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Bovine spongiform encephalopathy (BSE) cases born after the total feed ban

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Abstract

Sixty bovine spongiform encephalopathy (BSE) cases of Classical or unknown type (BARB-60 cases) were born after the date of entry into force of the EU total feed ban on 1 January 2001. The European Commission has requested EFSA to provide a scientific opinion on the most likely origin(s) of these BARB-60 cases; whether feeding with material contaminated with the BSE agent can be excluded as the origin of any of these cases and, if so, whether there is enough scientific evidence to conclude that such cases had a spontaneous origin. The source of infection cannot be ascertained at the individual level for any BSE case, including these BARB-60 cases, so uncertainty remains high about the origin of disease in each of these animals, but when compared with other biologically plausible sources of infection (maternal, environmental, genetic, iatrogenic), feed-borne exposure is the most likely. This exposure was apparently excluded for only one of these BARB-60 cases. However, there is considerable uncertainty associated with the data collected through the field investigation of these cases, due to a time span of several years between the potential exposure of the animal and the confirmation of disease, recall difficulty, and the general paucity of documented objective evidence available in the farms at the time of the investigation. Thus, feeding with material contaminated with the BSE agent cannot be excluded as the origin of any of the BARB-60 cases, nor is it possible to definitively attribute feed as the cause of any of the BARB-60 cases. A case of disease is classified as spontaneous by a process of elimination, excluding all other definable possibilities; with regard to the BARB-60 cases, it is not possible to conclude that any of them had a spontaneous origin.

Keywords: BSE, BARB, origin, feed, spontaneous

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Summary

From the data notified by European Union (EU) Member States (MS) to the EU transmissible spongiform encephalopathies (TSE) database, it appears that 60 bovine spongiform encephalopathy (BSE) cases (BARB-60 cases) of Classical or unknown type were born after the date of entry into force of the EU total feed ban (i.e. after 1 January 2001 for 'old Member States', and after 1 May 2004 for the central and eastern European countries which joined the EU on that date).

The European Commission requested the European Food Safety Authority (EFSA) to provide a scientific opinion on the following Terms of Reference: (1) What is or what are the most likely origin(s) of the EU BSE cases of Classical or unknown type born after entry into force of the EU total feed ban? (2) Can feeding with material contaminated with the BSE agent be excluded as the origin of any of these cases? If yes, is there enough scientific evidence to conclude that such cases had a spontaneous origin?

A literature review has been conducted regarding the possible origin/s of BSE and the epidemiology of the BSE epidemic, together with non-peer reviewed documents such as The Phillips report on the BSE inquiry, the Horn report and previous opinions of the Scientific Steering Committee (SSC).

Surveillance data have been extracted or collated as necessary for the estimation of the design prevalence at MS level applying the Cattle TSE Monitoring Model (C-TSEMM), already used in EFSA for previous scientific opinions. The objective of this analysis was: (a) to evaluate the ability of each individual MS to detect a case of BSE based on the power of its surveillance systems and (b) to detect any heterogeneity in the occurrence of the BARB-60 cases between MS.

A questionnaire survey was conducted in September 2016 targeting the 11 MS in which the BARB-60 cases had been confirmed. The survey was based on a short questionnaire containing eight questions (mixed closed/open) circulated to representatives of the EFSA BSE/TSE Network from the 11 MS with the aim of collecting information on the epidemiological investigations conducted at case level, the hypothesis/hypotheses that the authorities considered most plausible to explain the occurrence of these cases, and on the evidence to support these explanations.

A qualitative assessment of the traceability of ingredients used in the production/mixing of livestock feed has been conducted.

The 54 audit reports resulting from inspections conducted by the Food and Veterinary Office (FVO) for the period 2001–2015 to monitor different aspects of the TSE legislation have been reviewed for the 11 MS that have had at least one BARB-60 case. The objective was to summarise all available information regarding the implementation and the compliance of MS with the total feed ban, and to assess whether contaminated feed with BSE agent could be excluded as a source of infection for the BARB-60 cases.

Multiple hypotheses have been postulated for the origin of BSE, including spontaneous, genetic, cross-species, iatrogenic, animal feed or other, without conclusive evidence to support, or actively refute, any of them. Previous epidemiological investigations at population level conducted post-1996 in the UK and Ireland (IE), looking at a larger set of confirmed BARB cases that included most of the BARB-60 cases of these two MS, concluded that feed was a significant risk factor. Some evidence within these two MS of a geographically associated risk or a spatial correlation/clustering of BARB cases was also identified. This supports the hypothesis of a common source/s for at least some of these cases, consistent with a feed source. Data also offer some support for the possibility of maternal transmission or environmental contamination in an undetermined number of cases.

More than half of the MS have not had a BARB-60 BSE case: this may be due to an insufficient sensitivity of the surveillance systems, i.e. they have not tested sufficient cattle to detect it. This is consistent with the distribution of the power of surveillance calculated by the C-TSEMM model in the EU-28 in 2015: the BARB MS group accounted for 84.1% of the total power of surveillance, with 15.9% accounted for by the non-BARB MS. Being a BARB MS is significantly associated with a detectable design prevalence lower than 1 in 100,000 in 2015.

The heterogeneous occurrence of BARB-60 cases between MS could be affected by differences in the sensitivity of surveillance, but could also be also accounted for by differences in the exposure to as yet unidentified geographically associated risk factors, whether they are feed-related or not. The exponential decay of the BARB cases, as indicated by the applied mathematical model, is consistent with a EU-wide single epidemic declining to zero.

When focusing on feed controls, it can be concluded that despite the large number of feed samples tested in the EU and the high analytical sensitivity of the tests in place, in the context of the huge

volumes of ingredients used for the production of livestock feed, the feed surveillance system has limited sensitivity for the detection of low levels of contaminated material.

There was an overall effort to comply with the TSE legislation with regard to the enforcement of the total feed ban in MS. The deficiencies observed by the FVO teams in the early years of the implementation were progressively overcome by measures applied by MS. However, contaminated material was still present in the EU after the total feed ban, as documented in the FVO audit reports.

