal of SRM, none of the evidence from sheep and cervids suggests that satisfactory levels of sensitivity and specificity would have been achieved, even if bovine LRS had been found to be PrP^{res}–positive.

In sheep, sensitivities 85.3-99.4% have been cited (O'Rourke et al, 2000). Detection cannot however be guaranteed at early stages of incubation, other than in the most susceptible of genotypes, and therefore such approaches are more appropriate to flock screening in a certification/eradication context, rather than as a guarantee of freedom from infection for individual animals. There is little danger of false positive results. The situation in cervids is similar, with sensitivities and specificities ranging from 81% (farmed) to 91% (wild) (Keane et al, 2009).

The most sensitive approach to the examination of LRS tissue involves the use of immunohistochemistry, as this can control for the presence of sufficient lymphoid follicles, and can confirm infection in the presence of minimal staining that would generate negative results with rapid test formats. Attempts to investigate the potential of tonsillar biopsies for live testing of bovines failed not only because of the absence of immunostaining in the few samples tested, but also due to the relative absence of lymphoid tissue in biopsies when compared with small ruminants, coupled with the greater practical difficulties of collecting biopsy samples from cattle (VLA, unpublished observations).

I 4.6 Additional approaches

Methods that can be applied more easily in a clinical context with humans, either to samples or to the body, frequently involve the use of equipment that cannot be transported to the farm or other locations. Such approaches, involving nuclear-magnetic-resonance (NMR) (Bell et al, 1991; Vidal et al, 2006), magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) (Broom et al, 2007), in-vivo micro magnetic resonance imaging (Sadowski et al, 2003) are most unlikely to represent approaches to the screening of healthy animals on the grounds of both impracticality and diagnostic insensitivity during early stages of infection.

I.5. Consultations

I.5.1 Consultation with test-developers

A questionnaire was sent via the Veterinary Laboratories Agency (VLA) to 23 companies or institutes that had requested materials from the VLA TSE archive in the past for the purposes of test development or validation, with particular emphasis on live tests. Another company known to be involved in both research and continued test development, but which had not received materials from the VLA, was approached directly. In addition, some scientists funded by the FSA and Defra in recent years under the banner of "diagnostics" were sent an equivalent questionnaire, either via their funding body (FSA), or directly in the case of staff at the VLA and the Roslin Biocentre.

The number of responses received from the companies and institutes contacted by the VLA was disappointing, but not unexpected. Many had previously expressed their intention to VLA staff of impending withdrawal from the field. Others were expected to defer from responding due to commercial confidentiality.

Four detailed questionnaires, and one background note, were received from scientists contacted via the FSA or directly. Two commercial companies, currently involved in marketing post-mortem tests for BSE and scrapie, responded with a completed questionnaire. Three others confirmed their withdrawal from this field of development.

The following represents a summary of relevant detail provided in six questionnaires. All six confirmed a desire to develop a test for use on blood, while two also expressed an interest in testing milk, and one also targeted CSF. Four approaches involved the detection of PrP^{Sc}, one used proteomics while the fifth targeted up-regulation of ERAF. The latter approach has since been abandoned because of the considerable variability in results from healthy large animals and humans which confounded discrimination between infected and healthy individuals.

Only three of the six responses indicated a desire or scope to test bovines. The three did not include the commercial companies. All six were interested in the testing of sheep both as an end in itself and as a model for test development. Only three groups had an immediate interest in developing a test for humans, while one felt there was scope for testing cervids (but this was subsequently abandoned).

Test development had begun between 2003 and 2007, but this was in part determined by the availability of research funds. In other words, the start date coincided with the initiation of research projects. Attitudes regarding the time required to develop methods to the point of introduction were surprisingly, and perhaps unrealistically in the light of current approval procedures, optimistic, ranging from 15 months to five years. It was suggested that a test for BSE in bovines could possibly be available within two to three years, but only if it was firstly demonstrated that test methodology that worked on sheep could actually detect PrP^{Sc} in bovine blood.

While researchers were concerned that lack of funding was a key obstacle to future development, they also felt that the key deficiency was the lack of understanding of pathogenesis in any species. In other words, the route to identifying the best approach to live animal testing was thought to be resolution of underlying pathogenesis, and that failure to do so would inevitably hamper test development.

