Potential use of farm of origin information for more targeted inspection of *Cysticercus bovis* (FS517002)

Report on the results of a case-control study of UK cattle farms and proposals for a classification tool.

**Authors:**
Laura Marshall
Bhagyalakshmi Chengat Prakashbabu
Jorge Pinto Ferreira
Katharina Staerk
Javier Guitian

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EXECUTIVE SUMMARY

SCOPE AND OBJECTIVE
In line with the recommendations made by the 2010 European Food Safety Agency (EFSA) Scientific Report on monitoring and reporting of Cysticercus in animals and foodstuffs in the European Union, the Food Standards Agency (FSA) contracted the Royal Veterinary College (RVC) and Safe Food Solutions (SAFOSO) to carry out project FS517002, with the aim of gathering evidence that could inform more targeted and cost effective inspection activities, for the detection of Cysticercus bovis infection in UK cattle at slaughter. This report outlines the methods, results and conclusions of the epidemiological research carried out by SAFOSO and the RVC in order to fulfil this aim. Additionally, it makes a proposal for a ‘classification tool’ whereby meat inspection activities can be targeted at cattle with a higher risk of C.bovis infection at slaughter, within abattoirs in Great Britain.

KEY FINDINGS AND INTERPRETATIONS
The project initiated with a systematic review of published evidence to identify previously defined risk factors for C. bovis infection in cattle, in addition to previous research outcomes on the design and implementation of more targeted meat inspection of cattle for C. bovis infection; this review incorporated input from the direct consultation of experts in the field of C. bovis research. Secondly, between 1st January 2013 and the 31st January 2015, a sample of 2323 slaughter records was collected from cattle found to be infected and cattle not found to be infected with C. bovis at slaughter (1:3 ratio). These records were obtained from FSA approved abattoirs within Great Britain (GB): out of the total 187 FSA-approved ‘meat of domestic ungulates’ slaughter establishments in GB which deal with cattle, for which enrolment in the study was compulsory, 62 abattoirs (33%) had detected infected cattle at slaughter within the study period. Movement history data was available and acquired from the British Cattle Movement Service (BCM) for 98.5% of the total (2323) cattle slaughter records acquired during the study period; suggesting in itself that cattle movement data are very comprehensively recorded, and thus could support a potential classification tool based on movement history.

Using these cattle slaughter records and accompanying movement history data, three analytical approaches were employed in order to detect risk factors which are associated with C. bovis infection in GB cattle. The first analysis involved a case-control study, comparing detailed information on the characteristics of 226 GB cattle farms. These farms were derived from the results of a selection process carried out on the collective movement history data. Details on this selection process can be found later in this report, under the sections entitled ‘Initial Data Collection’ and ‘The Case Control Study’. The aim of the case control study was to identify whether farm characteristics are risk factors for C. bovis infection. The results of the analysis showed limited evidence of association between farm characteristics and risk of C. bovis infection. The second analysis assessed the network and sequence of inter-farm movements performed by 2270 C. bovis infected and uninfected animals, prior to slaughter. The results of this analysis showed that those animals with a history of being on a ‘high risk’
farm, which is defined as a farm featured in the movement history of a previously infected animal, have a 4 times higher odds of being found as positive for C. bovis at meat inspection; i.e. a movement history comprising one or more ‘high risk’ farms can be considered as a risk factor for C. bovis infection in cattle at slaughter.

The results of the case-control study and movement history network analyses were presented to key stakeholders in the cattle production industry, during a workshop with the aim of discussing the practicalities and potential challenges related to the design and implementation of a classification tool. Based upon the output from the workshop, a third analysis was carried out, which aimed to identify whether the individual animals' characteristics of sex and age serve as risk factors for C. bovis infection at slaughter. The results of this analysis showed that male cattle slaughtered at between 0-20 months of age were less likely to be infected with C. bovis. All female cattle and male cattle older than 20 months were therefore demarcated as a high risk group for C. bovis infection. The outputs from the analyses and consultations were then integrated to propose options for a classification tool whereby meat inspection activities can be targeted at cattle with a higher risk of C. bovis infection at slaughter in Great Britain. Simulation modelling was utilised for this purpose. The ability of different inspection regimes consisting of applying normal vs. enhanced inspection to different proportions of the total vs. the ‘high risk’ population of animals at slaughter was assessed. ‘High risk’ animals were defined on the basis of their movement history and the age-sex category to which they belong.

The outputs of the simulations showed that a classification tool which utilised:
- an enhanced inspection methodology (assumed sensitivity 30%) in animals with a movement history including one or more ‘high risk’ farms;
- current inspection methods enforced through Regulation (EC) No 854/2004 (estimated sensitivity 15%) in animals in the high risk sex-age group;
- no inspection with regard to C. bovis in all remaining animals not falling into these categories could lead to an 18.5% reduction in the total number of cattle undergoing meat inspection for C. bovis, whilst at the same time increasing the proportion of infected carcasses detected at inspection from 15%, in the current situation, to 21%.

CONCLUSIONS
- Cattle movement history data and inspection results are very comprehensively recorded in the UK. This supports the notion of a classification tool, based on such data, whereby inspection activities can be targeted at cattle with a higher risk of C. bovis infection at slaughter.
- Due to the specific epidemiological characteristics of C. bovis infection in UK cattle, that is, a very low prevalence resulting from what appear to be sporadic incidents involving a small number of farms, a classification tool based on farm characteristics is unlikely to offer the appropriate discriminatory power to discern and target cattle with a higher risk of C. bovis infection at slaughter.
Conversely, the high degree of connectivity of infected animals through farms common to their movement histories and the lower prevalence of infection in some age-sex groups, supports the potential use of previous *C. bovis* diagnoses at slaughter (Collection and Communication of Inspection Results), cattle movement history records (stored within the BCMS database) and the sex and age of slaughtered animals (FCI) to target inspection.

The simulation of hypothetical inspection scenarios strongly suggest that there is scope for modified inspection regimes that are at the same time less onerous in terms of number of inspections carried out and equally effective (or even superior) at protecting animal and public health. A hypothetical scenario in which a classification tool is used to target enhanced inspection activities at cattle with a high risk movement history; whilst animals in the high risk age-sex category (who are not also from high risk farms) are subject to current inspection methods and all remaining animals undergo no *C. bovis* inspection; is likely to result in an increase in the number of *C. bovis* infected animals detected annually and a decrease in the total number of inspections.

The estimates presented in this report are based on a number of assumptions and on imperfect field data. The risk factors identified (movement history and age-sex group) should be reassessed as more data (e.g. 2015 inspection results) become available. The feasibility and sensitivity of an enhanced inspection protocol should be trialed and compared with those of normal inspection to provide the empirical evidence that could eventually support the introduction of a more cost-effective inspection system.

**RECOMMENDATIONS**

Based on the results of FSA project FS517002, and to facilitate the potential translation of project findings into more cost-effective inspection protocols we recommend:

- That the strength of association between movement history and infection and between age-group and infection is re-assessed as more data (e.g. 2015 inspection results) become available. These are critical inputs in our simulations that should ideally be validated and refined.

- That the feasibility of implementing an enhanced inspection protocol is evaluated and that such a protocol is trialled in order to compare its sensitivity with that of normal inspection. It should be noted that the low prevalence of infection poses a challenge to the field evaluation of enhanced inspection. If such a trial were to be implemented it should target high risk groups to increase statistical power and should involve a control group also of high risk animals subject to “normal” inspection.

- The project has shown that data are comprehensively recorded; however, given how critical accurate data are for the follow-up of this project, the validation of its findings and its eventual translation into revised inspection, we recommend that all possible efforts are made to maintain and improve the systematic, accurate and complete recording of information such as inspection findings and age and sex of the animals. With regard to *C. bovis* inspection findings, there is potential for improved standardization of records.
BACKGROUND AND PROJECT OBJECTIVES

Bovine cysticercosis is caused by the larval stage of the human tapeworm *Taenia saginata*. Humans are the definitive host and hold the adult tapeworm (taeniosis), while cattle act as the intermediate host and harbour the larvae (cysticercosis). Humans become infected after ingestion of raw or undercooked beef containing infective cysticerci. The disease does not typically cause major health problems in humans, being characterised by mild symptoms, if any. Specifically, the adult tapeworm may cause mild inflammation at the site of its implantation on the intestinal wall, but substantial damage is generally not incurred. Nevertheless, the active discharge of proglottids from the anus, during the actively reproductive stage of the parasite, causes emotional stress and an unpleasant sensation in the vast majority of patients, making it unacceptable in most countries of the EU.

Cattle become infected through accidental ingestion of food or water which is contaminated with human faeces containing viable *T. saginata* eggs. These eggs can ‘remain viable for several weeks or months in sewage, water or on pasture’\(^5\). After 8-10 weeks the eggs have developed into larvae which establish in bovine skeletal and cardiac muscle, and less commonly in fat and visceral organs. They develop into cysticerci (viable cysts), remaining infective for approximately nine months before they eventually die and calcify, becoming non-infective (non-viable cysts)\(^6\). Both viable and non-viable cysts can be present in the same animal.

The apparent prevalence of *Cysticercus bovis* in cattle at individual animal level, based on post mortem inspection, ranges between 0.01% and 0.06% in European countries\(^1,4\). Although no cases were reported in the UK in the last EFSA report\(^1\), the apparent prevalence appears to be similar to that in other member states. The rates of detection from UK meat inspection data from 2008-2011 are 0.008% (15/190,493) and 0.032% (2674/8,484,371) for slaughtered calves and adult cattle respectively\(^20,21\). An outbreak of *C. bovis* was mentioned in project FZ2100 Q3 and Q4 reports\(^5\), and APHA, the Animal and Plant Health Agency, previously known as the Animal Health and Veterinary Laboratories Agency (AHVLA), further investigated four other farms in the same locality as the farm of the original outbreak\(^6\).

The presence of *Taenia saginata* cysts in cattle is determined during post-mortem meat inspection and enforced through Regulation (EC) No 854/2004. This current slaughterhouse regulation requires that meat inspectors evaluate all susceptible bovine carcases above six weeks of age, using the following palpation and incision techniques:

- visual examination and palpation of the tongue;
- two deep incisions in the external- and one in the internal- cheek muscles parallel to the mandible;
- visual examination of the heart (incised lengthwise to open ventricles and to cut through the interventricular septum);
- visual examination of the diaphragm and oesophagus.