Based on the limited qualitative data provided for the individual BARB-60 cases by the competent authorities (CA), and all other gathered evidence, a number of biologically plausible potential sources of infections (feed, maternal, environmental, genetic, iatrogenic) have been considered.

The source of infection cannot be ascertained at an individual level for any BSE case, including these BARB-60 cases. Thus, the uncertainty remains high about the origin of disease in each of these animals. However, compared with the other potential sources of infection, feed-borne exposure (i.e. associated with proprietary concentrates, milk replacers or cross-contamination with feedstuffs intended for other species on the farm) is the most likely source of infection.

In the investigation by the CA, feeding with material contaminated with the BSE agent was excluded for one case, reported by the UK in 2009. However, there is considerable uncertainty associated with the data collected through the investigation of all of the BARB-60 cases. This is due to factors such as the time span of several years between the potential exposure of the animal and the confirmation of disease, recall difficulty, and the general paucity of documented objective evidence available in the farms at the time of the investigation.

Given this uncertainty, feeding with material contaminated with the BSE agent cannot be excluded as the origin of any of the BARB-60 cases. However, this does not mean that feeding can be definitely attributed as the cause of any of the BARB-60 cases.

Spontaneous cases, interpreted as cases occurring without an apparent cause, are not predictable and may not be detectable either. The classification of a case as spontaneous is circumstantial and may change over time subject to additional information. It does not infer that there is no external cause; just that it could not be ascertained. A case of disease is classified as spontaneous by a process of elimination, excluding all other definable possibilities: with regard to the BARB-60 cases, it is not possible to conclude that any of them had a spontaneous origin.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

European Union (EU) prevention, control and eradication measures for Classical Bovine Spongiform Encephalopathy (BSE) are built on the understanding, based on scientific evidence, that Classical Bovine Spongiform Encephalopathy (BSE) is transmitted via feed contaminated with PrP^{Sc} given to a bovine animal during the first year of its life, or transmitted vertically. On this basis, a ban on the use of proteins derived from mammalian tissues for feeding ruminant animals was introduced in the EU legislation in June 1994, and extended to a wider ban on the use of processed animal proteins in feed for farmed animals (the so called 'total feed ban') in January 2001. Furthermore, EU eradication measures in case of detection of Classical BSE cases require the culling, testing for BSE and destruction of the cohort of BSE cases as well as of the progeny born within a period of two years prior to, or after, the clinical onset of the disease, where the disease was confirmed in a female animal.

From the data notified by EU Member States (MS) to the EU Transmissible Spongiform Encephalopathies (TSE) database, it appears that 61 BSE cases of Classical or unknown type were born after the date of entry into force of the EU total feed ban (BARB) (i.e. after 1 January 2001 for 'old Member States', and after 1 May 2004 for the central and eastern European countries which joined the EU on that date).

Given the total feed ban in force after those dates, the origin of those cases, and in particular of the five Classical BSE cases born between 2006 and 2011, is difficult to interpret.

In accordance with Article 29 of Regulation (EC) No 178/2002, the European Food Safety Authority (EFSA) is therefore requested to provide a scientific opinion according to the Terms of Reference (ToR).

In order to address these terms of reference, EFSA is kindly invited to contact at least the concerned MS in order to obtain all necessary information for this assessment, in particular information gathered during the epidemiological investigations on these cases.

Furthermore, in accordance with Article 22(7) of Regulation (EC) No 178/2002, EFSA is kindly invited to closely cooperate for this mandate with the competent bodies carrying out similar tasks to those of EFSA in at least the BARB MS, in particular with a view to discuss the risk assessment methodology of the EFSA scientific opinion.

Table 1: List of BSE BARB cases included in the mandate

Country (number BARB - 60 cases)	Animal birth year	Animal birth month	Target group	Sampling period year	Sampling month	Age	Case type	National case number
Czech Republic (1)	2004	6	Healthy slaughtered	2009	5	60	Classical	2/2009
France (3)	2001	1	Healthy slaughtered	2006	1	60	Classical	978
	2004	4	Fallen stock	2010	1	69	Classical	1013
	2011	4	Fallen stock	2016	3	59	Classical	1029
Germany (2)	2001	3	Healthy slaughtered	2005	6	51	Classical	DE22/2005
	2001	5	Fallen stock	2005	4	47	Classical	DE2005/13
Ireland (12)	2001	2	Fallen stock	2009	2	96	Unknown	1621
	2001	3	Suspects subject to laboratory examinationIexaminadon	2006	9	66	Unknown	1561
	2001	11	Suspects subject to laboratory examinationIexaminadon	2008	6	79	Unknown	1607
	2001	3	Fallen stock	2005	7	52	Unknown	IE2005/1501