Lack of available test materials (representative of strain, genotype, stage in time-course, and appropriate negative controls) was seen as an obstacle by all respondents. Only the commercial companies considered that the absence of national and international policy for the use of live tests was a factor in influencing test development. Because of the inability to define a future market, the costs of developing a test could not be compared with potential future income in order to offer confidence that there would be returns on investment.

In summary, none of the respondents indicated that a live test for BSE was likely to be available in the immediate future. Nevertheless, it is known that at least three commercial companies that were not amongst the respondents are still interested in marketing a test for CJD in humans, and can legitimately be considered to have a real interest in the testing of bovines. This is no longer a priority for them however, and will probably not be progressed if they fail to gain approval for the testing of human blood. Furthermore, there is no doubt that the lack of clear cut future markets and international policy on the use of live animal tests may prevent them from investigating this area. In addition, some of the issues arising from the stakeholder workshop may prove to be so challenging that the introduction of live animal tests for cattle will prove to be impossible in the foreseeable future.

I.5.2 Consultation with the European Commission/EFSA

The Commission was consulted with respect to its medium term expectations, in the context of the TSE RoadMap (EC, 2005), which acknowledges a need to de-regulate with time, and its open-call for test developers to submit tests for approval. The call, published in October 2007, offered a five year window within which companies could submit post- and ante-mortem tests for evaluation. It built on the Commission's record of evaluating post-mortem tests which began

in 1999, and established a benchmark of 100% sensitivity and specificity, by using test materials derived solely from clinically affected confirmed cases of BSE, and unexposed controls originating from New Zealand. All subsequent evaluations have had to demonstrate equivalence in performance prior to approval.

Such an approach fails to recognise the fact that post-mortem tests are only effective shortly before clinical onset in animals exposed to low doses of BSE (50% detectable 1.7 months before onset; Arnold et al, 2007), and they will inevitably therefore fail to detect cases slaughtered at earlier stages of incubation. This argument has been defended by the fact that the primary protective measure in the food chain is the removal and destruction of SRM from slaughtered cattle. Consumers therefore remain protected if false negative results occur.

Attempts to draft equivalent guidelines for ante-mortem tests have been difficult, driven primarily by an inability to establish the true infectious status of cattle that are in the preclinical stages of incubation. In other words, the absence of a definitive gold standard to confirm the status of preclinical animals remains a major obstacle to evaluating the true performance of ante-mortem tests. At present the EFSA guidelines for the evaluation of a live animal test are limited to a preliminary evaluation of sensitivity and specificity (EFSA, 2007b). How a formal evaluation of suitability for use on a wider scale will be conducted is partly dependent on the analyte to be measured, and whether appropriate test materials are available to enable such studies to be carried out. It is not however expected that rapid ante-mortem tests will ever be accepted as alternatives to the removal of SRM. It is also clear that tests will be expected to have diagnostic sensitivity of not less than (100% - 95% confidence limit not less than 98.5%)(EFSA 2007a).

A further complication to future evaluations is the need to take into account the need to detect atypical phenotypes of BSE (H and L-type) already identified at low prevalence through the EU active surveillance programmes. Despite their rarity, they have been detected by current post-mortem tests, and future evaluations will need to make use of experimentally produced material, where available, to ensure that new tests are also capable of detecting them (EFSA 2007a).

The Commission cannot offer a forward plan for the integration of live animal testing within the EU programme of statutory testing. The TSE RoadMap acknowledges the advantages of using such tests in the tracing and culling of exposed animals, especially in the case of small ruminants. It also raises the possibility of requiring all animals to be tested above an agreed age, and that historical practice with tuberculosis and brucellosis could form a model for the establishment of herd certification schemes.

The call for expressions of interest in 2007 (EC, 2005) generated seven applications for evaluation of tests, of which three have subsequently withdrawn (source – EFSA). For reasons of confidentiality it was not possible to determine if any of the tests remaining in the evaluation programme were ante-mortem tests, although given the current state of the science, based on published evidence, it is believed that this is most unlikely.