Carcasses with visible cysts (independent of the method of detection at meat inspection) are either downgraded (requiring extra handling and freezing to inactivate cysticerci) or
condemned, depending upon the amount and type of visible cysts, (i.e. whether they are infective or non-infective). Whilst this process is necessary for the protection of public health, it does lead to significant economic losses. The sensitivity of visual inspection is low (<30%) and positively correlated with the amount and size of the cysts\textsuperscript{1,2,3}, thus the need to develop more sensitive tests has been recognised \textsuperscript{1}. However, this would take time, and even if a more sensitive test was developed, it is likely that its use in all slaughtered cattle would not be practical or cost effective under the current meat inspection system.

In England, Collection and Communication of Inspection Results (CCIR), such as the details of those \textit{C. bovis} positive cases detected during post mortem inspection, is facilitated through record storage within the FSA national meat inspection database. Food Chain Information (FCI), such as the identification details of the animal and farm of immediate origin, are also recorded within this database. However, additional information pertaining to animal and farm characteristics is not noted. Therefore, any potential study investigating the risk factors for cysticercosis in cattle in the UK would require amalgamation of data held in different databases. Currently, the meat hygiene inspection system is under review. It has been suggested that stratified sampling of cattle is appropriate where the national prevalence is low (<0.1%) \textsuperscript{7}, and simulation models suggest that meat inspection could potentially be more cost-effective, without compromising public health \textsuperscript{8}.

The last EFSA report \textsuperscript{1}, in addition to highlighting the poor sensitivity of meat inspection methods, also emphasizes the need for more targeted, risk-based and cost-effective methods of \textit{C. bovis} surveillance in cattle at slaughter. In line with this, the Food Standards Agency (FSA) contracted The Royal Veterinary College (RVC) and Safe Food Solutions (SAFOSO) to carry out research project FS517002. Based upon EFSA’s recommendations, the aim of this project has been to provide evidence that could inform more targeted and cost-effective inspection activities. To fulfil this aim, the final project objectives comprised:

1. A systematic review of published evidence relevant for:
   a) The identification of risk factors for \textit{C. bovis} infection in cattle.
   b) The design and implementation of more targeted meat inspection of cattle for \textit{C. bovis} infection.
2. A farm-level case-control study, and analysis of the cattle movement network. These analyses were relevant for the identification of risk factors for \textit{C. bovis} infection of significance within the UK cattle population. An assessment was also made regarding the value of these risk factors, in terms of their use to target \textit{C. bovis} inspection. This objective utilized expert opinions from the field of \textit{C. bovis} research, in addition to feedback from stakeholders within the UK cattle industry.
3. The proposal of a classification system/tool, which could be used to target \textit{C. bovis} inspection, using FCI and CCIR. Again, this objective utilized feedback from stakeholders within the UK cattle industry.
This report includes the methods and results affiliated with completion of objectives 1, 2 and 3, in full. For further information regarding objective 1, please see the original report on the systematic review of published evidence, submitted to the FSA in January 2015 as ‘Project FSS17002: Deliverable 1’. For further information regarding objective 2, previously submitted reports include detailed information about the methods utilized to gather data, and the methods of the case-control study: ‘Deliverable 2: Interim Report on elicitation of expert opinion’; ‘Deliverable 3: Interim report on data gathered on the occurrence of cases of C. bovis across UK abattoirs’ and Deliverable 4: ‘Interim report on the yields from the selection of UK-wide ‘case’ and ‘control’ farms, in addition to subsequent farm-characteristic questionnaire response rates as of 04/06/15’.

**SYSTEMATIC REVIEW OF PUBLISHED EVIDENCE**

In order to provide an adequate and up to date epidemiological platform for this project, the aforementioned systematic review of published evidence was performed on three databases (Pubmed, CAB abstracts and ScienceDirect).

The following search terms were specified for, within the title of search result publications: ((bovine cysticercosis) OR (Cysticercus bovis) OR (C. bovis) OR (Taenia saginata) OR (taeniosis)) AND ((inspection) OR (risk-based) OR (risk factors) OR (husbandry) OR (practice) OR (surveillance) OR (indicators)). References were considered for inclusion within the review if they had been published in English, between 2000 and 2014. The search yielded 15 results on PubMed, 39 on CAB abstracts and 23 on ScienceDirect. Reasons for exclusion included not having full-text available online and being published in a non-English language.

Considering the previously described cycle and means of transmission between humans and cattle, it is perhaps not surprising that the majority of the previously identified risk factors involve cattle residing in close proximity to humans (or more precisely, human faeces) (Table 1). The “Risk assessment of a revised inspection of slaughter animals in areas with low prevalence of Cysticercus” report published in the EFSA Journal in 2004, recognizes “direct on-farm human excrement deposition” and “staff training and turnover” as risk factors.

The WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniosis/cysticercosis lists, as risk factors for the transmission from humans to cattle: “outdoor defecation in or near cattle rearing facilities or pastures”; “use of sewage effluent, sludge or untreated human faeces to irrigate or fertilize feed crops and pastures”; “human carriers involved in the rearing and care of cattle” and “indiscriminate deposition of feces on campgrounds, along highways, and along rail tracks”. Interestingly, Flütsch (2008), in a study conducted in Switzerland, also recognizes the “presence of a railway line or car park close to areas grazed by cattle” as a significant risk factor, together with “leisure activities around areas grazed by cattle”, “use of purchased roughage” and “organized public activities on farms attracting visitors”. Additionally, Calvo-Artavia in a case-control study in Denmark, reported “sharing machinery or hiring contractors” as a risk factor for bovine cysticercosis.
It is important to highlight that in the previously mentioned recent outbreak in the UK, the common link between the affected farms was the feeding of potatoes from a single supplier. However, these potatoes had been originally imported for human consumption, but were rejected after illegal immigrants were found occupying the transport vehicle. Therefore, the “feeding of potatoes” should not be recognized as a risk factor, but instead this case reinforces the significance of human faecal contamination of cattle feed as a risk factor for the development of cysticercosis in cattle.

A second group of risk factors is related to the water supply provided to farmed cattle. Boone et al. (2007) studied the distribution and risk factors of bovine cysticercosis in Belgian dairy and mixed herds, and identified “flooding of pastures”; “free access of cattle to surface water” and “proximity of wastewater effluent” as risk factors. Similarly, Calvo-Artavia et al. (2012) “found the access to risk water sources with sewage treatment plant effluent in proximity” to be a significant risk factor.

Besides animal biological factors ((older) age and (female) gender), other previously identified risk factors include: “organic wastes as fertilizers”, “farm location”; “roughage types”; “lack of effective fly and bird control around cattle facilities” and certain “grazing practices.”
**Table 1:** Previously reported risk factors for the infection of cattle with *C. bovis*; from a systematic review performed on three databases (Pubmed, CAB abstracts and ScienceDirect), concluded in January 2015.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Water supply for animals</td>
<td>1</td>
</tr>
<tr>
<td>- Organic wastes as fertilizers</td>
<td></td>
</tr>
<tr>
<td>- Roughage types (hay, silage, or crop by-products (e.g. potato by products), originating from locations contaminated with human waste)</td>
<td></td>
</tr>
<tr>
<td>- Farm location</td>
<td></td>
</tr>
<tr>
<td>- Direct on-farm human excrement deposition</td>
<td></td>
</tr>
<tr>
<td>- Staff training and turnover</td>
<td></td>
</tr>
<tr>
<td>- Calf age</td>
<td></td>
</tr>
<tr>
<td>- Outdoor defecation in or near cattle rearing facilities or pastures</td>
<td>14</td>
</tr>
<tr>
<td>- Lack of effective fly and bird control around cattle facilities</td>
<td></td>
</tr>
<tr>
<td>- Use of sewage effluent, sludge or untreated human faeces to irrigate or fertilize feed crops and pastures</td>
<td></td>
</tr>
<tr>
<td>- Human carriers involved in the rearing and care of cattle</td>
<td></td>
</tr>
<tr>
<td>- Indiscriminate deposition of faeces on campgrounds, along highways, and along rail tracks</td>
<td></td>
</tr>
<tr>
<td>- Flooding of pastures</td>
<td>9</td>
</tr>
<tr>
<td>- Free access of cattle to surface water</td>
<td></td>
</tr>
<tr>
<td>- Proximity of wastewater effluent</td>
<td></td>
</tr>
<tr>
<td>- Presence of a railway line or a car park close to areas grazed by cattle</td>
<td>10</td>
</tr>
<tr>
<td>- Leisure activities around areas grazed by cattle</td>
<td></td>
</tr>
<tr>
<td>- Use of purchased roughage</td>
<td></td>
</tr>
<tr>
<td>- Organized public activities on farms attracting visitors</td>
<td></td>
</tr>
<tr>
<td>- Gender</td>
<td>4,8,11</td>
</tr>
<tr>
<td>- Age</td>
<td></td>
</tr>
<tr>
<td>- Grazing</td>
<td></td>
</tr>
<tr>
<td>- Access to risky water sources with sewage treatment plant effluent in proximity</td>
<td></td>
</tr>
<tr>
<td>- Sharing machinery or hiring contractors</td>
<td></td>
</tr>
<tr>
<td>- Age</td>
<td>15</td>
</tr>
<tr>
<td>- Gender</td>
<td></td>
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</tbody>
</table>

Since farm characteristics such as type of production, water source, geographic location (proximity to places accessed by the general public) and biosecurity are likely to influence the likelihood of *C. bovis* infection⁹–¹¹; farm characteristics could therefore be used to classify farms according to the *a priori* risk of animals raised in them having cysticercosis; this
classification could be the basis for different, more targeted, types of inspection. In this project we aim to generate evidence that could inform more targeted and cost-effective inspection activities. Our project is therefore aligned with previous and ongoing activities within the UK (FSA project MC1001 for the development and adaptation of qualitative risk assessment framework on green offal inspection) and EU that pursue more targeted risk-based inspection\textsuperscript{1,12}. The farm-level risk factors identified through this systematic review of published evidence were utilised in the development of a questionnaire, designed to collect information relating to a range of farm characteristics, for use within a case-control study; see subsection ‘Case and Control Farm Data Collection’ within the section entitled ‘The Case Control Study’.
INITIAL DATA COLLECTION (IDENTIFICATION OF INFECTED ANIMALS)

The UK farm-level case-control study, and analysis of the cattle movement network, initiated with FSA contacting the 187 FSA-approved cattle abattoirs across Great Britain (GB) to enroll in the study. Abattoirs were requested to provide the RVC with cattle slaughter records for all C. bovis-infected cattle (‘viable cysts’, ‘non-viable cysts’ and ‘positive’ diagnoses made at meat inspection/ post mortem) detected at slaughter between January 2013 and January 2015. For each infected animal, the abattoir was requested to provide slaughter records for three uninfected animals, which underwent slaughter within subsequent batches on the same day. FSA required confirmation from abattoirs which had detected no C. bovis infected cattle at slaughter during the time period. Enrolment in the study was compulsory for those abattoirs which had detected C. bovis infected cattle within this time period, of which there were 62 (33% of FSA approved cattle abattoirs). This process yielded a total of 2323 cattle slaughter records, which were comprised of 612 infected bovines (134 viable cyst infected bovines plus 478 non-viable cyst infected or ‘positive’ bovines) and 1711 uninfected bovines.

