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Using an epidemiological framework and bovine spongiform encephalopathy investigation questionnaire to investigate suspect bovine spongiform encephalopathy cases: an example from a bovine spongiform encephalopathy case in Ireland in 2015

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In several EU member states, bovine spongiform encephalopathy (BSE) cases have been identified in cattle born after the reinforced ban (BARB cases), for reasons that are not entirely clear. Epidemiological investigation of these cases has proved challenging. The European Food Safety Authority recently recommended the collection of a predefined set of epidemiological data from BSE suspects and confirmed BSE cases to aid future investigations. In this study, we present an epidemiological framework and BSE investigation questionnaire to aid the investigation of suspect BSE cases, and illustrate its application during the investigation of a BSE case in Ireland in 2015. It is recommended that the framework and questionnaire are used concurrently: the framework provides structure and focus, whereas the questionnaire (with 135 questions) aids data collection. The framework focuses on confirmation and discrimination, estimating the date and location of exposure, and determining the method/source of exposure. The BSE case in Ireland in 2015 was a BARB case born in 2010. It was identified with classical BSE at an authorised knackery as part of Ireland's targeted active surveillance programme for BSE. No definitive source of infection with the BSE agent could be attributed in this case.

Introduction

Bovine spongiform encephalopathy (BSE) is a progressive fatal neurodegenerative disease of cattle, first recognised in 1986 in the UK.[1] An early study indicated that BSE was consistent with exposure of cattle to a scrapie-like agent via cattle feedstuffs containing ruminant-derived protein.[2] Transmission mainly occurs during calfhood, with the time of infection ranging between 0 and 18 months of age and a typical incubation period of five years.[3 4] In dairy cattle, the age-dependent risk of infection is highest during the first six months of life.[5] Since 1987, 28 countries in Europe, Asia and North America have reported cases of BSE. The International Organisation for Animal Health (OIE) assigns a BSE disease status to member countries, and in 2016 categorised 46 countries as having a negligible BSE risk and 8 as having a controlled BSE risk.[6]

In Ireland, the first case of BSE was diagnosed in 1989.[7] By December 31, 2016, 1660 cases of BSE had been confirmed in Ireland, including four atypical BSE cases, as discussed later. Most clinical BSE cases have been detected in cattle aged between four and eight years, with an age range from three to twelve years.[8 9] Since 1989, it has been a legal requirement for any person observing an animal with clinical signs consistent with BSE to inform the Department of Agriculture, Food and the Marine (DAFM). In line with EU legislation, all cohorts and progeny of BSE-positive animals are traced, killed and disposed of outside the food chain.

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In view of the link established between BSE and cattle feed containing ruminant-derived protein,[2] a ban on the feeding of animal protein to ruminants was introduced in Ireland in 1990 (the initial feed ban). Legislation banning the use of proteins derived from mammalian tissues for feeding ruminant animals was introduced at the EU level in June 1994. In 1996, enhanced controls on the production, sale or supply of mammalian meat and bone meal (MBM), as defined by the OIE,[10] were introduced in Ireland[11] and in the UK.[12] At the EU level, legislation was introduced in January 2001 to enforce a total ban on the feeding of processed animal proteins to farmed animals. A total feed ban was implemented on May 1, 2004 in the 10 central and eastern European countries that joined the EU on that date (the 'newer' EU member states). Therefore, a total feed ban has been in place in Ireland since 2001, or since 2004 in all member states, but reinforced feed bans had previously been implemented in Ireland and the UK in 1996. Given this context, it has been challenging to determine the aetiology for those BSE cases born after the reinforced bans (BARB), and in particular the five classical BSE cases born between 2006 and 2011 (3 UK, 1 France, 1 Ireland).

The aetiology of BSE remains contentious. Early evidence suggested that BSE was caused by a single major strain, commonly referred to as classical BSE.[13] More recently, two sporadic atypical forms of BSE have been identified (namely high-type (H-BSE) and low-type (L-BSE)), which differ from classical BSE.[14 15] The unusually old age of all H-BSE and L-BSE cases, and their apparent low prevalence in the population, could suggest that these atypical BSE forms are arising spontaneously.[16] However, there is no comprehensive information available on the aetiology and pathogenesis of atypical BSE in cattle. Serial passage of H-BSE in other species can lead to the emergence of a classical BSE phenotype, raising the possibility that classical BSE emerged from H-BSE.[17–19] Such passage can also lead to the emergence of new strains of BSE,[20] which underlines the lack of aetiological clarity for BSE and the challenges in determining controls suitable to deal with it. For BARB cases, it is suggested that the majority of cases have arisen due to continued exposure to contaminated feed.[21] This view is also supported by a more recent UK study that hypothesises that the continued occurrence of classical BSE is due to an exogenous feedborne source, as a result of an over-reliance on imported feedstuffs into Great Britain and the later introduction of a ban on the use of mammalian MBM in other EU member states in January 2001.[12] Milk replacer, containing extracted animal fats, has been implicated as a source of BSE for cattle in a number of countries.[22–26]