Although the Commission has no plans to extend the call any further than 2012, and cannot confirm its policy regarding the likely context in which such a test might be introduced, much will inevitably depend on timing, the prevalence of infection and costs of introduction at the time. If live animal tests are not available until such time as the EU testing programme has been reduced significantly, because of the low prevalence of BSE, the issue of equivalence with SRM removal will be less of an issue, and may enable a flexible and positive approach to application for herd certification if such a demand exists.

I 5.3 Consultation with the OIE

Although the OIE has established mechanisms for the evaluation of tests, for all diseases within its scope, the approach is more passive than that adopted within the EU for TSE tests. Companies or institutes submit dossiers of data for evaluation by experts, and if successful are registered on the OIE web site as fit for specific purposes.

The difficulties presented by the evaluation of tests for TSEs has inevitably resulted in few applications, with only two approved tests registered, both after thorough evaluation in Europe, and with the gathering of significant amounts of data in the course of surveillance in the EU which supported the submission of application dossiers to the OIE.

The OIE has confirmed that although it could accommodate live animal tests within guidelines for international trade, and for diagnosis of BSE, it has not yet drafted protocols for the evaluation of live-animal tests, and has no plans to draft any at present. Consequently it has no proposals for the context in which it may accept the introduction of live tests, and will no doubt await evidence of successful development before taking this issue forward.

I.5.4 Consultation with those involved in the assessment of in-vivo tests for humans

This evaluation is confidential and consequently individual details cannot be revealed. It is clear that the primary objective of each manufacturer remaining in this field is to obtain approval of a test for use on human blood. None have been approved yet, and evaluation on sheep-derived samples continues. Challenges of both sensitivity and specificity remain. While it may be anticipated that a breakthrough could lead to reconsideration of likely performance on animals, it would be premature to anticipate this at present. Successful approval will inevitably divert resources of test developers into an implementation phase for human blood. The absence of a recognised large market for the testing of animals may prove to be a disincentive to further development even if such approaches become theoretically possible.

I.5.5 Conclusion

The review of the state of the science identified no test for BSE that is currently ready for use or for approval. Taking into account the likely timescale for approval and modification of policy to enable the introduction of live animal tests, and the attitude of commercial companies to the challenges and risks involved, there appears to be little scope for their use in the foreseeable future.

An issue that has also been highlighted by the review and the stakeholder consultation is that test specificity is likely to be critical to the acceptance of live animal testing, by both industry and consumers. While test sensitivity is already recognised as important, where perception of risk is so important in determining the climate of national and international acceptability, prospects of substantial numbers of false positive results that perpetuate fear of undetected or unconfirmed infection may undermine any attempts to introduce such tests. Realistically there is a need for two tests that are capable of detecting infected animals at any stage of incubation, one for screening and one for confirmation. Failure to confirm the results of a primary positive result will critically undermine confidence in a screening programme.

I.6. Small Ruminants

With respect to small ruminants, addressed in many of the studies reviewed, it is clear that while there are grounds for greater optimism than with bovines, significant challenges remain. There is a need for clarity on the extent to which genotype confers resistance to infection with specific strains (absolute or relative) when exposed by natural routes. Within specific

breed/genotype/scrapie strain combinations there is a need to confirm the performance of putative tests. Finally there will be difficulties in confirming the effectiveness of a test with respect to all strain/genotype combinations present within a national flock. Where there may only be a limited number of strains involved, there may be scope for live tests to be used in specific scenarios, such as flock/herd certification or culling of exposed animals. At the moment there is little evidence of a test approach that can be used for scenarios equivalent to those discussed for cattle.

I.7. Scenarios for the use of in-vivo tests for TSEs

A number of possible scenarios for the introduction of live animal tests for TSEs, and specifically for BSE, were developed as a basis for the cost effectiveness assessment, based on past practice in the eradication of animal diseases. These formed the basis of discussion at the Stakeholders' workshop.