The Rural Payments Agency Planning and Performance Department then provided the movement histories (farm county parish holding number (CPH) and address) for at least the previous 3 years for all GB cattle slaughter records collected (the earliest record extended to 1997). Out of the original 2323 cattle slaughter records collected, 35 (1.5%) had movement histories unaccounted for. The main reason for this was the lack of a matching ID number in the movement history database, which could not be remedied through contacting the slaughterhouse Official Veterinarian who created the slaughter record, for example. This reduced the number of useable cattle slaughter records to 2288 (98.5% of the original records collected), comprised of 596 infected bovines (131 viable cyst infected bovines plus 465 non-viable cyst infected bovines) and 1692 uninfected bovines. The acquisition of movement history data for 98.5% of the cattle slaughter records initially collected is in itself a relevant finding, as it suggests that cattle movement data are very comprehensively recorded, and thus could support a potential classification tool based on movement history.

The Department of Agriculture and Rural Development of Northern Ireland (DARD NI) also provided slaughter records for all cases of C. bovis detected at slaughter in NI abattoirs over the same time period, with three associated controls per case as in GB. This yielded a total of 141 cattle slaughter records, comprised of 36 infected bovines (1 viable cyst infected bovine plus 35 non-viable cyst infected bovines) and 105 uninfected bovines; see Figure 2.

The British Cattle Movement Service (BCMS) advised that cattle at markets and auctioneers generally move on and off the premises on the same day; any additional length of time that cattle spend in association with the market or auctioneer tends to be within a separate and adequately equipped holding facility or farm, with a different CPH number. Those holdings on which cattle were resident for zero days (i.e. moved on and off on the same day) were therefore assumed to be markets, auctioneers and livestock show grounds, and were excluded from the movement history dataset. Slaughterhouse CPH numbers were also excluded. Those holdings which remained within the dataset were therefore assumed to be farms.
After this exclusion, the study sample contained 2270 cattle, composed of 592 infected bovines (130 viable cyst infected bovines plus 462 non-viable cyst infected bovines) and 1678 uninfected bovines. There were 3453 holdings in the combined movement histories of these animals; see Figure 1. It is suspected that this loss of 18 animals from the original 2288 may be due to imported cattle which, immediately upon entering GB, were moved to an abattoir lairage before slaughter on the same day.

DARD NI provided the movement histories (herd number (CPH)) for at least the previous 3 years, for all 141 NI cattle slaughter records collected. The NI codification system allowed direct exclusion of markets, slaughterhouses, slaughterhouse lairage and ports of embarkation from the NI movement histories.
THE CASE CONTROL STUDY

A case-control study, with the farm as the unit of interest, was carried out after identifying case and control farms from the data collected at individual animal level. The aim of the study was to assess the potential differences, in terms of farm characteristics, between those farms which were likely to have produced *C. bovis* infected animals (case farms), and the control farms.

‘CASE’ AND ‘CONTROL’ FARM SELECTION

This step involved the selection of case farms from the collected data on the movement history of infected animals. In parallel, a comparison group of control farms was identified from the farms that sourced non-infected animals slaughtered in the same day and abattoir as the infected animals. We enlisted the opinions of collaborating experts in the field of *C. bovis* research to define some practical criteria for case and control farm selection. These involved the following:

**Case Farms:**

a) A case farm is the only farm cited in the movement history of an infected bovine, or

b) A case farm is not the only farm cited in the movement history of an infected bovine, but it is cited in the movement history of at least one other infected bovine, or (rarely) it is cited more than once in the movement history of an infected bovine.

**Control Farms:**

a) A control farm is the only farm cited in the movement history of an uninfected bovine and is not found in the movement history of any infected animal in the dataset.

b) To increase the number of control farms available for selection (and enable a 1:3 ratio of case to control farms), farms selected through control criterion a) were combined with a random sample of those selected via the following criterion: a control farm is not the only farm cited in the movement history of an uninfected bovine, but it is found only in the combined movement histories of all uninfected animals in the dataset and it is not found in the movement history of any infected animal in the dataset.
Yields from Case and Control farm selection

Figure 1 diagrammatically depicts the final numbers of farms obtained from the GB and NI case and control farm selection process respectively.

Figure 1. Schematic to show a breakdown of the finalised study samples of cattle and the farms in their collective movement histories; in addition to the subsequent yields of case and control farms; for Great Britain (GB) and Northern Ireland (NI)
The geographic locations of GB case and control farms are presented in Figure 2. The figure shows that both the case and control farms were broadly distributed throughout GB. However, there were fewer farms in East Anglia, the Borders and Scotland, by comparison to other areas. This distribution of case and control farms probably reflects underlying patterns in the GB cattle farm population. The spatial scan statistic using a Bernoulli purely spatial model was implemented in SaTScan™ version 9.4.2 to assess whether there was spatial clustering of case compared to control farms. The size of the circular window was limited to 50% of the population at risk and the statistical significance of spatial clusters was assessed using 999 Monte Carlo simulations. No statically significant spatial clusters were detected, therefore case and control farms appear to be homogenously distributed across GB. Due to differing data security requirements, the RVC were not in direct receipt of postal code addresses for NI farms (only CPH numbers), therefore it was not possible to produce a similar distribution map to that presented in Figure 2, for NI farms.

Figure 2. Distribution of case (n=219) and control (n=666) farms included in the case-control study of risk factors for C. bovis infection in Great Britain.
CASE AND CONTROL FARM DATA COLLECTION

Each of the selected case and control farms received a postal questionnaire, designed to collect information relating to a range of farm characteristics, including the farm-level risk factors identified within our systematic review of published evidence on C. bovis; see Table 1. Farmers were also given the alternative option of completing an online electronic version of the questionnaire; however, none chose this method. Farmers were awarded a voucher for return of their completed questionnaire. Questionnaires were received over a time period of 2 months, with a reminder questionnaire sent to those farms who had not replied midway through this period. We attained a return of 229 adequately completed ‘useable’ farm questionnaires, out of the 885 GB case and control farms selected. Data from these 229 farms was collated combined, cleaned, condensed and coded in order to produce the final dataset for the case-control analysis. Table 2 gives a breakdown of the adequately completed (‘useable’) questionnaire replies received for GB. The highest proportion of responses were received from beef farms, with very few from hobby farms. The replies maintained a ratio of controls to cases, which was close to the optimum 3:1 ratio (an exact 3:1 ratio would have entailed 174 controls). Forty five ‘non-useable’ questionnaires were also received, comprised of thirty one incomplete questionnaires, in addition to six electronic questionnaire replies and eight complete questionnaire replies received after closure of the two month receipt period. Including both adequately completed ‘useable’ questionnaires and ‘non-useable’ questionnaires, the total response rate for GB farms was 31%.

Table 2: Number of adequately completed questionnaire replies received for GB, according to farm status and production system type.

<table>
<thead>
<tr>
<th>Farm status</th>
<th>Production system</th>
<th>Beef n (%)</th>
<th>Dairy n (%)</th>
<th>Beef and Dairy n (%)</th>
<th>Hobby n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case farms</td>
<td></td>
<td>37 (63.8)</td>
<td>13 (22.4)</td>
<td>7 (12.1)</td>
<td>1 (1.5)</td>
<td>58 (25.2)</td>
</tr>
<tr>
<td>Control farms</td>
<td></td>
<td>111 (64.9)</td>
<td>34 (19.9)</td>
<td>24 (14.0)</td>
<td>2 (1.17)</td>
<td>171 (74.7)</td>
</tr>
<tr>
<td>Total farms</td>
<td></td>
<td>148 (63.6)</td>
<td>47 (20.5)</td>
<td>31 (13.5)</td>
<td>3 (1.3)</td>
<td>229 (100)</td>
</tr>
</tbody>
</table>

For NI, 11 adequately completed 'useable' farm questionnaires were returned, out of the 60 NI case and control farms selected. Table 3 gives a breakdown of the adequately completed ‘useable’ questionnaire replies received; only one of these was from a case farm. Seven ‘non-useable’ questionnaires were also received, comprised of four incomplete questionnaires and three replies received after closure of the two month receipt period. Including both adequately completed ‘useable’ questionnaires and ‘non-useable’ questionnaires, the total response rate for NI farms was 30%.

However, Northern Irish farms were not incorporated in the case-control analysis given that complete questionnaire data were available from one farm only and considering that NI farms have a high level of farm fragmentation by comparison to GB farms \(^{17}\), which may make them quite different in terms of the analysis of farm characteristics.
Table 3: Number of adequately completed questionnaire replies received for NI, according to farm status and production system type.

<table>
<thead>
<tr>
<th>Farm status</th>
<th>Beef n (%)</th>
<th>Dairy n (%)</th>
<th>Beef and Dairy n (%)</th>
<th>Hobby n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case farms</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Control farms</td>
<td>7 (70)</td>
<td>2 (20)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Total farms</td>
<td>7 (63.6)</td>
<td>3 (27.3)</td>
<td>1 (9.1)</td>
<td>0 (0)</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

Due to the low rate of response from hobby farms, and due to their comparative variability and intrinsic differences to commercial cattle production systems, the 3 GB hobby farms from which complete replies were received (Table 2) were also excluded from the case-control study analysis. The final dataset for analysis thus contained 226 farms in total, comprised of GB beef, dairy and beef/dairy farms, of which 58 were case farms, and 171 control farms (Table 2).
**DATA ANALYSIS**

The analysis of the final set of animal and farm level data included three components. Firstly, univariate and multivariate (logistic regression) analysis was conducted to identify which farm characteristics (risk factors) are associated with a higher risk of a farm being a “case”. Secondly, the networks of infected and uninfected animal movements between farms were characterized using social network analysis. Thirdly, univariate and multivariate (logistic regression) analysis was conducted to identify whether the individual animal characteristics of sex and/or age were associated with a higher risk (risk factors) of an animal being infected with *C. bovis*.