Country (number BARB - 60 cases)	Animal birth year	Animal birth month	Target group	Sampling period year	Sampling month	Age	Case type	National case number
	2001	9	Fallen stock	2005	5	44	Unknown	IE2005/1486
	2002	5	Fallen stock	2007	10	65	Classical	1587
	2002	11	Healthy slaughtered	2009	10	83	Classical	1625
	2003	2	Eradication measures	2008	10	68	Unknown	1613
	2003	3	Fallen stock	2008	9	66	Unknown	1612
	2003	3	Fallen stock	2011	4	97	Classical	1631
	2004	4	Healthy slaughtered	2009	11	67	Classical	1626
	2010	1	Fallen stock	2015	6	65	Classical	1637/1/2015
Italy (1)	2001	1	Clinical signs at AM	2006	2	61	Classical	01
Luxembourg (1)	2001	2	Healthy slaughtered	2005	11	48	Classical	LU2005/1
Netherlands (1)	2001	2	Clinical signs at AM	2005	12	58	Classical	NL2005/3
Poland (3)	2004	8	Healthy slaughtered	2007	4	32	Classical	52
	2005	3	Healthy slaughtered	2008	9	42	Classical	63
	2005	11	Healthy slaughtered	2012	7	80	Classical[(a)]	3/2012
Portugal (2)	2001	2	+N86[(b)]	2007	12	82		02/2008
	2002	10	Fallen stock	2005	6	32		PT2005/23
Spain (7)	2001	1	Fallen stock	2006	5	64	Classical	26
	2001	1	Healthy slaughtered	2008	2	85	Classical	2008/7
	2001	5	Suspects subject to laboratory examination	2008	6	85	Classical	2008/13
	2001	9	Fallen stock	2008	11	86	Classical	2008/21
	2002	7	Fallen stock	2008	6	71	Classical	2008/10
	2002	1	Fallen stock	2005	6	41	Unknown	ES2005/48
	2004	10	Fallen stock	2010	6	68	Classical	2010/8-ES050402116335
United Kingdom (28)	2001	0	Fallen stock	2008	9	93	Classical	000800054
	2001	1	Fallen stock	2006	2	61	Classical	000600032
	2001	1	Fallen stock	2006	3	62	Classical	000600063
	2001	3	Fallen stock	2006	5	62	Classical	000600100
	2001	4	Fallen stock	2007	3	71	Classical	2174
	2001	4	Fallen stock	2014	10	162	Classical	001400003
	2001	6	Fallen stock	2006	12	66	Classical	000600216
	2001	10	Emergency slaughter	2005	1	39	Classical	GBUK12005/24
	2001	9	Eradication measures	2005	5	44	Classical	GBUK12005/166
	2002	1	Fallen stock	2013	10	141	Classical	001300004
	2002	4	Fallen stock	2007	11	67	Classical	000700102
	2002	5	Eradication measures	2008	11	78	Classical	000800067
	2002	5	Fallen stock	2007	12	67	Classical	000700110
	2002	7	Suspects subject to laboratory examination	2008	9	74	Classical	000800050
	2002	8	Healthy slaughtered	2006	9	49	Classical	000600175
	2002	9	Emergency slaughter	2009	12	87	Classical	000900030
	2002	10	Fallen stock	2008	1	63	Classical	000700116

Country (number BARB - 60 cases)	Animal birth year	Animal birth month	Target group	Sampling period year	Sampling month	Age	Case type	National case number
	2002	5	Eradication measures	2005	5	36	Classical	GBUK12005/167
	2003	1	Healthy slaughtered	2008	7	66	Classical	000800044
	2003	4	Fallen stock	2009	3	71	Classical	2187
	2003	6	Fallen stock	2009	2	68	Classical	2186
	2003	8	Fallen stock	2008	4	56	Classical	2182
	2003	9	Fallen stock	2009	11	74	Classical	000900028
	2004	10	Fallen stock	2010	4	66	Classical	001000009
	2004	11	Fallen stock	2010	12	73	Classical	001000033
	2006	7	Fallen stock	2012	12	77	Classical	001200014
	2007	2	Fallen stock	2013	7	77	Classical	001300003
	2009	5	Fallen stock	2015	9	76	Classical	001500004

(a): This case has been recategorised as Atypical BSE. See Section 1.2.
(b): According to the CA, this case was identified in the surveillance stream 'Suspects subject to laboratory examination.'

If it appears that there are other origins of Classical BSE cases than contamination via the oral route or vertical transmission, risk management measures, other than the eradication measures currently required in the case of detection of a Classical BSE case, will have to be considered by the European Commission and the MS.

Based, in particular on the existing scientific literature and on the results of investigations carried out by the concerned MS, EFSA is requested to provide a scientific opinion on the following questions:

1) What is or what are the most likely origin(s) of the EU BSE cases of Classical or unknown type born after entry into force of the EU total feed ban?
2) Can feeding with material contaminated with the BSE agent be excluded as the origin of any of these cases? If yes, is there enough scientific evidence to conclude that such cases had a spontaneous origin?

1.2. Interpretation of the Terms of Reference (if appropriate)

- Following the review of the EU BSE cases of Classical or unknown type born after entry into force of the EU total feed ban as in the EU TSE database, the case number 3/2012 reported by Poland initially as Classical has been reviewed and changed to Atypical BSE. Accordingly and as agreed with the requestor, the total number of cases to be considered in the mandate is 60.
- Spontaneous disease is 'a disease without apparent cause or occurring without external influence'.[1] When addressing the answer to ToR 2, the concept of 'spontaneous origin' is interpreted as the occurrence of cases without an apparent cause. However, this may change over time subject to additional information,and it may not infer that there is no external cause This does not mean that a case necessarily has no cause, just that it could not be ascertained.
- The 60 cases included in the mandate as reported in Table 1 will be referred to in this scientific opinion as BARB-60 cases, and not as BARB cases. BARB is a term that has been traditionally used to describe cases of classical BSE in cattle that were 'born after the reinforced ban'. However, the EU-wide reinforcement of the feed ban was implemented differently by the UK and Ireland: the cases born between April 1996 and January 2001 in the UK and between October 1996 and January 2001 in Ireland are called BARB cases, but are not included in the mandate. The 11 MS in which BARB-60 cases were confirmed are referred to in this scientific opinion as BARB MS.

[1] http://medical-dictionary.thefreedictionary.com/spontaneous

2. Data and methodologies

2.1. Data

2.1.1. Questionnaire survey data

Data and information were provided by the MS participating in the questionnaire survey (see Section 2.2.2), in the form of answers to the questions included in the questionnaire (see Appendix A), individual case reports and other ad hoc reports. Relevant data and information contained in these sources were transferred to an Excel grid in a matrix format (case x indicator of potential source of infection) that includes the indicators of potential sources of infection of each BARB-60 case, either historical sources of BSE infection, even if they should be currently totally prevented, or other potential sources such as the historical presence of BSE on the farm. The potential sources included in the template were: feeding (milk replacer), feeding (proprietary concentrates), feeding (presence of feed intended for other species on farm that might contribute to cross-contamination), maternal transmission, previous cases of BSE on farm, environment (farmyard disposal of carcasses), environment (farmyard application of manure/sewage) and other (iatrogenic source, genetic peculiarity, proximity to potential external sources of infection like meat-and-bone stores, rendering plants, knackeries, feed mills, etc.).