Basic assumptions that need to be to taken into account include:

- **Attribution of costs** - Current responsibility and cost sharing for BSE in Great Britain
  - Slaughtered for human consumption – Industry pays for sampling and testing (with EU co-financing) and laboratory approval. Government pays for official controls via the Meat Hygiene Service (MHS) and Animal Health (AH – hide control). Government pays compensation for any positives.
  - Fallen stock – Industry pays for collection and disposal of fallen stock. Government pays for sampling and testing and official controls (Animal Health).
  - Clinical Suspects – Government pays for all costs including compensation.
  - Cohorts – Government pays for all costs including compensation.
- **Notifiable diseases** – private testing is not normally permitted other than for certification purposes (such as pre-export, or potentially pre-movement). There is a legal obligation to report suspicion of disease arising from a positive result to the competent authority. Otherwise costs fall to government.
- **Criteria for the acceptance of tests** - as described in the body of the report, these are established for the whole EU, by the Commission, aided by EFSA, who will set up expert groups to evaluate individual tests.

If BSE is accepted as no longer representing a significant human health risk, by virtue of low prevalence, it is conceivable that lower standards could be accepted for ante-mortem tests. Low sensitivity in a low prevalence scenario would render the tests virtually useless. Low specificity could give rise to false positive results, and incur high costs/case in dealing with animals as if they were positive, because a confirmatory post-mortem test would not be possible until after slaughter.

While tests with relatively low sensitivity/specificity have historically been used in the past for other diseases, in the absence of better options, they have not been without risk – most notably with TB. BSE is different, because it is not contagious and there is no evidence of a wildlife reservoir that challenges TB eradication.

The following eight scenarios were identified and presented to the Expert Workshop for review and evaluation:

1. The testing of clinically affected animals (BSE suspects)
2. The testing of BSE cohorts associated with index cases

3. The testing of healthy cattle, pre-slaughter, on farms
4. The testing of healthy cattle, pre-slaughter, at abattoirs
5. The testing of healthy cattle intended for export
6. The testing of healthy cattle for the purposes of herd certification
7. The testing of healthy cattle for the purposes of national accreditation
8. The testing of healthy cattle to detect resurgence of BSE

The following considerations and comments were prepared as a basis for the discussion at the Expert Group Meeting on the 10th September 2009. The outcome of the meeting and the scenarios selected for evaluation are presented in Section 4 of the Main Report.

Scenario	Considerations	Comments
1. Clinically affected animals – BSE suspects	Could require private vet to test animal before reporting to Animal Health as part of the process of arriving at a differential diagnosis. This could however delay resolution if not a cow-side test (i.e., Samples sent to lab). Unlikely that the regulatory requirement to report to Animal Health on first suspicion could be relaxed.	Likely to be of value for use by Animal Health, albeit at low volume (39 suspects in 2008), as a means of minimising cost of disposal and compensation for cattle that prove to be BSE-negative.
Scenario	**Considerations**	**Comments**
2. Cohort slaughter	When a feed or birth cohort is identified, testing on farm could result in considerable savings by avoiding the unnecessary slaughter of uninfected animals and allowing affected farmers to continue to trade normally. Numbers of cohorts to be tested will be related to index cases detected, so relatively low volume	Likely to be of benefit to Animal Health. Apart from savings involving reduced compensation and disposal costs, there would be an advantage in that culling would be more visibly dependent on science than current cohort policy.

3. Healthy cattle – pre-slaughter – on farm	Could test on farm before transport. Provided test results remained valid for a period of months it would enable testing to be done in batches to anticipate future slaughtering. It would be too expensive to apply to single animals. Could consider two testing scenarios. The first - to be applied to cattle currently tested post-mortem. Alternatively test prime beef to monitor for resurgence of BSE, or appearance of variants. Unless a test was 100% **specific** a large number of investigations to resolve false positive results could generate resistance and uncertainty.	Accepted as the most easily delivered option. Likely to be considerable industry resistance for two reasons. Firstly, the likely lack of benefit to farmers, and secondly due to uncertainty over the consequences of false positive results. Resolution of inconclusives or false positives would need to offer sufficient certainty regarding the status of the animal to ensure acceptability to the food industry and consumers. Although environmental contamination is not currently an issue, as it is with scrapie, this option could be of advantage should concerns arise retrospectively.