**Farm-level risk factor identification**

By identifying risk factors and quantifying the strength of their association with the status of a farm as a “case farm” it would be possible to categorize farms in the movement history of a given animal according to their probability of being case farms. We surmised that such an assessment could then be regarded as a proxy for potential exposure or non-exposure to *C. bovis* infection. A bovine with a case farm (i.e. potential exposure to *C. bovis* infection) in its movement history could therefore be regarded as more likely to be infected with *C. bovis* than a bovine with no ‘case’ farms in its movement history. A bovine that is more likely to be infected may warrant a more intensive meat inspection than one that is less likely to be infected: thus enabling targeted *C. bovis* surveillance in cattle at slaughter, and efficiency in resource use.

In order to produce such a statistical model, data collected from the 226 GB farms described in Table 2 was used to carry out a logistic regression analysis, to define those farm characteristics that gave good evidence for their being associated with a farm being a case farm. This association was defined as a high ‘odds’ of a farm being a case farm (versus a control farm) if it had that characteristic. The final statistical model contained those characteristics that, in combination, gave the best evidence for an association with a farm being a case farm. The questionnaire contained 134 questions spanning general management practices, feeding and water systems, transport and destinations for animals leaving the farm, land management practices and the interactions between cattle and people visiting or working on the farm. The majority of these questions were focused on those farm characteristics found, or theorized, to be associated with greater risk of *C. bovis* infection in cattle, within current literature; see section entitled ‘Systematic Review of Published Literature’. The categorization and selection of a restricted list of those questions/farm characteristics to take through to the statistical analysis incorporated both descriptive data analysis and biological plausibility, involving the following steps:

1) Consideration of the biological causal relationships between farm characteristics and the lifecycle of *C. bovis* (as described in current literature). Farm characteristics for which no evidence of causal link to *C. bovis* infection in cattle was found in the current literature were excluded from the chosen variables.
2) Characteristics that were considered to be correlated to another characteristic (which met subsequent criteria more effectively) reflecting the same biological pathway for infection (e.g. the use of straw as bedding was met more effectively by the use of straw as a forage feed) were also excluded.

3) In many cases, a number of related characteristics were combined into a single index variable, based on their theorized biological importance, as in point 1.

4) Consideration of the validity of the farm characteristic with regards to application within the final classification tool. This involved asking questions such as, “is this characteristic reliable and easy to measure?” Characteristics that were highly variable or very subjective were not included in the selection for the statistical analysis.

5) Only characteristics that could be adequately combined across beef, dairy and dairy/beef production systems, with minimal effect of missing values, were included in the selection. Ideally, separate analyses could have been carried out for these different production systems, however, due to the numbers of farms involved in the dataset, it was thought best to combine the data to make more reliable predictions.

Thirty farm characteristics remained after the categorization and restriction steps; these were taken through to the second level of predictor variable selection.

The second level of farm characteristics selection involved:

1) Checks for missing data (a frequent issue with questionnaire-based data collection). Characteristics with, for example, most missing values situated within either the case or control group, were excluded.

2) Univariable logistic regression to assess whether each individual characteristic had evidence of a statistical association with farm status i.e., whether a farm was a case or control. Any farm characteristics for which P<0.2 (by univariable logistic regression) were then assessed against one another in the same manner, to check for multi-collinearity (i.e. a high level of association between two characteristics). If any such multi-collinearity was found, the variable with the strongest evidence for an association with farm status or the most superior variable during the categorisation and restriction steps was selected. Five farm characteristics remained after selection through univariable analysis (see Table 4, ‘Results’ section), these were taken through to the next step: multivariable analysis.
Multivariable logistic regression was used to assess which, if any, of the five farm characteristics selected through univariable analysis has good evidence of an association with farm status once any inter-relationships with the other four characteristics had been accounted for. The final output from the multivariable logistic regression consists of a logistic model, comprising all farm characteristics which have good evidence of an association with farm infection status, when they are considered together. Comparative models were created using a ‘stepwise forwards selection’ method. This involved adding each of the five farm characteristics to the model, one at a time. If the addition of a new farm characteristic caused a substantial change to the evidence for, and measures of, the association between farm status and the characteristics already present in the model (and also the results of the univariable analyses for these characteristics), in addition to those for the new characteristic itself, it was kept in the model. If, after the addition of this new characteristic, the evidence for an association between any characteristic and farm status in the new model became weak (adjusted Wald P value below 0.05), such a characteristic (whether new or not) was not kept in the model. Subsequent to each change within the model, the overall ‘fit’ of each model to the data, pre and post the addition or removal of characteristics, was also compared using a ‘likelihood ratio test’. If this test gave evidence that, for example, the patterns within the data were unlikely to have arisen without the combined effect of both the characteristics already present in the model and the new characteristic, then this was taken as reason to keep the characteristic in the model. The process was then repeated for the next characteristic, and so on. Additional models built using the opposite method: ‘stepwise backwards selection’ were also tested, where all variables are put into the model initially, with variables subsequently removed one at a time, whilst assessing any changes to the evidence for, and measures of, the association between farm status and the characteristics still present in the model; in combination with the ‘fit’ of the reduced model to the data at each step. Subsequent to the consideration of all such statistical tests and criteria, the model which singularly gave the strongest evidence of having adequately described the patterns within the data when produced via both ‘stepwise forwards association’ and ‘stepwise backwards association’ was selected as the final version, this final model comprised 2 farm characteristics (see RESULTS section, Table 5).

**Social network analysis**

Network analysis was also carried out to visualize and describe the movement networks involving case and control animals, with farms as nodes (connection points) and animal movements as the edges (the relationship between nodes). R version 3.1.1 (igraph package) was used to create directed networks to show the movement of animals. In order to measure the number of connections between farms we used in-degree, which is a measure of the edges coming to a node (Figure 3).
Additionally, animal movement histories were used to create a new binary variable reflecting whether in the period 1st January 2014-31st December 2014 a given animal had inhabited a farm in which one or more animals were found to be infected with *C. bovis* the year before (1st January 2013- 31st December 2013). The new variable was tested in a similar univariable analysis as used in the case-control study. The dataset used for the network analysis comprised the same 2270 GB cattle from whose movement histories the case and control farms were selected for the case-control study. i.e., imported animals and movements to markets and slaughterhouses were omitted from the analysis. However, for the purpose of network analysis, movement data from only one *C. bovis* uninfected animal per infected animal was used (as opposed to the 3:1 ratio of uninfected to infected animals used previously).

**Individual animal risk factor identification**

Following on from farm risk factor identification and social networks analysis, a workshop was held to present the interim results of our analyses to key stakeholders in the cattle production industry (see section entitled STAKEHOLDER WORKSHOP). One of the suggestions made was that the relationship between age and gender demographics of cattle found and infection should be explored. The first component to this assessment included a statistical analysis, similar to that conducted to assess farm risk factors, to identify whether the age and/or sex of the individual animals in the dataset was associated with their *C. bovis* infection status. The dataset used for this age and sex analysis comprised the same 2270 GB cattle used for the social networks analysis, and from whose movement history farms were selected for the case-control analysis. Univariable logistic regression techniques were utilised as for the farm risk factor identification, this time assessing the relationships between sex, age and infection status, (see RESULTS, Table 7). Age was measured in months at the time of slaughter, and was categorised according to both biological significance (based upon the age ranges significant to UK cattle production systems) and the age distribution of the animals in the dataset (e.g. the very small number of animals in the <9 months category precluded consideration of this age group). Multivariable logistic regression was used in a similar manner to their use for farm risk
factor identification, this time assessing the relationship between infection status and both age and sex. The final model was built using the ‘stepwise backwards selection method’ (see RESULTS, Table 8).

Assessment of the potential of a herd classification tool based on movement history and individual animal risk factors
One of the main conclusions alighted on during the stakeholder workshop, was that “the classification tool proposal will not be based on individual farm characteristics” (see section entitled STAKEHOLDER WORKSHOP). This decision was based on the fact that the case-control study found limited evidence of an association between farm characteristics and farm infection status (see RESULTS section); therefore the ability of a farm classification tool, based on farm characteristics, to discriminate between high and low risk animals was thought to be limited. In addition, workshop attendees concluded that the collection of information from farms pertaining to these risk factors, in a reliable and cost effective fashion, would be unmanageable within current farming and data recording systems. Workshop attendees were therefore in agreement that a proposal for the classification tool should entail the use of movement history and/ or individual animal risk factors for targeted meat inspection to detect C. bovis infection in UK cattle.

The outputs from the social network analysis, individual animal risk factor identification and stakeholder discussions as described were integrated to propose options for a classification tool whereby meat inspection activities can be targeted at cattle with a higher risk of C. bovis infection at slaughter, within Great British (GB) abattoirs. An initial input of the evaluation was the proportion of the slaughtered cattle population which had a ‘high risk’ farm in their movement history and which also featured within the high risk group for sex and age. As per the results of the movement history network and individual animal characteristics analyses, these animals had been demarcated as at higher risk of C. bovis infection at slaughter.
A number of different scenarios for the utilisation of a classification tool, combining and not combining current and ‘enhanced’ inspection methods that would be targeted at animals belonging to this high risk sub group were simulated in @Risk version 7.0. The sensitivity of current meat inspection was assumed to be 15%; it was assumed that enhanced inspection could achieve 30% sensitivity.