2.1.2. FVO audit reports

In December 2000, the Commission Implementing Decision 2001/9/EC concerning control measures required for the implementation of Council Decision 2000/766/EC (i.e. the Decision that introduced the total feed ban)) was enforced. According to Article 2, MS shall prohibit 'the feeding of processed animal proteins to farmed animals which are kept, fattened or bred for the production of food'. The prohibition was included in the EU Regulation 999/2001 (The TSE Regulation), where in Article 7 it is stated that 'the feeding to ruminants of protein derived from animals shall be prohibited and extended to animals other than ruminants and restricted, as regards the feeding of those animals with products of animal origin'.

Following the entry into force of the TSE Regulation, and in order to monitor the BSE control measures, different inspections/visits/missions were conducted by the Food and Veterinary Office (FVO) in the EU MS in order to evaluate the compliance with the TSE Regulation.

A total of 88 FVO reports on audits carried out in different MS were downloaded,[2] with review restricted to the 54 reports involving the 11 BARB MS (Table 2) and the sections related to the total feed ban reviews. FVO audit reports were of different types depending on the main objective of the mission: (a) to evaluate protective measures against BSE or the reinforced protection measures against BSE, (b) to evaluate controls over the feeding of swill to farm animals and waste food from prohibited sources, (c) to evaluate certain measures aimed at the eradication, control and prevention of TSE, (d) to evaluate the total feed ban and organic fertilisers or for organic fertilisers and soil improvers, (e) to evaluate the production and use of certain proteins of animal origin in feed for aquaculture animals.

For BARB-60, MS joining the EU after 2001 (CZ[3] and PL), inspections took place soon after accession.

Table 2: Number of Food and Veterinary Office reports reviewed by country and year for the 11 Member States with BARB-60 cases

Country	Year															Sum
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	
CZ							1			1						2
DE	1	1		1		1								1		5
ES	1	1	1	1			1	1	1		1			1		9
FR	2	1				1			1							5

[2] http://ec.europa.eu/food/audits-analysis/audit_reports/index.cfm
[3] Two-letter codes for EU and EFTA countries haves been used throughout the scientific opinion. See Glossary.

Country	Year															Sum
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	
IE	2	1						1	1					1		6
IT	1	1				1										3
LU	1	1	1	1		1				1					1	7
NL	1	1														2
PL	1	1														2
PT					1	1	1	1			1					5
UK	2	2		1	1	1				1						8

Shaded cells: years of EU membership.

2.1.3. Data for the C-TSEMM and the analysis of surveillance data

2.1.3.1. Data on the standing cattle population

Data on the cattle population at MS level for the EU-25 was originally collated in 2012 when a model (Cattle TSE Monitoring Model (C-TSEMM)) was developed for the evaluation of different options for the monitoring of Transmissible Spongiform Encephalopathies in cattle in the European Union (EFSA, 2012). This model has been periodically updated with the last version updated for this opinion (EFSA 2013a,b, 2016). Data for the standing cattle population by MS for the age intervals 0–11 months and 12–23 months were obtained annually from Eurostat from 2001–2015, while MS completed a questionnaire for EFSA for the adult population (> 24 months), with dates of 2008–2012 for the EU-25, 2012–2014 for HR, and 2008–2013 for BG and RO, as C-TSEMM has been periodically updated to include additional new MS. In the absence of available recent data for 2013, 2014 and 2015, the populations recorded for the most recent year were used.

2.1.3.2. Data on the number of cattle tested and test results

The numbers of cattle tested, and positive test outcomes by surveillance stream for each MS, for the period 2002-2015, were obtained from the EU TSE database hosted by the European Commission (data accessed by EFSA) (Appendix B).

The original version of C-TSEMM (Adkin et al., 2012) applied by EFSA (2012, 2016) used data reported by MS up to December 2012. Surveillance results up to 31 December 2015 were added to the dataset for all EU-28 MS. In some cases, the age of the animals tested was no longer collected in the same intervals as previously. For example, some MS are now reporting data from cattle classified as above or below 72 months of age. In these cases, it was assumed that the proportion of animals tested in each of the age intervals was the same as the proportion tested in the previous two years for which more detailed data were available.

2.1.3.3. Data on the number of cattle dead or slaughtered and not tested

Previously (EFSA, 2012, 2013a,b), data on the number of cattle dead/slaughtered and which were not required to be tested under the legislation (i.e. below the age for testing) were elicited through an EFSA questionnaire sent individually to each MS, and were current up to July 2012. For the updated C-TSEMM, it was assumed that the populations of dead and slaughtered cattle which were not tested in each MS for the most recent years were stable. Therefore, the proportion of animals dead/slaughtered and not requiring to be tested in each age interval was assumed to be the same as the proportion of animals dead/slaughtered in the most recent 2 years, for which data were available. These assumed proportions were only used to estimate the number of animals that may be tested in theoretical surveillance scenarios (Adkin et al., 2012), so this assumption does not affect the design prevalence estimated by the model for the tested scenarios.

2.1.3.4. Data on BSE case types

The case types have been updated, taking into account the results of the retrospective classification exercise of BSE cases dating back to 2003 in which positive cases were retested and classified into the following case types: Classical BSE (C-BSE), Atypical H-type BSE, Atypical L-type BSE or unknown. Only C-BSE and unknown types were included in the analysis, according to the definition of BARB.

2.2. Methodologies

Given the small number of BARB-60 cases and the limited and variable information available for each one obtained by the questionnaire survey, it is not possible to conclude anything about the possible origin of these cases using these data alone. A variety of approaches have therefore been used to assess these cases, both individually (the questionnaire data) and in the wider context of BSE epidemiology (literature review and direct analysis of surveillance data). Evidence to support or refute the likelihood of feed-based origin being relevant in these cases has been sought through the assessment of individual animal information, and in the wider context, the evaluation of the implementation of the feed ban and of the robustness of the controls in place for feedstuff manufacture and testing in the BARB MS.