Other routes of transmission, such as maternal, environmental and iatrogenic, have also been proposed. In a review of the epidemiological features of cases of BSE born after July 31, 1996 in Great Britain, Wilesmith and others[12] concluded that there was no evidence of a maternally associated risk factor. However, these authors also state that the nature of the risk of maternal transmission remains uncertain. In the same paper, the authors considered two means of environmental contamination to be possible: first, that in the early years of the epidemic in the UK, a proportion of clinically infected animals were buried; and secondly, the potential excretion of the BSE agent in faeces. Nonetheless, the authors concluded that there was no evidence that a substantial number of BARB cases in the UK occurred as a result of environmental contamination (other than from feedstuffs). Also, of significance regarding environmental contamination is spatial information regarding the location of the index farm in relation to previously infected premises or other local features of potential interest, for example, knackeries. Iatrogenic transmission of Creutzfeldt-Jakob disease (CJD) has been demonstrated in human beings. This was linked to human growth hormone therapy,[27] donated tissues,[28] surgical instruments[29] and blood.[30] Iatrogenic transmission of scrapie through vaccines prepared from ovine material has also been documented.[31 32] Posterior pituitary extract was used in veterinary practices at the start of the BSE epidemic in the UK, but no association between its use and the occurrence of BSE was found.[2] A spontaneous origin has been proposed for a number of transmissible spongiform encephalopathies (TSEs), including atypical BSE as mentioned above, atypical scrapie[33 34] and sporadic CJD.[35] Because of the long interval between the implementation of the total feed bans and the time of occurrence of BSE cases in some EU member states such as Ireland, UK and France, consideration must also be given to the possibility that some cases of classical BSE occur due to spontaneous mutation of prions. As yet, however, this hypothesis is speculative and there is currently no supporting scientific evidence.

The investigation of BSE cases, including BARB cases, can be particularly difficult due to the time lag between exposure to the BSE agent and the onset of clinical signs, and the small number of cases, thereby making attribution of source difficult. Furthermore, the relevant data required to investigate BARB cases are sometimes unavailable, due to the passage of time, the loss of paper records and changes in farm management.[11] Epidemiological studies of BSE have mainly been conducted using quantitative methods based on national data sets. Such studies seek to address a range of objectives, including an improved understanding of disease epidemiology[11 36] and the spatiotemporal distribution of cases.[37 38] There has been limited information on field-based epidemiological methods to investigate suspect cases of BSE, in contrast to other diseases such as foot and mouth disease where applied (field-based) epidemiological methods are available.[39] This last concern has been further highlighted in a recent scientific opinion from the European Food Safety Authority (EFSA), which recommended the creation of a predefined set of epidemiological data to be collected across the EU for the investigation of future BSE cattle suspects and new confirmed BSE cases.[40] This approach would also be of potential value to countries with no previous BSE experience, when faced with a first or single case of BSE.

In this study, in line with recent EFSA recommendations, we present an epidemiological framework and BSE investigation questionnaire to aid the investigation of suspect BSE cases, and illustrate its application during the investigation of a suspect BSE case in Ireland in 2015. Suspect BSE cases are defined in accordance with the definition in Article 3 of Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain TSEs.

Materials and methods

Development of the epidemiological framework and BSE investigation questionnaire

Considerable expertise has developed over many years among DAFM staff in Ireland regarding the investigation of suspect BSE cases. During this period, a questionnaire was informally and progressively developed by DAFM staff to guide the investigation of BSE, and particularly BARB, cases. The questionnaire, currently with 135 questions, is presented in online supplementary appendix 1. For ease of data collection, the questions are grouped by topics to aid data collection, including case animal details (*Q1–25*), herd details (*Q26–41*), parents (*Q42–67*), compliance with identification requirements (*Q68–75*), general farm management practices (*Q76–92*), feed (*Q93–117*), farm management practices when case animal was present on the farm (*Q118–131*) and history of on-farm deaths (*Q132–135*).