The potential for an “enhanced” inspection procedure to achieve a significantly higher sensitivity was highlighted by workshop participants with anecdotal evidence of individual inspectors contributing to a high proportion of the cases identified probably as a result of more time available to inspect individual animals. These participants suggested that such meat inspectors were in some cases very experienced and highly motivated to follow meat inspection protocol “to the letter”. A working environment with a low throughput was also cited as enabling a longer and more intensive inspection of each carcass than is required in the current legislation (e.g. more cuts to the masseter).
For the purpose of our simulations, the specificity of both inspection methods was assumed to be 100%. Other input parameters where the apparent prevalence of infection (assumed to
be 0.012% based on data for the period 1st January 2013 to 31st January 2015) and the probabilities of animals belonging to the high risk group conditional to their infection status (obtained from the movement histories and age-sex categories of case and control animals used in this study). The outputs of each simulated scenario include estimates for the annual number of infected cattle which would remain undetected at slaughter, number of animals detected at slaughter and number of inspections needed to detect one infected carcase. Scenarios tested included the current inspection regime (enforced through Regulation (EC) No 854/2004), and five hypothetical scenarios:

i) A situation in which 100% of the animals from high risk farms (based on movement history) and 100% of the animals in the high risk age-sex category are subject to current inspection; the remaining animals undergo no inspection for *C. bovis* detection.

ii) Animals from high risk farms are subject to current inspection; none of the remaining animals are subject to inspection for *C. bovis* detection.

iii) Animals from high risk farms are subject to enhanced inspection; animals not from high risk farms but in the high risk age-sex category are subject to current inspection; all remaining animals undergo no inspection for *C. bovis* detection.

iv) Animals from high risk farms are subject to enhanced inspection; in addition a random sample of 20% of the animals that are not from high risk farms but belong to the high risk age-sex category are subject to current inspection; all remaining animals undergo no inspection for *C. bovis* detection.

v) Animals from high risk farms are subject to enhanced inspection; none of the remaining animals are subject to inspection for *C. bovis* detection.
RESULTS

FARM LEVEL RISK FACTORS

The results of the univariable and multivariable analyses of farm characteristics associated with the risk of animals originating from the farm being found to be infected with *C. bovis* at slaughter are presented in Tables 4 and 5, respectively.

Table 4. Results of the univariable analysis to assess the association between farm status (case vs. control farm) and each individual farm characteristic, in 226 GB cattle farms between January 2013-2014.

<table>
<thead>
<tr>
<th>Farm characteristic</th>
<th>Farm characteristic categories (number of farms in each category)</th>
<th>Number (%) of case farms in each farm characteristic category</th>
<th>Odds Ratio (OR)</th>
<th>95% Confidence Interval (CI)</th>
<th>Wald's tests P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle production type on farm (n=226)</td>
<td>Beef (148) BASELINE Dairy (47) Beef and dairy (31)</td>
<td>37 (25) 13 (27.7) 7 (22.6)</td>
<td>1 1.15 0.87</td>
<td>0.55-2.4 0.35-2.2</td>
<td>0.77 0.71</td>
</tr>
<tr>
<td>Farmer has prior knowledge about <em>C. bovis</em> (n=220)</td>
<td>Yes (70) No (150) BASELINE</td>
<td>22 (31.4) 35 (23.3)</td>
<td>1.51 1</td>
<td>0.8-2.83 -</td>
<td>0.21 -</td>
</tr>
<tr>
<td>Farm rears its own replacements² (n=224)</td>
<td>Yes (135) No (89) BASELINE</td>
<td>29 (21.4) 28 (31.4)</td>
<td>0.6 1</td>
<td>0.32-1.09 -</td>
<td>0.09 -</td>
</tr>
<tr>
<td>Method used to source roughage for cattle feed (n=218)</td>
<td>Home grown (96) Not Home grown (122)</td>
<td>22 (23) 30 (24.5)</td>
<td>1 1.1</td>
<td>0.58-2.06 -</td>
<td>0.774 -</td>
</tr>
<tr>
<td>Source farmer uses to obtain drinking water for cattle (n=225)</td>
<td>Public supply only (110) Private supply only (38) Private and public (46) Uses natural sources (31) BASELINE</td>
<td>28 (25.4) 11 (28.9) 9 (19.5) 9 (29)</td>
<td>0.83 0.99 0.59 1</td>
<td>0.34-2.03 0.35-2.8 0.21-1.72 -</td>
<td>0.68 0.99 0.34 -</td>
</tr>
<tr>
<td>Cattle have access to a natural/ground water source, such as a river (n=225)</td>
<td>Yes (133) No (92) BASELINE</td>
<td>34 (25.6) 23 (25)</td>
<td>1.03 1</td>
<td>0.56-1.9 -</td>
<td>0.92 -</td>
</tr>
<tr>
<td>Herd health plan enlisted on farm (n=222)</td>
<td>Yes (171) No (51) BASELINE</td>
<td>44 (25.7) 11 (21.6)</td>
<td>1.26 1</td>
<td>0.59-2.67 -</td>
<td>0.54 -</td>
</tr>
<tr>
<td>Fly control methods enlisted on farm (n=220)</td>
<td>Yes (50) No (170) BASELINE</td>
<td>10 (20) 42 (24.7)</td>
<td>0.76 1</td>
<td>0.35-1.65 -</td>
<td>0.5 -</td>
</tr>
<tr>
<td>Bird control methods enlisted on farm (n=219)</td>
<td>Yes (65) No (154) BASELINE</td>
<td>15 (23) 40 (30)</td>
<td>0.85 1</td>
<td>0.43-1.69 -</td>
<td>0.65 -</td>
</tr>
<tr>
<td>Land ownership</td>
<td>Own (79) BASELINE</td>
<td>19 (24)</td>
<td>1</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Farm characteristic (total number of farms with information for this characteristic)</td>
<td>Farm characteristic categories (number of farms in each category)</td>
<td>Number (%) of case farms in each farm characteristic category</td>
<td>Odds Ratio (OR)</td>
<td>95% Confidence Interval (CI)</td>
<td>Wald's tests P</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Rent and/or shared (n=141)</td>
<td>38 (26.9)</td>
<td>1.17</td>
<td>0.62-2.2</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Land prone to flooding (n=223)</td>
<td>Yes (53) No (170) BASELINE</td>
<td>16 (30) 41 (24.1)</td>
<td>1.36</td>
<td>0.69-2.7</td>
<td>0.377</td>
</tr>
<tr>
<td>Land used for any activities other than cattle production, such as arable cropping (n=210)</td>
<td>Yes (56) No (154) BASELINE</td>
<td>16 (28.5) 38 (24.7)</td>
<td>1.22</td>
<td>0.62-2.43</td>
<td>0.57</td>
</tr>
<tr>
<td>Rent and/or shared equipment to produce feed or bedding for cattle (n=217)</td>
<td>Yes (57) No (160) BASELINE</td>
<td>10 (17.5) 43 (26.9)</td>
<td>0.58</td>
<td>0.27-1.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Farmer uses own cattle manure for pasture fertilisation (n=219)</td>
<td>Yes (185) No (34)</td>
<td>45 (24.3) 9 (26.5)</td>
<td>0.89</td>
<td>0.39-2.05</td>
<td>0.79</td>
</tr>
<tr>
<td>Farmer uses own and/or bought in manure from animals other than cattle (including treated human sewage) for pasture fertilisation (n=219)</td>
<td>Yes (23) No (196)</td>
<td>10 (43.5) 44 (22.5)</td>
<td>2.66</td>
<td>1.09-6.47</td>
<td>0.03</td>
</tr>
<tr>
<td>Farmer uses artificial fertiliser for pasture fertilisation (n=220)</td>
<td>Yes (64) No (156)</td>
<td>41 (64.1) 13 (8.3)</td>
<td>0.71</td>
<td>0.35-1.45</td>
<td>0.35</td>
</tr>
<tr>
<td>Land is accessible to the public (n=224)</td>
<td>Yes (167) No (57) BASELINE</td>
<td>40 (24) 17 (30)</td>
<td>0.74</td>
<td>0.38-1.45</td>
<td>0.38</td>
</tr>
<tr>
<td>Farm is close to a permanent potential source of human faecal contamination, such as a sewage plant (n=224)</td>
<td>Yes 74 No 150 BASELINE</td>
<td>24 (32.4) 33 (22.6)</td>
<td>1.7</td>
<td>0.91-3.17</td>
<td>0.09</td>
</tr>
<tr>
<td>Farm has an organic production system (n=198)</td>
<td>Yes (16) No (182) BASELINE</td>
<td>3 (18.8) 48 (26.4)</td>
<td>0.64</td>
<td>0.18-2.36</td>
<td>0.507</td>
</tr>
<tr>
<td>Farm has a history of being an ‘open farm’, i.e. holds open days for the public (n=219)</td>
<td>Yes (21) No (198) BASELINE</td>
<td>6 (28.6) 47 (23.7)</td>
<td>1.29</td>
<td>0.47-3.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Farm stockmen are given formal training in farm</td>
<td>Yes (87) No (112) BASELINE</td>
<td>22 (25.3) 27 (24.1)</td>
<td>1.07</td>
<td>0.56-2.04</td>
<td>0.85</td>
</tr>
<tr>
<td>Farm characteristic (total number of farms with information for this characteristic)</td>
<td>Farm characteristic categories (number of farms in each category)</td>
<td>Number (%) of case farms in each farm characteristic category</td>
<td>Odds Ratio (OR)</td>
<td>95% Confidence Interval (CI)</td>
<td>Wald’s tests P</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Hygiene practices (n=199)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm stockmen are shared with other farms, temporary or seasonally employed (n=221)</td>
<td>Yes (44)</td>
<td>9 (20.5)</td>
<td>0.71</td>
<td>0.32-1.59</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>No (177) BASELINE</td>
<td>47 (26.6)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Average number of people accessing the farm on a monthly basis (n=223)</td>
<td>1-3 (83) BASELINE</td>
<td>16 (19.3)</td>
<td>0.6</td>
<td>0.31-1.15</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>&gt;3 (140) BASELINE</td>
<td>40 (28.6)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Average number of people in contact with cattle on the farm on a weekly basis (n=220)</td>
<td>1-3 persons (153) BASELINE</td>
<td>38 (25)</td>
<td>0.9</td>
<td>0.47-1.73</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>4 persons (67) BASELINE</td>
<td>18 (26.8)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Average daily number of vehicular movements on/off farm (n=207)</td>
<td>1-5 (168) BASELINE</td>
<td>38 (23)</td>
<td>0.66</td>
<td>0.3-1.42</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>&gt;5 (39) BASELINE</td>
<td>12 (30.8)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Average length of time taken to walk to farm toilet facilities from cattle housing and pasture (n=205)</td>
<td>1-5 minutes (183) BASELINE</td>
<td>46 (25)</td>
<td>0.77</td>
<td>0.3-1.98</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>&gt;5 minutes (23) BASELINE</td>
<td>7 (30.4)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Number of separate toilets on farm (n=202)</td>
<td>1-2 (172) BASELINE</td>
<td>44 (25.6)</td>
<td>0.8</td>
<td>0.34-1.88</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>&gt;2 (30) BASELINE</td>
<td>9 (30)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Number of dwellings on farm (n=222)</td>
<td>0-1 (96) BASELINE</td>
<td>23 (24)</td>
<td>0.5</td>
<td>0.15-1.69</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>2-5 (113) BASELINE</td>
<td>29 (25.7)</td>
<td>0.55</td>
<td>0.17-1.82</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>&gt;5 (13) BASELINE</td>
<td>5 (38.5)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Shaded variables are those for which there is either strong evidence (P<0.05) or limited evidence (P<0.2), for an association with farm status.