2.2.1. Literature review

To retrieve data on the possible origin/s of BSE and the epidemiology of the BSE epidemic, a literature search in the Pubmed database was undertaken. The time of publication was restricted to the period 1/1/2007–31/12/2016. The publication of a comprehensive review of the epidemiology and dynamics of BSE (Ducrot et al., 2008) was used to set the starting date of the literature search, with a buffer of one extra year. The search was restricted to English language. The resulting search string was used: ((BSE OR bovine spongiform encephalopath*) AND (cattle OR bovine) AND ("risk factor" OR epidem* OR origin OR transmi* OR risk* OR "feed ban" OR BARB OR feed*). These terms were searched in the titles and abstracts of books and documents, case reports, classical articles, clinical trials, comments, comparative studies, data sets, editorials, electronic supplementary materials, English abstracts, introductory journal articles, journal articles, news, newspaper articles, randomised controlled trials, reviews, scientific integrity reviews, systematic reviews, technical reports and validation studies. No exclusion was applied based on potential explanations of the origin of the BSE epidemic, study design, geographical location or analytical method. Eligible criteria for selecting references included any reference to the possible origin/s of BSE and the epidemiology of the BSE epidemic. A total of 486 references were retrieved and a double screening (two reviewers independently screened the full list) looking for potentially relevant references was conducted. Discrepancies were discussed between the two reviewers until a final shortlist of references was agreed. A subset of 62 relevant references was selected and considered in this assessment by reviewing the full papers.

In addition, it was agreed to carry out a qualitative evaluation for the origin and risk factors of BSE (Sections 3.1 and 3.2) by means of literature reviews based on the knowledge and expertise of the Working Group (WG) members, taking into account the comprehensive reviews of the Phillips report (Phillips et al., 2000) and previous opinions of the Scientific Steering Committee (SSC) of the European Commission. In these cases, the experts in the WG selected relevant references starting from scientific papers, including review papers, books chapters, non-peer-review papers known by the experts themselves or retrieved through non-systematic searches, until the information of the subject was considered sufficient to undertake the assessment by the WG.

2.2.2. Questionnaire survey

A questionnaire survey was conducted in September 2016 targeting the 11 MS countries in which the BARB-60 cases had been confirmed. The survey was based on a short questionnaire (Appendix A) containing eight questions (mixed closed/open) circulated to representatives of the EFSA BSE/TSE Network from the 11 MS with the aim of collecting information on: (a) the epidemiological investigations conducted at case level; (b) on the hypothesis/hypotheses that the authorities considered most plausible to explain the occurrence of cases; (c) and on the evidence to support these explanations. MS were also asked to provide case reports or any other output of the investigations for each individual case.

For each of the BARB-60 cases, an assessment for each indicator of potential source of infection was performed with the aim of answering the ToRs. For ToR1, which focuses on the most likely origin of the cases, the aim was to exclude as many possible sources, to end up with the indicators of potential sources of infection that cannot be excluded or cannot be supported by data. ToR2 specifically questions if feeding with material contaminated with BSE can be excluded as the origin of any of the cases, so the answer to this ToR will also be provided with the results of this exercise.

A list of indicators of potential source of infection (risk factors) and an associated set of decision rules was defined *a priori* (Table 3), based on the data collected for the UK cases, which represents almost 50% of the cases. The rules were adapted iteratively to accommodate alternative data observed in the other case reports. For each source and case, one of the following three categories was assigned for each risk factor: (a) 'presence of risk factor not supported by data', (b) 'risk factor cannot be excluded', (c) 'insufficient data to take a decision'. For example, in the case of maternal transmission, the following criteria were applied: (a) 'presence of risk factor not supported by data' if dam was still alive 2 years after the birth of the case or died and tested negative for BSE, (b) 'risk factor cannot be excluded' if the dam died within 2 years of the birth of the case without testing, (c) 'insufficient data to take a decision' if no data available on the dam. Option (c) 'insufficient data to take a decision' was applied when there were not enough data to conclude, for example on milk replacer when the dam was a dairy cow and there was no indication about the use of milk replacer. The decision rules were applied for each case based on the data collected on the cases by the competent authorities (CA) and provided in the questionnaires.

To maximise the consistency of interpretation, the categorisation of data from each individual case report was conducted by a WG member, and the classification of a subset of cases (the 28 cases from the UK) was independently reviewed by a second WG member. Discrepancies were discussed until the final allocation of status to each indicator of potential source of infection was agreed. The categorisation of the other 34 cases was then revisited by the first assessor. Details of the categorisation for each indicator of potential source of infection and case are presented in Appendix B.

Table 3: Decision rules applied based on the data collected on the cases by the CA of the MS

Indicator of potential source of infection	(a) Presence of risk factor is not supported by data	(b) Presence of risk factor cannot be excluded
FEEDING (Milk replacer)	• Dairy cow plus mention that milk replacer was not used • Suckler cow without any indication about milk replacer	• Use of milk replacer
FEEDING (Proprietary concentrates)	• When no proprietary concentrate used (until adult age)	• Any kind of proprietary concentrate used
FEEDING (Other species on the farm)	• No other species • Other species than pig and poultry • Pig or poultry, but totally isolated in another unitPossibility of a few backyard chickens or pigs for the farmer's consumption, and dogs and cats	• Pig or poultry on the farm (with no indication of a clear separation in another unit)
MATERNAL TRANSMISSION	• Dam still alive 2 years after birth • Dam dead/culled within 2 years after birth but tested negative for BSE	• Dam died within 2 years after birth of the BARB-60 case and not tested • Dam died of BSE
ENVIRONMENT (BSE on the farm before)	• No previous cases of BSE recorded on the farm	• BSE cases recorded on the farm
ENVIRONMENT (Disposal of carcasses on the farm)	• Records showed that the disposal of carcasses did not occur on farm	• Records showed that carcasses had been disposed of on farm even for a short period of time
ENVIRONMENT (Farmyard application of manure/sewage)	• When only manure from ruminants from the farm has been applied	• Manure from external sources has been applied
ENVIRONMENT (Other potential sources of infection in the vicinity of the farm)	• No other potential sources of infection in the vicinity of the farm	• Other potential sources of infection in the vicinity of the farm

The third option (c) 'insufficient data to take a decision' has not been included.

2.2.3. FVO report analysis

Relevant data and information contained in the audit reports were extracted and a narrative report with the main conclusions was produced.