The BSE investigation questionnaire was developed to aid field-based data collection in Ireland. As part of the current study, we have adapted this questionnaire to maximise its usefulness as an investigative tool for suspect BSE cases. Specifically, drawing on current understanding of BSE and on experiences gained with BSE investigations in Ireland, we have developed an epidemiological framework to be used in conjunction with

BOX : Epidemiological framework to investigate suspect BSE cases.*

Confirmation and discrimination

a. Tools available
   i. Confirmatory tests (OIE-approved immunoblot method) (see online supplementary appendix 2)
   ii. Discriminatory tests (two-blot protocol)
   iii. Clinical history
b. Information required
   i. Laboratory confirmation of presence of BSE agent (Q1–2)
   ii. Identification of BSE type (classical, atypical H-type or L-type) (no further investigation is conducted if atypical BSE is confirmed) (Q3–6)
   iii. Description and timeline of clinical signs (Q7–Q11, Q2)

Estimating the date and location of exposure

a. Tools available
   i. BSE investigation questionnaire (see online supplementary appendix 1)
   ii. National identification and movement database
   iii. Additional data gathering (personal interview, inspection of farm records)
b. Information required
   i. Details of case animal, including identification and movement history (Q12–Q14, Q15–Q18, Q19, Q20, Q21–Q28)
   ii. Herd/farm details (Q29–Q31)
   iii. Past BSE and scrapie history of farm (Q29, Q32, Q33)
   iv. Likely infection window (encompassing the most likely period of infection of the case animal) (Q15, Q16, Q11)
   v. Progeny/cohort tracing (Q34, Q29, Q30)

Determining the method/source of exposure

Possible sources of exposure are:

- Maternal transmission (dam and progeny)
- Feedborne details and feed management/storage
- Environmental exposure
- Iatrogenic transmission

a. Tools available
   i. Epidemiological investigations on index farm (the herd of residence of the case animal at the time of diagnosis) (and on previous farms if deemed necessary) (farm visit, interview, BSE investigation questionnaire (online supplementary appendix 1))
   ii. Maps (other infected premises)
   iii. Backward tracing information (animal movement, identification of cohorts and progeny)
   iv. Additional data gathering (personal interview, farm records, feed company records, medicines, BSE status of parents/cohorts/progeny/disposal of carcases)
b. Information required
   i. BSE status of parents of index case (Q35–Q38, Q39-Q64)
   ii. Spatial relationship between index and other known infected premises (Q65–Q67, Q68, Q69)
   iii. Location of index farm in relation to other features of potential interest (including neighbours, abattoirs, feed mills, roads, water courses, etc) (Q70–Q75, Q69)
   iv. Source and storage of feeds and fertilisers (including milk replacer and proprietary calf feed) (Q76–Q100)
   v. On-farm animal movement during tracing window (progeny, cohorts and other movements) (Q29, Q101–Q103)
   vi. Human and other movement during tracing window (Q104–Q108)
   vii. Husbandry/medicinal practices (Q109–Q123)
   viii. General farm management practices (Q124–Q135, Q104–Q108)

The relevant questions in the associated BSE investigation questionnaire (online supplementary appendix 1) are included in brackets (in italics).
*Suspect BSE case is defined in accordance with Article 3 of Regulation (EC) 999 of 2001.
BSE, bovine spongiform encephalopathy; OIE, International Organisation for Animal Health.

the BSE investigation questionnaire, focusing on (1) confirmation and discrimination; (2) estimating the date and location of exposure; and (3) determining the method/source of exposure (Box). At each step of the framework, we describe the tools available, information required and the relevant questions from the supplementary questionnaire. The steps to be taken during the investigation are guided by the type of BSE (classical or atypical) identified, as determined by OIE-approved discriminatory testing. If atypical H-type or L-type BSE is confirmed, the investigation does not progress beyond '1. Confirmation and discrimination'. If classical BSE is confirmed, the investigation continues to include '2. Estimating the date and location of exposure' and '3. Determining the method/source of exposure'. Decisions with respect to '3. Determining the method/source of exposure' should be made using a legal standard of proof of at least 'on the balance of probabilities' or 'on the preponderance of the evidence',[41 42] after considering all data relevant to the biological plausibility of each alternative. The framework has been developed to be suitable for use during on-farm epidemiological investigations of suspect BSE cases.