Scenario	Considerations	Comments
4. Healthy cattle – pre-slaughter, at abattoir	This scenario could build on the current infrastructure for post-mortem testing. It would enable all cattle, or specified age groups, to be tested. Ideally tests should work cow-side, or with minimum of 2 hour turn-round, and conducted at the abattoir. Some abattoirs would need to adapt premises to enable sampling, although if the test could be carried out on blood collected post-mortem it could be feasible. The establishment of a laboratory at the abattoir may have significant cost implications, particularly if the nature of the test requires high containment facilities, and ISO certification. Unless a test was 100% **specific** it could trigger a large number of investigations to resolve false positive results.	Likely to be significant industry resistance unless clear benefits accrue (eg. through re-categorisation of waste, and reduction of disposal costs). A significant number of false positives, with consequential precautionary action regarding disposal of carcases and offal while gold standard tests are applied, would be severely disruptive. Considerable resistance expected by operators. False positives and inconclusive would undermine confidence in test performance and safety of meat. Resolution of inconclusives or false positives would need to offer sufficient certainty regarding the status of the animal to ensure acceptability to the food industry and consumers. Compulsory testing of specified age groups would probably be required. Although environmental contamination is not currently an issue, as it is with scrapie, this option could be of advantage should concerns arise retrospectively.

Scenario	Considerations	Comments
5. Healthy cattle – pre-export	Costs borne by farmer/exporter with financial advantage if this enabled exportation of live animals. Dependent on such rules being relaxed, and acceptance of test validity beyond the EU.	Little effect on public health in UK unless it led to the next two scenarios.
6. Herd certification	National trade in cattle could be seen to be more secure if cattle were tested before movement to other farms. This is less relevant if the purchasing farm is not "tainted" if a purchased animal dies of BSE. This is most likely to be a basis for facilitating international trade, and declaration of national freedom. Farmers might be expected to cover costs.	This is unlikely to be attractive unless there is evidence of ongoing sub- clinical BSE, and possible alternative strain types, where feed and SRM controls are considered insufficient or too expensive to prevent transmission. There is no obvious short term gain, as this could not be a substitute for SRM removal. Although environmental contamination is not currently an issue, as it is with scrapie, this option could be of advantage should concerns arise retrospectively.

7. National accreditation scheme	Although environmental contamination is not currently an issue, as it is with scrapie, this option could be of advantage should concerns arise retrospectively. Repeat testing every few years might be required.	This is a long term scenario, with high volume and if followed likely to involve other countries too, so attractive to companies. The biggest benefit is that it could enable a return to definition of country status based on testing rather than failure to detect positive cattle by what will inevitably be smaller active surveillance programmes. It is not however seen as a likely scenario given the current state of knowledge, the absence of clear cut benefits, and the relaxation of trade rules already enables exportation of both animals and beef/beef products under current OIE guidelines.
8. Surveillance for a resurgence of BSE or another variant	Could possibly be used to detect a resurgence, possibly arising from relaxation of controls, or from a new variant of BSE that spreads horizontally, sooner than would be possible if reliant on passive surveillance and post-mortem testing. Assuming that resurgent BSE behaved like classical BSE, it would be possible to target young stock (18-30 month beef) to detect infected animals in the early stages of incubation.	Not considered to be a likely scenario as it is difficult to envisage how such surveillance could be appropriately targeted, as the likely risk population might vary with the cause (feed or horizontal transmission). A national programme to detect a resurgence early would have to be disproportionately large if it was to be sensitive enough to detect cases and trends. Clear benefits or significant risk would need to be defined to consider a re-direction of surveillance on such a scale.

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Appendix II Infectivity of Bovine Tissues

A key input to the risk assessment is the amount of infectivity present in the material to which people may be exposed. There are four factors that need to be assessed in order to determine the potential infectivity of any bovine tissue to humans:

- The infectivity of central nervous system (CNS) tissues from an animal with clinical BSE to another bovine
- The relative infectivity of non-CNS tissues in an animal with BSE;
- The development of infectivity through the incubation period of the disease; and
- The cattle-human species barrier.