The FVO inspections focused on the collection of information covering multiple aspects, from the transposition of the EU into national legislation to the enforcement of legal obligations and the implementation of monitoring programme, as follows:

- Transposition of EU legislation into the national legislation
- Responsibilities for the application of the legislation
- Instructions from Central Competent Authority (CCA)
- Production of animal feed
- Official controls on total feed ban
- Procedures in case of non-compliance
- Approval of the manufacturing plants producing derogated processed animal protein
- Approval of feed mills using derogated processed animal protein
- Programme of official controls (monitoring and management of the sampling programme and on the spot visits)
- Laboratory network and laboratory proficiency
- Results by CCA
- Withdrawal of processed animal protein from the market
- Importation/exportation of processed animal protein including controls of the imported processed animal protein.

The audit reports resulting from inspections conducted by the FVO in MS for the period 2001–2015 to monitor different aspects of the TSE legislation have been reviewed for those MS that have had at least one BARB-60 case. The review had a twofold objective: (a) to summarise all information available regarding the implementation and the compliance of EU MS with the total the feed ban during 2001–2015; (b) and to assess whether feed contaminated with BSE agent could be excluded as a potential source of infection for the BARB-60 cases.

2.2.4. The analysis of surveillance data

The objective of this analysis was to evaluate the ability of MS to detect a case of BSE based on the power of their surveillance systems, exploring the possibility that MS is a proxy for a geographical risk factor associated with the occurrence of BARB-60 cases. This exploratory analysis has been conducted by calculating the annual detectable design prevalence for each MS and year for the period 2001–2015 (Appendix B) considering the cattle tested by all surveillance streams, on the assumption that the prevalence of BSE in cattle can be described in terms of a binomial distribution (or Poisson approximation) (SSC, 2001), and by applying the C-TSEMM model. The power of surveillance was also estimated at group level, comparing the BARB MS and the non-BARB MS using the outputs of the C-TSEMM model.

The estimation of the C-TSEMM (Adkin et al., 2012) was originally developed to evaluate the performance of different BSE monitoring regimes in cattle in the EU. For full details of the model and its assumptions, see Adkin et al. (2012). Broadly, the C-TSEMM requires that, for modelling scenarios applicable to a group of countries, it must be possible to merge them together as a unique epidemiological unit, as was considered the case with the EU-25 in the previously mentioned assessment, and as such to estimate the design prevalence that the surveillance regime can detect when applied to the entire cattle population of the unit.

The C-TSEMM uses individual MS BSE case data and the number of animals tested between 2002 and 2015. Following the assumptions described in Section 2.1.3 of Adkin et al. (2012), there are four surveillance components included in the model: animals clinically suspected of being infected by BSE, healthy slaughtered, fallen stock and emergency slaughter (including animals with clinical signs at *ante mortem* inspection.

The C-TSEMM requires annual historical information on the standing population, slaughter/death of animals in each surveillance stream, those animals which have been tested, and test results by case type (Classical BSE, Atypical H-type BSE, Atypical L-type BSE or unknown). These data are required for each MS, so when individual country estimates were not available, an EU average was used.

The data imported into the C-TSEMM only include the BSE test data according to the EU legislation in terms of surveillance stream and age thresholds for testing. Any additional data from MS that tested

beyond the EU requirements are disregarded. For example, in 2014, FR continued testing healthy cattle, slaughtered for human consumption, older than 72 months of age. For any MS implementing a testing programme that exceeds the legal requirement, the C-TSEMM model will underestimate the sensitivity of the surveillance system.

The model provides different outputs for the evaluation of alternative surveillance scenarios at either individual MS level or aggregated level. For non-BARB MS, an alternative estimate of prevalence is required by the C-TSEMM. For MS in that category, prevalence has been assumed to be the average prevalence of groups of MS with BSE cases. Three groups have been used previously by the C-TSEMM: EU-25, EU-17 and the EU-8 (for more details, see Adkin et al., 2012; EFSA, 2016).

Results are provided based on the detectable prevalence (prevalence of cases) for the adult standing population. The monitoring regime applied is the compulsory testing of emergency slaughter and fallen stock older than 48 months of age and the testing of all clinical suspect animals. Voluntary testing of animals outside these bounds is not included in the calculations. Results are expressed as 1 in X, so a result of 100,000 indicates that we would expect the current system to detect a prevalence in adult cattle (> 24 months) of 1 in 100,000. For the main results, the outputs are calculated with a confidence level $\tau = 0.95$, but to show the uncertainty surrounding these estimates, two extra τ levels have been reported: $\tau = 0.925$ and $\tau = 0.975$.

3. Assessment

3.1. Nature of BSE and hypotheses of its origin

BSE was first identified in the mid-1980s and described as a 'scrapie-like' disease of cattle, based on the distinctive spongiform lesions in the brain resembling those described for scrapie (Wells et al., 1987). Initial epidemiological investigations (Wilesmith et al., 1988) defined the disease as an extended common source epidemic in which all affected animals were index cases, and no link could be established with the usage of drugs (including bovine pituitary extracts) or agricultural chemicals, nor was it obviously inherited. This paper concluded that the epidemic was consistent with the exposure of cattle to a scrapie-like agent via cattle feedstuffs, natural cross-species transmission being ruled out do to the historical absence of sheep on many farms that reported BSE.

It is widely accepted that BSE and scrapie are prion diseases, sharing common features, in particular the presence of the abnormal protease-resistant isoform PrP^{Sc} (or prion protein) that is a pathognomonic feature of these diseases, and the target of the majority of current diagnostic methods. Abnormal protein accumulates in cells by causing the misfolding of a normal cellular protein (PrP^{C}), a fundamental component of disease pathogenesis. This has been shown by the complete resistance to infection of animals which are null for the *PRNP* gene, and it is this *PRNP* gene which underpins the genetic susceptibility to disease in a range of species. In 2000, the Phillips BSE Inquiry (Phillips et al., 2000) concluded that 'all plausible theories must accommodate a central role for PrP in TSE's, while 'theories which fail to acknowledge a place for PrP^{Sc} in the causation of TSEs remain unconvincing'.