1 Odds ratios (OR) are an estimate of the effect of the association between the specified farm characteristic category and farm status. Also shown are the 95% confidence intervals for the odds ratios, which give a range in which we are 95% confident that the true value for the effect of the specified association (the OR) lies, in the UK cattle population. The P values for the Wald’s test describe the probability of the observed data arising, if the null hypothesis that: “there is no association between the farm characteristic in question and farm status” lies, in the UK cattle population. Therefore, a small P value of <0.05 gives a low probability that the null hypothesis is true, based on the data observed, and therefore gives good evidence that the opposing hypothesis is true, i.e. that: “there is an association between the farm characteristic in question and farm status”.

2 For example, farms which answer ‘yes’ to rearing their own replacements have 0.6 times the odds of being a case farm (i.e. this characteristic is protective), when compared to those which do not rear their own replacements. We can be 95% confident that the true odds ratio for this relationship lies between 0.32 and 1.09. This range includes an odds ratio of 1, which would indicate no difference in the odds of being a case farm, when comparing farms which rear their own replacements and those that do not. In addition, the Wald’s Test P value of 0.09 indicates weak evidence against the hypothesis that this association could be exhibited in our results by chance alone. This association is therefore unlikely to exist in the true UK cattle farm population.
The results of the univariable analysis (Table 4) show that only one of the farm characteristics analysed: “Farmer uses own and/or bought in manure from animals other than cattle (including treated human sewage) for pasture fertilisation”, had good evidence of an association (P<0.05) with farm status. Those farms that use manure from animals other than cattle to fertilise their pasture have a 2.66 times higher odds of being a case farm (i.e. potentially producing cattle infected with C. bovis at slaughter). Four other farm characteristics showed some limited evidence of an association with farm status (P<0.2): farms rearing their own replacements and farms sharing or renting equipment to produce feed or bedding for their cattle, were associated with a reduced odds of producing cattle infected with C. bovis at slaughter; whilst farms which were in close proximity to a permanent potential source of human faecal contamination, such as a sewage plant and farms accessed on average by more than 3 people every month, had a higher odds of producing cattle infected with C. bovis at slaughter.

In the multivariable analysis (Table 5), once any inter-relationships with the other farm variables expressing good to limited evidence for an association with farm status had been accounted for, only two variables had good evidence of an association with the outcome (P<0.05). Farms which were situated close to a permanent potential source of human faecal contamination (e.g. sewage works) have a marginally higher odds (1.77 times higher) of producing cattle infected with C. bovis at slaughter, by comparison to farms which are not. Farms which use their own and/or bought in manure from animals other than cattle to fertilize their pasture, which includes treated human sewage, have a 2.74 times higher odds of producing cattle infected with C. bovis at slaughter, by comparison to farms that do not (e.g. farms that use their own cattle manure).

Table 5. Results from the final statistical model produced via multivariable analysis considering those farm characteristics with at least limited evidence of an association with farm status together within the same model, in 226 GB cattle farms between January 2013-2014.

<table>
<thead>
<tr>
<th>Farm characteristic (number of farms)</th>
<th>Farm characteristic category (% of farms in that category which are case farms)</th>
<th>Crude odds ratio^3 (95% CI)</th>
<th>Adjusted odds ratio (95% CI)</th>
<th>Wald’s test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm close to a permanent potential source of human faecal contamination (n = 201)</td>
<td>Yes (44.9)</td>
<td>1.77 (0.91,3.41)</td>
<td>2.04 (1.03,4.04)</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>No (55.1) BASELINE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm uses own and/or bought in manure from animals other than cattle (n = 201)</td>
<td>Yes (20.4)</td>
<td>2.74 (1.12,6.73)</td>
<td>3.25 (1.29,8.22)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>No (79.5) BASELINE</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^3 The crude odds ratio is defined as in Table 3^1. In table 5, the crude odds ratio for each farm characteristic differ slightly from those in Table 4. This is because within the multivariate model, those farms with missing values for the farm characteristics selected were deleted. The adjusted odds ratio describes the effect of the association between the farm characteristic and farm status, once the farm characteristic in question is considered in combination with the other farm characteristics in Table 5, i.e. when they are added into the model.
Overall, the results of the case-control study show relatively limited evidence of an association between farm characteristics and the risk of a farm producing cattle infected with *C. bovis* at slaughter. It was therefore hypothesized, a theory agreed upon at the stakeholder workshop (see section entitled STAKEHOLDER WORKSHOP), that in the current low prevalence level in GB, the ability of a farm classification tool based on farm characteristics to discriminate between high and low risk animals, for *C. bovis* infection at slaughter, would be limited.

**MOVEMENT NETWORKS**

The networks of farms linked by movements of animals found to be infected versus not infected with *C. bovis* at slaughter are presented in Figure 4. The raw dataset used for the network analysis comprised the same 2270 GB cattle from whose movement histories the case and control farms were selected for the case-control study. The total number of infected animals within the dataset (592), and an equal number of uninfected animals were selected for the movement networks analysis. Those animals which had made no movements between farms in their lifetime (i.e. animals with only one farm in their movement history) were removed for the purpose of this analysis, leaving a final study dataset of 2262 animals. Analysis of the separate movement networks of infected animals and uninfected animals respectively revealed 899 farms in the infected animal network or ‘case network’ and 875 farms in the uninfected animal network or ‘control network’. The range of in-degree in the case network was 0-49, whilst it was 0-18 in the control network. In the case network 6 farms were receiving animals from more than 10 farms, however in the control network there was only one such farm. Among these 6 farms we have received questionnaire information from only 2 farms. Both of these are beef farms who buy store cattle for fattening from large regional markets, whilst one farm buys-in cattle from another country within the UK.

**Figure 4:** Network diagram to show the between-farm movements of animals found at slaughter to be infected
or uninfected with *C. bovis* between 1st January 2013 and 31st January 2015.

Among the 2262 animals within the dataset, 40% of the infected animals and 14% of the uninfected animals slaughtered in 2014 had inhabited a farm in which an animal, diagnosed as infected at slaughter in 2013, had previously resided (1471 animals were slaughtered in 2014, or 64.8% of the total dataset). Those animals with a history of being on a farm which appears in the movement history of a previously infected animal have a 4 times higher odds of being found as positive for *C. bovis* at meat inspection (odds ratio =4.27, p value = <0.001: Table 6).

**Table 6.** Results of the univariable analysis of the association between an animal having resided on a farm in which an infected animal had previously resided and the *C. bovis* infection status of that animal at meat inspection, within the sub-sample of 1471 animals slaughtered in 2014.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categories</th>
<th>No. positive animals in 2014</th>
<th>Odds ratio (95% CI)</th>
<th>P (Wald’s test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal had resided on a farm which had been inhabited previously by an infected animal</td>
<td>Yes (343)</td>
<td>172(50%)</td>
<td>4.27 (3.3-5.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>No (1164)</td>
<td>222(19%)</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

The results suggest that there is a strong potential for using movement history (of which Food Chain Information provides the last farm the animal inhabited prior to slaughter, whilst an animal’s full movement history is stored within the BCMS database) and previous *C. bovis* diagnoses (Collection and Communication of Inspection Results) to target meat inspection at slaughter. An animal’s movement history appears to have more discriminatory power than the characteristics of the farm of origin and may be more readily available for its potential use to inform inspection.

**STAKEHOLDER WORKSHOP**

A workshop was held on 14th September 2015, to present the interim results of our analyses of farm characteristics and movement networks to key stakeholders in the cattle production industry. The aim of this workshop was to promote discussion and formulate conclusions with regard possible industry challenges related to the design, transfer and implementation of a classification tool. The primary aim of the proposed classification tool would be to enable targeted surveillance for *C. bovis* infected cattle at meat inspection. These conclusions, and further themes which attendees highlighted for consideration, are laid out below:

- The classification tool proposal will not be based on individual farm characteristics.
- The classification tool proposal will be based on cattle movement history data (which is already recorded by the British Cattle Movement Service (BCMS)), and Collection and Communication of [Meat] Inspection Results (CCIR). This will enable the periodic identification of ‘high risk’ farms which are more likely to feature in the movement histories of animals found to be infected at slaughter. Such farms may be the true origin of infection for infected animals, or they may simply buy in and sell large numbers of
animals (e.g. intensive beef farms). Animals arriving at the slaughter plant which have ‘high-risk’ farms in their movement history may be at higher risk of \( C.\ bovis \) infection, and may therefore be targeted for a more intensive meat inspection.

- New ‘high-risk’ farms will not be identified via this method. Therefore, the classification tool should form one component of a two part system. The second component could be designed with the objective of maximizing the probability of infection being detected in animals entering the food chain (risk-based) or, alternatively, with the aim of estimating the prevalence of infection in the UK cattle population (representative). It would be important to assess the performance of such a hypothetical second inspection component for different scenarios of sample size and sensitivity of inspection.

- The identification of risk factors for \( C.\ bovis \) infection should also be considered in light of the age and gender demographics of cattle found to be infected at slaughter. These demographics may be compared to those of the general UK cattle slaughter population.

- Workshop attendees also suggested further consideration of methodologies by which the sensitivity and specificity of meat inspection for \( C.\ bovis \) may be improved.

- Workshop attendees also suggested that it would be interesting to look into the source, and pattern, of human cases of \( C.\ bovis \) (aka \( Taenia saginata \)) in the UK; in light of the current research outcomes in cattle.