II.1 Infectivity of CNS tissues from an animal with BSE

The Veterinary Laboratory Agency (VLA) in the UK has carried out experiments to identify the effect on cattle of oral doses of BSE infected cattle brain. In this "attack rate" experiment groups of 10 calves were each fed 300g, 100g, 10g and 1g of an homogenate made from the brain stems from clinically sick animals. All animals in the two higher dose categories came down with BSE, and 7 out of 10 in both the 10 g and 1g trials. The range of incubation periods for both the 1g and 10g trials were similar (44 – 71 months). An extension of the attack rate experiment has recently been completed with doses of 1g 100mg, 10mg and 1mg (Wells *et al*, 2007). The results show 3 of 5 in the 1g trial group, 7 out of 15 animals in the 100mg group, 1 out of 15 in the 10mg group, and 1 out of 15 in the 1mg group, positive for BSE. Incubation periods for the positive results in both the 1 and 10mg groups were similar to those for the 1 g trial, but two of the animals in the 100mg group had incubation periods in excess of 90 months.

Wells *et al* (2007) report that the ID_{50} estimate from these experiments is equivalent to 0.20 g of the brain homogenate used (i.e. 5 ID50/g) with a 95% confidence interval of 0.04 – 1.00g. Interim results from these experiments were considered by an EFSA working group in preparing a quantitative risk assessment on bovine derived products (EFSA, 2005). The Working Group noted that "*with higher titres of BSE affected brain the range could extend to 300 ID50/g"* and decided to take a precautionary view and to assume that the infectivity titre in brain of a clinically BSE infected bovine follows the following distribution:

Log normal distribution with
Median (50 percentile): 5 cattle oral ID_{50}/gram
Higher 99 percentile: 100 cattle oral ID_{50} ($CoID_{50}$)/gram

It is considered that this distribution remains a reasonable representation of the infectivity taking into account the more recent results from the attack rate experiment and the same distribution is used in this assessment.

II.2 Development of infectivity through incubation period

In its Opinion and Report of the 16 May 2002 (SSC, 2002) the Scientific Steering Committee of the EC has provided an interpretation of the pathogenesis study to try and determine the time after exposure at which infectivity can be detected in the central nervous system and spinal cord. It highlights the problems in interpreting the data and concludes that the assumption made by the SSC in its opinion of the 12 January 2001, - i.e "that in general, as a reasonable worst case assumption, the dorsal root ganglia and the spinal cord are considered to pose a higher risk as from the second half of the incubation period" - remains valid.

Analysis of the data from the VLA Pathogenesis experiment for the OTM review risk assessment (Comer and Huntly, 2004) resulted in an estimate of a 2 month doubling time. This would indicate that the infectivity at 50% and 70% of the incubation period is 4.5 logs and 2.5 logs less than the clinical value at the end of the incubation period. An unpublished analysis of the VLA data (Arnold, personal communication) indicates that the doubling time may in fact be slightly less than this, with a most likely value of 1.3 and a 95% range of 1.0 to 1.9 months. It is proposed that the 2 month doubling time will still be used in this study for consistency with the previous work but that a value of 1.3 will used in the sensitivity assessment.

II.3 Infectivity in non CNS tissues

Infectivity has been found in only a limited range of tissues in an animal with clinical BSE. In the pathogenesis study a large range of tissues were tested at various stages through the incubation of the disease using mouse bioassay and subsequently a more limited range of tissues were also tested by intracerebral inoculation into calves. The only non CNS tissues shown to harbour infectivity in these experiments are the distal ileum and tonsil. The distal ileum was found to be infectious from early in the incubation period with infectivity titres only slightly less than those in the CNS. However, in the abattoir the distal ileum is removed together with the entire abdomen and there is effectively no chance for the carcase to be contaminated with distal ileum.

Infectivity was detected in tonsil 10 months post exposure, but only one of 5 calves inoculated intracerebrally became infected (Wells *et al,* 2005). It has been estimated (EFSA, 2008) that the infectivity in the tonsil tissue was less than 1 bovine i.c. ID_{50}/g or $10^{-6.5}$ bovine oral ID_{50}/g. There were no other positive results for tonsil at subsequent time points, but for this study it will be assumed that this low level of infectivity could persist through the incubation period.