Even at the height of the epidemic, only a small proportion of cattle on any given farm developed disease, despite being exposed to the same batches of infective feedstuffs as their cohorts (Hörnlimann et al., 2006), and it has been shown that only very small amounts of infective material (as little as 1 mg) may be needed to produce disease under experimental challenge conditions (Wells et al., 2007; Konold et al., 2012). This may suggest that other factors are needed to ensure successful infection and/or modulate host susceptibility, but the very long incubation periods seen in these diseases, measured in years, also make the retrospective study of potential origins and contributing variables very difficult. This has given rise to many and varied hypotheses about the nature and origin of BSE, while at the same time making it difficult, if not impossible, to prove or disprove them unequivocally.

Over the years, and particularly following the identification of variant Creutzfeldt–Jakob disease (CJD) in man and its link to BSE, these theories have been reviewed and discussed in great detail by a number of expert groups (Phillips et al., 2000; Horn, 2001; SSC, 2001). Theories that have been proposed, but rejected by the wider scientific community on the basis of either insufficient supporting evidence, or evidence opposing the hypotheses, include the role of organophosphates (Purdey, 1992, 1996a,b, 1998; Gordon et al., 1998), an autoimmune reaction (Ebringer et al., 2005, 2007), dysregulation of carbohydrate metabolism (Frey, 2005), a mineral imbalance in the soil affecting copper and manganese levels, (Phillips et al., 2000; Purdey, 2000, 2001; SSC, 2001) and methyl

bromide poisoning (Phillips et al., 2000). Bacterial or other toxins have been proposed (Stockdale, 1997) as have chemicals used in the rendering process (Parish and Parish, 2001, as cited in SSC, 2001).

The prion protein is now widely accepted as being the sole, or at least the primary component of the disease agent for BSE. Another theory, particularly in relation to scrapie, is that the infectious agent is a virus (Darcel, 1995; Manuelidis, 2007), particularly that it is one of the family of 'slow viruses' or a 'virino' (Schreuder, 1994; SSC, 2001) or at least that it has an independent genome, such as ssDNA associated with a protein 'coat' (Narang, 2002) or nemavirus particles (Narang, 1994) but no virus has ever been isolated or visualised. This hypothesis has been argued against by studies looking at the inactivation of TSE infectivity using radiation (Alper, 1985, 1993) which indicated that if the agent was composed of nucleic acid, it would be too small to code for even a single protein.

However, unlikely these hypotheses might be as a single cause, the potential for such factors to modulate host susceptibility in some way, possibly even transiently, cannot be fully excluded (SSC, 2001; La Bonnardière et al., 2007).

3.1.1. Cross-species transmission of sheep scrapie

The proposal that the initial infection in cattle was a result of the cross-species transmission of sheep scrapie is one of the hypotheses that has been most regularly revisited over the years (Eddy, 1995; Taylor, 1995; Narang, 2001), not least because the UK had the largest sheep and third largest cattle population in the EU, and the highest ratio of sheep to cattle (Horn, 2001), but no successful conclusion has been reached. The Horn report (2001) said that 'it was not tenable to exclude an unmodified scrapie agent in sheep being responsible for BSE' while the Philips Inquiry stated that 'scrapie agents were not responsible for BSE' (SSC, 2001). Sheep scrapie occurs as several distinct strains, and none of these strains is the same as BSE, although not all strains have been characterised in the same way, or at the same time. Sheep challenged with BSE produce a disease which is similar to, but distinct from, scrapie (Jeffrey et al., 2001; Ligios et al., 2002; Konold et al., 2008), and cattle challenged with scrapie develop a disease distinct from BSE (Cutlip et al., 1994; Konold et al., 2006, 2015), but these challenges cannot represent the full diversity of natural scrapie, and the numbers of experimental challenges are too small to allow definitive conclusions (Baron et al., 2004). Moreover, there is little or no information on whether strain characteristics can 'drift' over time, so these studies do not comprehensively represent scrapie sources contemporary with the start of the epidemic, although the UK cattle challenges were carried out with two 'pooled' sources from different time periods. It was also suggested that a new strain might arise as a 'sporadic event' that could not be predicted, or identified retrospectively (SSC, 2001). There is increasing evidence that scrapie isolates can sometimes 'mutate' following experimental transmission (Simmons et al., 2015), so an event of this nature cannot be ruled out. The thermostability of isolates is also variable (Somerville et al., 2002), and could lead to unpredictable phenotype changes, or to the sub-selection of strains with different thermostability during the rendering process.

3.1.2. Contamination of animal feed with undetected TSE from other species

Contamination of animal feed with an undetected TSE from another species (e.g. cats, goats, exotic ungulates) entering the animal feed chain has also been postulated (SSC, 2001), with the same hypothetical possibilities for modulation or mutation as a consequence of the rendering process. It has even been argued that the agent could have been present in animal feed containing mammalian raw materials contaminated with human remains from the Indian subcontinent, that were imported in large quantities during the relevant time period (Colchester and Colchester, 2005). While the methods for the preparation of calcium diphosphate can reduce TSE infectivity by several logs in spiked bone samples, they do not completely remove it (Grobben et al., 2006).

It has also been speculated (SSC, 2001) that initial feed contamination could have been from a bovine source, either a low prevalence, previously undetected naturally occurring disease of cattle, or possibly a 'spontaneous' case (e.g. as a result of genetic mutation, possibly triggered by an extraneous insult such as a toxin) but there was no evidence to support this hypothesis (Fraser, 2000). The more recent identification of atypical BSE cases (EFSA, 2014) and the experimental evidence that these distinct cattle TSE may, under some experimental transmission circumstances, 'mutate' to a strain with properties indistinguishable from the epidemic strain of BSE (Capobianco et al., 2007; Torres et al., 2011) reopens these lines of enquiry.