**INDIVIDUAL ANIMAL CHARACTERISTICS**

The results of the analysis of individual animal characteristics (sex and age), to identify if either or both of these are associated with the \( C.\ bovis \) infection status of animals at slaughter, are presented in Table 7. There is strong evidence for an interaction between sex and age and their relationship with infection status. This means that the effect of the association between infection status and sex varies between different age groups; and the effect of the association between infection status and age varies between the sexes. Using the sex-age category associated with the lowest odds of \( C.\ bovis \) infection in cattle (young males) as a reference, young females were found to have a 3 times higher odds of infection at slaughter (OR 3.00; 95% CI 1.87-4.83); old males were found to have a 3.16 times higher odds of infection at slaughter (OR 3.16; 95% CI 2.24-4.46); and old females were found to have a 3.19 times higher odds of infection at slaughter (OR 3.19; 95% CI 2.29-4.45).

**Table 7.** Results of the final statistical model produced via multivariable analysis considering the association between infection status, sex and age at slaughter in months. The association between infection status and the sex-age interaction within all sex-age category combinations is depicted. The total study sample included 2270 cattle, slaughtered between January 2013-2014.

<table>
<thead>
<tr>
<th>Age-Sex categories</th>
<th>Odds ratio (95% CI)</th>
<th>Wald’s test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males 0-20 months</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Females 0-20 months</td>
<td>3.00 (1.87-4.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Males 21-194 months</td>
<td>3.16 (2.24-4.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Females 21-194 months</td>
<td>3.19 (2.29-4.45)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
In interpreting these results it should be noted that we have categorized animals into two groups, according to their age (in months) at slaughter. After experimenting with various cut-off points for division of these groups, a cut-off of 20 months of age was chosen, since this provided proportions of both males and females within the older age category and the younger age category, which were adequate for performing statistical testing (Figure 5). This grouping also bore relation to the organisation of UK cattle production, since production systems fattening dairy calves for meat are known to generally finish this process and send animals to slaughter once they are between 14-20 months of age. Production systems fattening beef calves for meat are known to generally finish this process and send animals to slaughter once they are between 18-30 months of age, a range which only marginally straddles our age grouping cut-off point. It was also assumed that breeding dairy and beef cows, and breeding bulls, tend to be of greater than 20 months of age when they are sent to slaughter (due to illness or infertility for example). A cut-off point of 16 months of age was also considered as it may better discriminate between animals that have spent time at grass vs. those that have not. When this cut-off point was used, males between 17 and 20 months of age appeared to be at higher risk than those younger than 17 months, suggesting that consideration should be given to the use of a different age threshold. However, the regrouping of the age groups in this manner resulted in a very small number of animals in the two lowest age groups (only 25 females exist within the age group of 0-16 months). The results produced from analyses of such small numbers of subjects are generally less reliable than those produced from larger groups.

The relationship between age, sex and infection can therefore be summarized as males younger than 20 months of age having 3 times lower odds of infection than the remaining 3 categories which have similar odds of infection between them. To help with the interpretation of this finding the age distribution of males and females is presented in Figure 5.
Figure 5: Stacked bar graph to show the proportion of male and female animals within each group for age at slaughter (in months). Data labels depict the actual number of male or female animals, for example, it can be seen that the 400 male animals slaughtered between the ages of 11-20 months comprised approximately 75% of the total animals slaughtered at ages within this range. The total study sample included 2270 cattle, comprised of 1148 females and 1122 males, slaughtered between January 2013-2014. Also shown is the cut-off point which divided the sample into the two age groups used for the multivariable analysis of sex and age.

Considering the spread of the data exhibited in Figure 5, it can be seen that males comprise the greater proportion of the younger age range, with this proportion gradually decreasing until 31-40 months, where an abrupt change leads to females comprising the greater proportion of the older age range. The demographic and dynamics of the young male cattle population is therefore quite different to that of the young female population: overall, males tend to be younger than females (i.e. males comprise a higher proportion of the youngest age groups, than females). This may explain our finding that the young male age group (0-20 months) comprises the lowest risk group for *C. bovis* infection. We hypothesise that young males of 0-20 months, which, in the UK cattle population tend to be dairy calves stored for fattening for beef, may be more commonly reared indoors and fed a diet largely comprised of concentrates rather than roughage. Their young age additionally means that they are theoretically less likely to have been exposed to potential infection as frequently over their lifespan, by comparison to older animals; making them less likely to have contracted infection from these exposures. According to the results of our systematic review of published literature, and knowledge of the biology of *C. bovis*, this would indeed put them at lower risk of infection. Additionally, our results concur with those of other studies, which found that female gender and old age were both risk factors for *C. bovis* infection in cattle. Based on these observations, we therefore concluded that all females (0-194 months of age at
slaughter) and old males (20-194 months of age at slaughter) should be combined and considered as the most appropriate sex-age category for cattle at high risk of *C. bovis* infection at slaughter.

**FARM CLASSIFICATION TOOL**

The results from the simulation of different inspection scenarios making use of a classification tool targeted at animals belonging to the final selected high risk sub-groups of the GB cattle population (based on the cattle movement history and age-sex risk factors) are shown in Table 8.

The results presented in Table 8 show the potential impact of introducing changes to the current simulation regime (baseline), for which the low ability to identify infected carcases is reflected in an estimated 85% (1711) infected carcases missed per year and the need to conduct more than 8000 inspections to detect one infected carcase. If inspection was conducted in the same way as it currently is but excluding low risk animals (Scenario 1) the total number of inspections could be reduced by almost 20% with a very minor impact on the proportion of carcases infected/carcases missed (86% vs. 85% in the baseline). If inspection is targeted further to animals from high risk farms only (Scenario 2) the total number of inspections would be greatly reduced to 16% of the current number but the proportion of infections missed would raise to 93%; in this scenario, the number of inspections needed to detect an infection drops to less than 3000.

The potential impact of enhancing inspection is analysed in scenarios 3, 4 and 5. Scenario 3 shows that if the sensitivity of the inspection could be raised to 30% and this improved inspection was applied to animals from high risk farms, with normal inspection maintained in animals from the high risk group it would be possible to reduce the proportion of infected carcases not identified (to 79%) even in the absence of inspection for animals in the low risk group (i.e. reducing the total number of inspections by almost 20%). Maintaining enhanced inspection in all animals from high risk farms while reducing further the number of normal inspections (in only 20% of animals of the high risk group; scenario 4) or eliminating normal inspection altogether (scenario 5) would result in a very large decrease in the number of inspections with minor impact on the proportion of infected carcases missed compared with the baseline scenario.
Table 8: Results of the simulation of different inspection scenarios: baseline scenario five scenarios representing combinations of ‘enhanced’ and current meat inspection activities carried out in the selected high risk subgroups of the GB cattle population. Numerical outputs, are annual estimates based on the number of cattle slaughtered annually within GB FSA approved abattoirs.

*The sensitivity of ‘enhanced’ meat inspection was assumed to be 30%, whilst the sensitivity of normal meat inspection was assumed to be 15%. The specificity of both meat inspection methods was assumed to be 100%.

**High risk farm group (HRF) refers to those animals with a history of being on a farm which appears in the movement history of a previously infected animal (the high risk group for movement history). High risk group (HRG) refers to all female animals (between 0-194 months of age at slaughter) and male animals between 21-194 months of age at slaughter (the high risk group for sex and age). Lower risk group (LR) refers to all animals which do not fall into the HRG and HRF.

Baseline: Current meat inspection regime, where all slaughter animals are subject to “normal” inspection
Scenario 1: Current meat inspection for all animals from HRF or HRG; no inspection for all LR animals.
Scenario 2: Current meat inspection for all animals from HRF; no inspection for any other animals.
Scenario 3: Enhanced inspection for all animals from HRF; current inspection in HRG; no inspection in LR animals.
Scenario 4: Enhanced inspection for all animals from HRF; current inspection in 20% of HRG; no inspection in LR.
Scenario 5: Enhanced inspection for all animals from HRF; no inspection in any other animals.

NB: ‘no meat inspection’ in the text above refers specifically to meat inspection methods to detect *C. bovis* infection in cattle carcases (incision of the masseter muscle etc.), any such animal may still undergo inspection to detect other diseases.
DISCUSSION
Between 1st January 2013 and 31st January 2015, a total of 612 C. bovis infected animals were identified in FSA-approved abattoirs in Great Britain. These results are indicative of a very low prevalence of infection (0.08% or 1 out of every 1250 animals; assuming a sensitivity of current meat inspection of 15% and 100% specificity). Movement history data were available and acquired from the British Cattle Movement Service (BCMS) for 98.5% of the 2323 cattle slaughter records acquired for this study. This degree of synchrony suggests that cattle movement data are very comprehensively recorded, and thus could support a potential classification tool based on the combined outputs of CCIR and BCMS data.

The case-control study of 226 GB cattle farms, comprised of 57 case farms and 169 control farms derived from this movement history data, found some evidence of association between farm characteristics and risk of infection. Animals from farms which were situated close to a permanent potential source of human faecal contamination (e.g. sewage works), and farms which use their own and/or bought in manure from animals other than cattle to fertilize their pasture (which included treated human sewage) may be at higher risk of C. bovis infection. These findings concur with known biological facts regarding the life cycle of C. bovis, or Taenia saginata, the adult stage of the parasite in humans. However, such evidence relating to farm characteristics as risk factors for C. bovis infection in cattle is complex, highly variable and occasionally conflicting, as described by the results of our systematic review. For example, a Danish study involving 77 case farms and 231 control farms, carried out between 2006-2010 found that “sharing machinery or hiring contractors” was more frequently observed in case farms; a finding which our study in GB found weak opposing evidence for. A study in Switzerland involving 119 case farms and 66 control farms, carried out between May 2005 and April 2006 found that variables defining “the presence of a railway line or car park close to areas grazed by cattle”; “leisure activities around these areas”; “use of purchased roughage” and “organized public activities on farms attracting visitors” were ‘positively associated with the occurrence of C. bovis’ on farms. Although our study comprised similar proxies for all of these variables, we did not find similar significant relationships. This may have been due to differences in the case and control farm selection process, or in the re-categorisation of variables. However, it may also be due to national differences, for example: it was noted that in Switzerland the majority of Swiss trains ‘still have carriages with open toilets which release sewage directly onto the tracks’ 10. Also, on 64% of the farms involved in this study, ‘domestic effluent was drained into a manure storage tank for pasture fertilization’; this would be illegal in the UK if the sewage was untreated 18 and is therefore unlikely to have occurred in the farms selected for our study.