Application of the epidemiological framework and BSE investigation questionnaire to the 2015 BSE case in Ireland

The epidemiological framework and associated BSE investigation questionnaire were used to guide the investigation of a BSE suspect identified in Ireland in 2015. A number of data-gathering methods were used during this investigation. The index herd (the herd of residence of the case animal at time of diagnosis) was visited by DAFM staff to examine the farm, herd

movement records and farm records. The field investigation began once a positive screening test result was received, noting the high specificity of these tests.[43] The likely infection window (encompassing the most likely period of infection of the case animal) was determined to be 2010, the year of birth. The case animal was born and died in the index herd. The identification of the cohort group, in line with DAFM protocols, took account of animals born in the index herd during 2010, and also those born in the previous (2009) and subsequent (2011) years. Data with respect to animal feed were gathered, including receipts and records in relation to animal feed and feeding systems. All feed business operators (FeBOs) that supplied proprietary feed to the index farm were contacted by DAFM to obtain details of all animal feed supplied to this farm during October to December (Q4) 2009 and throughout 2010. In addition, all questions in the BSE investigation questionnaire were completed, providing a summary of each line of investigation outlined in the epidemiological framework.

Results

Confirmation and discrimination

Details of the diagnostic methods are outlined in online supplementary appendix 2. Final confirmatory test results were received from both the national and EU reference laboratories on June 25, 2015, confirming the case as classical BSE. The case animal was a female Rotbunt aged 65 months at the time of death. The case animal had calved normally on February 24, 2015. During May 2015, milk recording data showed a drop-off in the animal's milk yield, and some decline in body condition was noted by the herd owner. On June 6, 2015, the case animal fell and remained recumbent until it was euthanased on June 8, 2015, followed by disposal at an authorised local knackery. In Ireland, knackeries are authorised intermediate plants used for the collection and assembly of carcases before disposal by rendering or incineration.

Estimating the date and location of exposure

Examination of DAFM's national bovine registration and movement data showed that the case animal was born in the index herd and had remained there throughout its life. The index herd was a dairy enterprise consisting mainly of Rotbunt animals. The index herd was situated in an area of mixed grazing (cattle and sheep) and tillage enterprises.

During the investigation, the year 2010 was identified as the likely infection window. During tracing, 63 cohort animals were identified, born in 2009, 2010 and 2011. At the time of the investigation, the case animal also had four progeny still alive. The cohorts and progeny were located in seven herds, including the index herd. All cohorts and progeny were immediately flagged on DAFM's Animal Identification and Movement IT system to prevent their further movement. All 67 animals were removed and slaughtered on June 22, 2015. Samples of brain tissue from all these animals were subjected to BSE testing. All of these tests were negative.

Determining the method/source of exposure

Relevant to maternal transmission

The grand-dam of the case animal had been imported from Germany in 2002 and its dam was born in the index farm in 2005. The dam and grand-dam of the case animal had each been sampled for BSE when slaughtered as healthy animals in 2006 and 2013, respectively, and each tested BSE-negative.

Relevant to feedborne transmission

Five FeBOs, four in Ireland and one in Northern Ireland, had supplied proprietary feeds to the index farm during the fourth quarter (Q4) of 2009 and during 2010. All of these FeBOs were, and are still, registered with /approved by their relevant competent authority, in line with Regulation (EC) 183/2005, which regulates feed hygiene and is subject to official controls. No other farmed species have been kept on the index farm, and it is therefore highly unlikely that non-bovine and bovine feeds have been mixed. There was no evidence that feed was ever acquired from an unlicensed source.

There was no proprietary feed from Q4 2009 or 2010 available for sampling, either on the index farm or at any of the FeBOs supplying the farm during that time. Samples of two proprietary feeds on the farm during inspection were sampled and analysed in accordance with official feed control methods (EU Regulation 152/2009 as amended) and were negative for any constituents of animal origin. In Ireland during 2009 and 2010, DAFM's feed controls with respect to the ban on the use of MBM comprised 2021 inspections and the analysis of 1279 samples in 2009, and 1783 inspections and 1180 analysed samples in 2010. All samples tested negative for the presence of constituents of animal origin. Specifically, 52 samples related directly to the four FeBOs based in Ireland that supplied feed to the index farm and these all tested negative. The Department of Agriculture and Rural Development (DARDNI) had conducted eight and seven MBM inspections in 2009 and 2010, respectively, on the single FeBO based in Northern Ireland that had supplied animal feed to the index farm. DARDNI sampled and analysed 10 samples of animal feed in both 2009 and 2010. All samples tested negative for the presence of constituents of animal origin.