II.4 Infectivity in the Peripheral Nervous System

There have been a number of recent reports of very low levels of infectivity found in some peripheral nervous system tissues using highly BSE sensitive mice (Buschmann & Groschup, 2005; Iwata et al, 2006). Buschmann & Groschup (2005) estimate that the infectivity in the sciatic nerve was about 6 logs lower than that in the brain, and Iwata et al (2006) showed infectivity in the femoral and lumbar nerves of naturally infected cattle estimated to be 1,000 to 1,400 fold less than in the spinal column. This range of infectivity will be combined with an estimate of the amount of PNS tissue in cattle from the VLA (personal communication, 2008).

II.5 Total Infectivity in Clinical case

The total infectivity in a clinical case of BSE is summarised in Table II.1. The weights of the various tissues are mainly taken from the LFRA (1997) report and the infectivity values are as discussed above, with the infectivity for whole brain taken to be 5 bovine oral ID50/g. It can be

seen that 90% of the infectivity is associated with central and peripheral nervous system tissues, with about 10% associated with the distal ileum.

Table II.1: Infectivity in a Clinical Case of BSE (Bovine oral ID_{50})

Tissue	Weight	Infectivity		%
	kg/animal	ID50/g	ID50/animal	
Brain	0.5	5	2500	60.2%
Spinal cord	0.2	5	1000	24.1%
Dorsal root ganglia	0.03	5	150	3.6%
Trigeminal ganglia	0.02	5	100	2.4%
Lingual Tonsil	0.05[1]	0.00005	0.0025	<0.01%
Distal ileum	0.8	0.5	400	9.6%
PNS	0.96[2]	0.0004	0.15	<0.01%
TOTAL	**1.6**		**4150**	

Notes:

1. The LFRA (1997) report gives the total weight of the tonsil as 200g. 50g is an estimate of the weight of the lingual tonsil.

2. Estimate of total PNS weight from VLA

II.6 Significance of Exposure Estimates

The infectivity of BSE for humans is believed to be lower than in cattle due to the species barrier. The species barrier in this context is defined as the factor by which the effective infectivity in one species is reduced when given to a second species. Thus, if the cattle–human species barrier was 100, it would mean that 100 times more infective material would be required in order to have a similar probability of infecting a man compared to a bovine.

As part of the work for the review of the Over Thirty Month rule, Comer and Huntly (2004) estimated the total exposure of the UK population to BSE infectivity through food. They estimated that a total of 54 million bovine oral ID_{50} units entered the human food chain from 1980 to 2001. However, at the time of that study the latest data from the attack rate experiment was not available and a different assumption for the infectivity of bovine tissues was used that was a factor of 10 higher. Thus to be consistent with this present study the estimate should be divided by 10 and a value of 5 million bovine oral ID_{50} units consumed by the UK population should be used. This would indicate that the average exposure of the population (assumed to be 60 million) over the 20 year period would have been 0.004 bovine oral ID_{50} units per person per year.

There have been a total 166 cases of vCJD in the United Kingdom (April 2008), and it now seems that the epidemic reached a peak in 2000 and there has been a subsequent decline in the numbers of vCJD cases (CJD Surveillance Unit, 14th Annual Report, 2005). Current estimates of the total size of the vCJD epidemic have reduced significantly from the high numbers thought possible a few years ago to an upper limit of 550 in a recent report (Clake & Ghani, 2004). In fact Clarke and Ghani give a best estimate of 70 future deaths, and state that *"even in the worst case scenario, when non-MM homozygous individuals are equally susceptible but have longer mean incubation period than MM homozygous individuals, the best estimate of the potential scale of the epidemic is unlikely to exceed 400 future cases."*

An indication of the significance of the exposure estimates may then be obtained by comparing the total exposure estimate over the BSE epidemic (5 million bovine oral ID_{50} units) with the upper limit of 550 total cases of vCJD. If the exposure is factored by 40% to allow for the proportion of methionine homozygous individuals then it is calculated that the estimated exposure per expected vCJD case is in the order of 4,000.

II.7 BSE prevalence

II.7.1 Model results

Updated estimates of the proportion of cattle slaughtered with BSE infection in 2009 within the last 12 months of the incubation period have been provided by the VLA for four age groups:
1. Cattle born before 1 August 1996
2. Cattle born after 1 August 1996 that are over 48 months of age
3. Cattle that are over 30 months of age but less than 48 months
3. Cattle under 30 months of age.