Importantly, it must also be noted that, due to the low prevalence of C. bovis in the UK (i.e. the EU in general) isolated one-off events that place an individual farm or a small number of farms at high risk at a particular point in time may have a large contribution to the overall risk of infection at national level. This is illustrated by the 2013 outbreak event investigated by the Animal and Plant Health Agency (APHA, then AHVLA) in August 2013 in which the source of C. bovis infection was thought most likely to be potatoes, from a single source, that were used as cattle feed. Our results and the weak associations identified between farm
characteristics and odds of infection are compatible with a low prevalence situation and the sporadic emergence of new case farms. In this situation, where a clear demographic pattern is lacking, the usefulness of a classification tool based on farm characteristics will be limited. Additionally, information on farm characteristics are not currently routinely collected, and, as concluded within the stakeholder workshop held to discuss the interim results of the project: farm characteristics such as manure use for pasture fertilization are frequently dynamic (varying with the seasons on any particular farm, for example) whilst they also vary to a huge degree between farms (in type, source, storage, application etc.). Such characteristics would therefore prove exceptionally challenging to reliably measure across the entire UK cattle farm population. For all of these reasons, it was concluded that a classification tool based on farm characteristics that are readily available to inform inspection at abattoirs would not be able to offer the appropriate discriminatory power to efficiently and effectively target cattle with a higher risk of \textit{C. bovis} infection at slaughter.

The results of the social network analysis of 2270 animals selected from the initial 2323 cattle slaughter records and associated movement history data acquired for this study show heterogeneities and linkages in the movement histories of animals found to be infected vs. non-infected at meat inspection, highlighting that some farms may play a key role as a source of \textit{C. bovis} infection. Our systematic review also found evidence which concurred with this finding: the Swiss study\textsuperscript{10} mentioned previously found that ‘29.4% of all case farms had previously experienced cases of cysticercosis’. Our results show that animals with a history of being on a farm which appears in the movement history of a previously infected animal have a 4 times higher probability of being found positive for \textit{C. bovis} at meat inspection. It may be that infection cycles within and between these subsets of the farm network, however, it must also be taken into consideration that large farms moving large numbers of cattle onto and off farm may be more likely to appear in the movement network of infected animals since they are more likely to buy them in. The analysis aimed at identifying whether the individual animal characteristics of sex and age serve as risk factors for \textit{C. bovis} infection at slaughter, showed that males younger than 20 months are at a lower risk of infection. In interpreting this finding it is important to take into consideration the different age structure of males vs. females at slaughter, with males overrepresented in the younger groups. These findings are concurrent with those of other studies discussed within the systematic review of published literature.

The differences in risk associated with i) having a farm with previous cases in the movement history and ii) being in the age-sex category associated with higher risk of infection (i.e. not being a young male) were used to simulate the potential performance of inspection regimes in which high risk animals are targeted by means of “enhanced” inspection. The scenarios were compared with the existing baseline situation in which all animals are subject to “normal” inspection. The results of the simulations provided further evidence of the potential for using previous \textit{C. bovis} diagnoses at slaughter (Collection and Communication of Inspection Results), cattle movement history records (stored within the BCMS database) and the sex and age characteristics of cattle (FCI) to efficiently and effectively target meat inspection at cattle with a higher risk of \textit{C. bovis} infection at slaughter. The outputs of the
simulations showed that a hypothetical inspection regime (scenario 5 in table 8) in which only animals originating from farms deemed to be of high risk are subject to an enhanced mode of inspection could detect almost the same proportion of infected carcases as the current system with a dramatic reduction in the total number of inspections conducted per year (to 16% of the current number). However, such a system would concentrate inspection on the same subset of farms and would fail to detect new incidents of infection in previously uninfected farms. To address this limitation, a second inspection tier could be added as showed e.g. in scenarios 3 (Table 8); in this scenario, a superior ability of detection of infected carcases is achieved compared to the current situation while the number of inspections is markedly reduced (to 80% of the current number, with around 20% of them being enhanced). The protocol utilised in scenario 3 also allows for the detection of infection in animals from farms which were previously not defined as ‘high risk’.

The simulation of hypothetical inspection scenarios strongly suggest that there is scope for modified inspection regimes that are at the same time less onerous in terms of number of inspections carried out and equally effective (or even superior) at protecting animal and public health. However, the estimates are based on a number of critical assumptions, such as the potential increase of the sensitivity of inspection from its current (assumed) 15% to an expected 30%. The estimates are also sensitive to the strength of the associations between history of movement through a high risk farm and infection and between age-sex category and infection. Such estimates have been derived from imperfect field data and should ideally be validated. A study conducted in Denmark, with a similar aim to our own, used a simulation model approach to test targeted meat inspection methods based on three predefined risk factors. These factors were gender (female cattle had been shown to have a higher risk of being infected with C. bovis, the same finding as presented within our results from the sex age logistic regression analysis), access to grazing on farm (animals which were grazed had been found to have a higher risk of infection) and access to risky water sources. In this case, the models of systems based on targeting more intensive surveillance methods for C. bovis at those animals having any one of the three risk factors all had a lower sensitivity than the current system. Despite this, the main conclusion from this Danish study was similar to our own, in that the authors recommended that the sensitivity of the actual inspection methods themselves should be improved, as a first line approach in improving the current C. bovis surveillance system for cattle at slaughter.

**STUDY LIMITATIONS**

When considering the overall results of this study, a number of limitations and assumptions must be taken into account. These included potential variation in the definitions of C. bovis infection at meat inspection between abattoirs and meat inspectors – i.e. ‘positive’, ‘non-viable cyst’, ‘viable cyst’, which may have led to some misclassification of animals and farms in the study. Equally, due to poor sensitivity, a considerable proportion of truly infected animals are missed by current inspection methods resulting in the potential inclusion of farms that produced infected animals as controls in our study. However, given the very low
prevalence of infection this should have had minimal effect on the results of the case-control study. Additionally, the ‘uninfected’ animal records selected retrospectively by slaughterhouses were generally from animals whose carcases were condemned for other reasons than *C. bovis* infection, such as peritonitis or pleurisy as opposed to “normal” animals. Although this may have introduced some bias, a large proportion of the dataset has been collected prospectively, and thus many of the uninfected bovine records belong to normal animals, whose carcases were not condemned and who have no other detected health condition, so this effect may be minimal.

The relatively high proportion of non-respondents within the case-control study could also have introduced bias if non-respondents systematically differ from respondents with respect to the farm characteristics.

The outcomes of simulated inspection scenarios are presented to illustrate trade-offs between the intensity of inspection in subgroups of animals with different *a priori* risk of infection and the ability of the system to identify infected animals. The validity of the outcomes relies on a number of assumptions such as the sensitivity of enhanced inspection, for which validation was not possible due to lack of data. Variable selection, coding and re-categorization pre-statistical analysis was a highly subjective process, although based on previous literature and sound biological principles. This must also be taken into account when considering the conclusions made from the case-control study.

**CONCLUSIONS**

- Cattle movement history data and inspection results are very comprehensively recorded in the UK. This supports the notion of a classification tool, based on such data, whereby inspection activities can be targeted at cattle with a higher risk of *C. bovis* infection at slaughter.
- Due to the specific epidemiological characteristics of *C. bovis* infection in UK cattle, that is, a very low prevalence resulting from what appear to be sporadic incidents involving a small number of farms, a classification tool based on farm characteristics is unlikely to offer the appropriate discriminatory power to discern and target cattle with a higher risk of *C. bovis* infection at slaughter.
- Conversely, the high degree of connectivity of infected animals through farms common to their movement histories and the lower prevalence of infection in some age-sex groups, supports the potential use of previous *C. bovis* diagnoses at slaughter (Collection and Communication of Inspection Results), cattle movement history records (stored within the BCMS database) and the sex and age of slaughtered animals (FCI) to target inspection.
- The simulation of hypothetical inspection scenarios strongly suggests that there is scope for modified inspection regimes that are at the same time less onerous in terms of number of inspections carried out and equally effective (or even superior) at protecting animal and public health. A hypothetical scenario in which a classification tool is used to target enhanced inspection activities at cattle with a high risk movement history, whilst
animals in the high risk age-sex category (who are not also from high risk farms) are subject to current inspection methods and all remaining animals undergo no C. bovis inspection, is likely to result in an increase in the number of C. bovis infected animals detected annually and a decrease in the total number of inspections.

- The estimates presented in this report are based on a number of assumptions and on imperfect field data. The risk factors identified (movement history and age-sex group) should be reassessed as more data (e.g. 2015 inspection results) become available. The feasibility and sensitivity of an enhanced inspection protocol should be trialed and compared with those of normal inspection to provide the empirical evidence that could eventually support the introduction of a more cost-effective inspection system.

- More analysis of different age cut-off points is recommended, as further data become available, in order to make more reliable judgements regarding those groups of animals which may be at higher risk of C. bovis infection.

**FINAL RECOMMENDATIONS AND PROPOSAL FOR VALIDATION OF THE HERD CLASSIFICATION TOOL**

Based on the results of FSA project FS517002, and to facilitate the potential translation of project findings into more cost-effective inspection protocols we recommend:

- That the strength of association between movement history and infection and between age-group and infection is re-assessed as more data (e.g. 2015 inspection results) become available. These are critical inputs in our simulations that should ideally be validated and refined. In line with this, the RVC and FSA are currently in the process of organising the sharing of similar data derived from cattle slaughtered in the first six months of 2015, to enable the validation and further refinement of these results.

- That the feasibility of implementing an enhanced inspection protocol is evaluated and that such a protocol is trialled in order to compare its sensitivity with that of normal inspection. It should be noted that the low prevalence of infection poses a challenge to the field evaluation of enhanced inspection. If such a trial were to be implemented it should target high risk groups to increase statistical power and should involve a control group also of high risk animals subject to “normal” inspection.

- The project has shown that data are comprehensively recorded; however, given how critical accurate data are for the follow-up of this project, the validation of its findings and its eventual translation into revised inspection, we recommend that all possible efforts are made to maintain and improve the systematic, accurate and complete recording of information such as inspection findings and age and sex of the animals. With regard to C. bovis inspection findings, there is potential for improved standardization of records.
REFERENCES


17. Personal communication via email 04/08/15: Xenofon Schizonicas, Department of Agriculture and Rural Development, Northern Ireland.


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