Feed was stored in a single multipurpose shed in bulk or bagged form. This shed was also used to house calves. The bay containing feed and the bay containing calves were separated by a gate.

All calves were housed in three separate areas (calving boxes, individual calf pens, group calf pens) in the multipurpose shed from birth until they were moved out to grass. These three separate areas were all located under a single roof and shared a common airspace. All calves were born in a dedicated calving area that consisted of a number of calving boxes. Calves remained in the calving boxes with their dams for approximately 24 hours before being removed to individual pens located in an adjacent area of the same shed. Calves did not receive any supplementary feeding while housed in the calving boxes. Calves remained in individual calf pens until they were able to feed on their own. While in individual pens calves were fed pooled milk from the index herd and given access to a cereal-based calf ration. Calves never received milk replacer. Calves were then moved into group pens. From entering the group pens until three months of age, calves were offered a proprietary feed in the form of a cereal-based calf ration. Calves also had access to water and forage (hay or straw). Calves were then moved to a grass paddock. From 3 months to 24 months of age, the animals were offered home mixed feed, with the vast majority of feed materials being sourced from a single local FeBO.

Most calf feed was purchased in bags and supplied to calves using troughs. Proprietary calf feed was delivered using a bucket dedicated to feeding calves. This bucket was replaced regularly due to wear and tear. Bulk feed for adults was mixed and moved out of the multipurpose shed using a tractor mounted front end loader. This front end loader was used to handle feed in 1994 and also in 2010. The herd owner reported that the front end loader was used for a number of other activities on the farm, such as handling forage, cleaning sheds and carrying bedding and other equipment.

Relevant to environmental exposure

A case of BSE had previously been diagnosed on the index farm, in a fallen animal that had tested positive in a knackery in 2002. This animal had been born on the index farm in 1994 and had never moved off the farm. The herd was de-populated in 2002,

following this diagnosis, and an extensive DAFM-supervised cleaning and disinfection programme was completed.

Animal movement records were available for the herd from 2002 to the present. All fallen animals had been sent to the local knackery. Oneadult bovine animal was buried in a paddock beside the farmyard around the period 1999/2000. The burial site was not disturbed during a subsequent extension of the farmyard.

The positive BSE animal detected on this farm in 2002 was disposed of through incineration, following detection as a fallen animal at a knackery.

The index herd is situated approximately 5 km from the nearest knackery premises, where the assembly and preparation of adult bovine carcases for BSE sampling takes place on a daily basis. The knackery is audited, at least twice yearly, by a veterinary inspector from DAFM's local Regional Veterinary Office. These audits indicate that the knackery has a high level of compliance with requirements with EU (EU Regulation 1069/2009 and its predecessor) and national legislation (Statutory Instrument 187/2014 and its predecessors). The last positive BSE case detected by active surveillance at the knackery was in 2006.

Relevant to iatrogenic transmission

From farm records, as corroborated by the herd owner, vaccination (blackleg, bovine viral diarrhoea, leptospirosis, rotavirus and corona virus) and anthelmintic treatments were administered to the case animal as part of routine herd husbandry and disease control measures. An interrogation of medicine records from 2010 to 2015 showed no record of administration of other medicines to the case animal. The attendant private veterinary practitioner (PVP) confirmed that there are no veterinary practice records of any medicinal treatment for the case animal. Inspection of medicine records and on-farm medicines used on the index herd by the investigation team confirmed compliance with regulations.

Discussion

This study describes the use of an epidemiological framework and associated BSE investigation questionnaire to structure the investigation of suspect BSE cases, detailing tools and information required to confirm and discriminate BSE, to estimate the date and location of exposure, and to determine the method/source of exposure. The framework and questionnaire provide a systematic approach to investigating suspect BSE cases, and subsequently confirmed classical BSE cases, based on the experience of the competent authorities in Ireland developed over the last 25 years. The epidemiological framework provided structure and focus to the BSE investigation questionnaire, noting that the latter has been used extensively to aid data collection during BSE investigations in Ireland.

Based on the results of our field investigation of the 2015 classical BSE case in Ireland, no source of exposure of the case animal to the BSE agent could be determined. Nonetheless, a number of tentative conclusions can be drawn in relation to this case, based on the results of the field investigation.