The calculations were performed using the back-calculation model of Arnold and Wilesmith (2003). The culling rate for human consumption was estimated from 2004 age at slaughter data, assuming an adult population of 4.2 million and that 20% of cattle previously designated as casualty slaughter would enter the food chain. Animals over 48 months are assumed to undergo a post mortem diagnostic test with a test sensitivity as defined in Arnold (2007). This results in higher estimates of infection in the older animals than in previous versions. The

proportion of infected animals in the last 12 months of the incubation period is estimated using an assumed age-at-onset distribution (derived from data on clinical cases 1984-1995).

It has been assumed that there is a reducing trend of infection in the post 2002-03 birth cohorts (approximately a 40% reduction per successive birth cohort). This results in lower estimates of infection in animals under 30 months old than the previous assumption of constant prevalence in all birth cohorts.

Results are given in Table II.2 for both before and after post mortem BSE testing, and for three time periods: less than 3 months before onset, 4 to 6 months before onset and 7 to 12 months of onset. The last column in Table II.2 gives a weighted mean value, where the proportion with infection is weighted by the infection level for the incubation stage assuming a 2 month doubling time. It is these weighted mean values that are used in the assessment.

Table II.2 : Proportion of Cattle slaughtered with BSE Infection for 2009

a) No BSE testing

Age at slaughter	Incubation stage (months before onset)			Weighted mean
	0 – 3 months	4 – 6 months	7 – 12 months	
Born before 1 August 1996	2.31E-05	2.27E-05	4.42E-05	2.15E-05
>48m but born after 1 August 1996	5.78E-06	5.38E-06	9.7E-06	5.25E-06
30 to 48 months	5.04E-08	1.01E-07	4.91E-07	7.80E-08
Under 30 months	2.19E-11	3.82E-11	9.48E-10	6.94E-11
Infectivity reduction (2 month doubling time)	62%	22%	5%	

b)After BSE testing

Age at slaughter	Incubation stage			Weighted mean
	0 – 3 months	4 – 6 months	7 – 12 months	
>48m but born after 1 August 1996	2.43E-06	5.37E-06	9.7E-06	3.17E-06

Appendix III Expert Workshop

An Expert Workshop was held at Aviation House, London on the 10th September 2009. The objective of the workshop was to review and agree the set of scenarios to be used as a basis for the study and to elicit views and data, where there are information gaps in the scenario definitions

Workshop Attendees

- Philip Comer, DNV Project Manager
- Danny Matthews, DNV – TSE Expert
- Kate Huxtable, DNV Team member
- Irene Hill, FSA Project Officer
- Jennifer Heigham, FSA Economist
- Darren Cutts, FSA TSE Section
- Jill Wilson, FSA TSE Policy
- Patrick Burke, Defra
- Dave Harris, Animal Health (TSE Expert)
- Bill Reilly, BVA (President from Sept)
- Karen Lancaster, BCVA
- Angel Ortiz Pelaez, VLA
- Angus Wear, VLA
- Rona Barron, The Roslin Institute –Neuropathogenesis Division
- Peter Hewson, AIMS
- Jane Downes, Meat Hygiene Service

Apologies

- Roland Kao, Epidemiologist – Glasgow University
- Fiona McCormack, Animal Health Veterinary Services Manager
- John Mercer, National Farmers Union, Chief Livestock Advisor
- Stuart Roberts, BMPA
- Roland Salmon, Director Communicable Disease Surveillance Centre (CDSC) Wales

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DNV
Veritasveien 1
N-1322 Hovik
Norway
Phone: +47 67 57 99 00

DNV
Businesspark
Essen - Nord
Schnieringshof 14
45329 Essen
Germany
Phone: +49 201 7296 412

DNV
Duboisstraat 39 – Bus 1
B-2060 Antwerp
Belgium
Phone: +32 (0) 3 206 65 40

DNV
Palace House
3 Cathedral Street
London SE1 9DE
United Kingdom
Phone: +44 20 7357 6080

DNV
Highbank House
Exchange Street
Stockport
Cheshire SK3 0ET
United Kingdom
Phone: +44 161 477 3818

DNV
Cromarty House
67-72 Regent Quay
Aberdeen AB11 5AR
United Kingdom
Phone: +44 1224 335000

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