There is uncertainty as to whether maternal transmission of BSE from infected dam to offspring in bovines can occur.[44 45] In this case, there was no evidence to support this hypothesis given that the dam of the index case never exhibited any clinical signs of BSE during her life and tested negative at healthy slaughter.

Although widely accepted as the main source of BSE prion transmission for cases of classical BSE, there was no evidence in this case to indicate that the feed supply chain was a contributory factor, or that the case animal had been fed with feed containing MBM. As well as carrying out a thorough investigation of the feed supply chain for the index herd, the investigation team evaluated the potential for contaminated feed to have been supplied to the farm. In 2009 and 2010, few BSE cases were detected in Ireland (9 in 2009, 2 in 2010) compared with a high of 333 in 2002. Also, comprehensive control measures had been put in place in 2009 and 2010, including active and passive surveillance, removal and destruction of dead-on-farm animals, effective rendering systems, and controls with regard to the potential for cross-contamination at mills. Feed imported into Ireland was also subject to routine inspection and testing, which did not identify any bone spicules. These controls led to a substantial reduction in the likelihood of contaminated feed acting as a source of the BSE agent. However, because of the passage of time, it was not possible to be certain that we obtained complete information on all possible sources of contamination of feed for the index herd. It has been shown that an animal can be infected by a very low oral dose of the BSE prion, with the attack rate and incubation period dependent on the dose.[46 47] Findings in the UK have highlighted the possibility of persistence of traces of contaminated feed in on-farm feed stores and the need for special care in the cleaning and maintenance of feed bins and silos and other feed storage facilities.[36] Consequently, inadvertent exposure to the BSE agent in residues of old particles of feed cannot be definitively ruled out.

Deposition of BSE prions in the environment may occur due to burial of carcases or through biosolids from water treatment plants processing infected animals.[48] However, the risk of such transmission is extremely low, with no evidence to support environmental contamination as a relevant infection route.[36] There was no evidence in our field investigation that the case animal was exposed to the BSE agent through an environmental source.

While there is evidence of iatrogenic transmission of the BSE prion,[49] this investigation did not support the hypothesis that the case animal was exposed to the BSE agent via medicinal products or vaccines. All of the products used on the farm were routine medicinal products or vaccines and there was no evidence that they could contain BSE prion material. We had no reason to doubt the reliability of information obtained from farm records, from the herd owner and from the PVP.

The identification of BARB cases is not unprecedented, but continues to be challenging from an epidemiological perspective. The epidemiological framework and BSE investigation questionnaire overcome some of these challenges, in particular the use of methodology to facilitate data collection that is comprehensive and consistent. Further, the framework and questionnaire are underpinned by best available science. Nonetheless, several challenges remain that contribute to the difficulty in attributing cause to recent BSE cases. By its nature, a case study does not allow definite conclusions to be drawn on the source of disease. Further, the role of any specific putative source cannot be directly tested as no information is available on suitable controls. Finally, because of the passage of time between exposure and the development of clinical signs, there are inevitable information gaps that hamper attribution of the source of the BSE agent. Ireland's 2015 BSE case was identified through existing surveillance mechanisms, and the investigation provided evidence that all BSE controls are operating as intended. Based on the investigation, no definitive source of infection with the BSE agent was identified.

Overall, the epidemiological framework and associated BSE investigation questionnaire provide structure, focus and detail to the field investigation of BSE cases in Ireland, and may be useful in other settings. We note that the main objective of the epidemiological framework is to hypothesise, or rule out, possible BSE sources on the balance of probabilities rather than beyond reasonable doubt. The framework provides an epidemiological logic to the assembly of evidence and allows the different steps of the investigation to be fully documented. The framework and associated questionnaire uses the best information available at the time of investigation of BSE cases. Given the rarity of BARB cases and uncertainty surrounding the source of infection for these cases, particularly as the interval between the implementation of the reinforced feed ban and the occurrence of new cases increases, it is essential that a comprehensive and thorough investigation of each

new BARB case is carried out. It is hoped that the investigation procedure described here will be of benefit in that regard and that the information provided by the investigations will assist veterinary authorities in ensuring that the measures in place to eradicate BSE continue to be relevant, appropriate and fit for purpose.

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Veterinary Record

Using an epidemiological framework and bovine spongiform encephalopathy investigation questionnaire to investigate suspect bovine spongiform encephalopathy cases: an example from a bovine spongiform encephalopathy case in Ireland in 2015

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