3.1.3. Spontaneous origin

How the conversion of the cellular PrP^C protein to the abnormal prion protein is triggered is still unknown. It has been proposed that this might be a spontaneous event (Sulkowski, 1992) in which post-translational protein becomes misfolded, or as a consequence of spontaneous (somatic) or inherited genetic mutations such as those associated with TSE in man (Weissmann, 2004; Sikorska and Liberski, 2012). Spontaneous events, by definition, will not be predictable and may not be detectable either, if they occur at the cellular level. In addition, attempting to identify such an event after an incubation period of several years is unlikely to be successful. The classification of a case as 'spontaneous' is therefore circumstantial. It occurs by a process of elimination, excluding all other definable possibilities. There is a parallel in the field of human TSE, where CJD cases that cannot be categorised as iatrogenic, familial, or linked to the consumption of infective material are considered to be 'sporadic'. This classification still accounts for 85% of all CJD cases reported each year[4].

3.2. Source of infection of the BSE agent

3.2.1. Transmission via feed

At the time of the initial work on the epidemiology of BSE, a feed-borne source was supported by the fact that changes to the rendering processes for animal-derived protein had been introduced over the previous decade (Wilesmith et al., 1991) with a move from batch to continuous rendering, and a reduction in the use of hydrocarbon solvent for the extraction of tallow. These production method changes coincided with changes in feeding practices which saw the introduction of meat-and-bone meal (MBM) into calf rations in the UK (Horn, 2001).

A subsequent case–control study (Wilesmith et al., 1992) provided evidence of a higher risk of disease in dairy herds where concentrated proprietary feed had been extensively used. Further evidence supportive of this hypothesis has been accumulated over the years (Wilesmith et al., 2000; La et al., 2004; Ducrot et al., 2005; Ru et al., 2007).

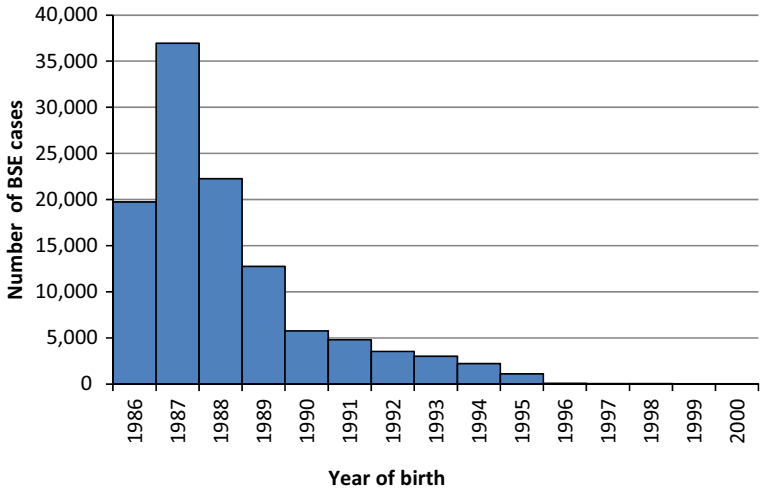
In a number of countries, milk replacer, containing extracted animal fats, has also been implicated although epidemiological and risk assessment studies (Paisley and Hostrup-Pedersen, 2004; Pottgiesser et al., 2006; Ovelhey et al., 2008; Tsutsui et al., 2008; Yoshikawa, 2008).

The bans put in place to prevent the feeding of animal protein to ruminants were very successful in controlling the epidemic (Wilesmith et al., 2010), but after peaking in 1992 the rate of decline of the epidemic in the UK was not as fast as expected. It was realised that the use of MBM or other protein in the feed of other species (e.g. pigs, poultry) facilitated accidental feed cross-contamination (Wilesmith, 1996; Stevenson et al., 2005).

In a case–control study in the Netherlands, there were meaningful differences in the level of infectivity in MBM from different origins, i.e. MBM originating within or outside the Netherlands at a time when cross-contamination was still possible. No other risk factors, either farm or cow related, were significantly associated with the occurrence of BSE (Heres et al., 2007). In France, similar studies suggested that both cross-contamination by MBM in bovine concentrates and, to a lesser extent, on-farm cross-contaminations, i.e. consumption by cattle of feedstuffs initially intended for other animals and which could legally contain MBM, have probably also existed, since the presence on farms of purchased feed for poultry increased the risk of BSE (Jarrige et al., 2007; Paul et al., 2007). Similar conclusions were reached by studies in Germany (Campe et al., 2013), and Switzerland (Schwermer and Heim, 2007; Schwermer et al., 2007).

It is widely accepted that the initial feed bans, while very effective at reducing numbers of cases (Figure 1), were not as robust as they needed to be. After the identification of the role of cross-contamination, reinforced bans were implemented in several MS until the total feed ban was implemented in the EU from 2001 (see Section 1.2). These greatly reduced the sources of infection for cattle.

[4] http://www.who.int/zoonoses/diseases/Creutzfeldt.pdf



Data from DEFRA (2005) (https://data.gov.uk/dataset/confirmed-cases-of-bse-in-gb-by-year-of-birth-where-known/resource/7c492f44-b28c-412f-85e5-85b900e42ab5)

Figure 1: Confirmed number of BSE cases in Great Britain by year of birth where known

3.2.2. Maternal transmission

During the height of the epidemic, there were no data to support the occurrence of maternal transmission in the absence of a feed-borne source (Hau and Curnow, 1996; Braun et al., 1998; Fatzer et al., 1998). Maternal transmission (defined in this instance as transmission before or immediately after birth, since there is some difficulty in separating possible horizontal and vertical components to transmission involved with the dam–offspring relationship (Simmons et al., 2008), was a theoretically possible route of transmission, and although it was not unequivocally demonstrated, there was some statistical support for it. However, it was calculated that it could not account for more than 10% (95% CI: 5–15%) of the offspring of all cases with BSE and probably less if transmission to calves occurred only if the dam was in the late stage of BSE incubation (Wilesmith et al., 1997). Doubt was cast on the statistically calculated figure of 10% for maternal transmission, as the number of cases in the cohorts at 5 years of age was low compared with the number expected (Donnelly et al., 1997). Bradley and Wilesmith (1993) reported that in no year between 1988 and 1993 (when the epidemic was at its height) did the actual incidence of BSE in the offspring of confirmed cases exceed the expected incidence of BSE from the feed-borne source alone. However, it must be remembered that in MS the offspring of BSE cases are traced and compulsorily slaughtered, which may bias the data away from evidence of maternal transmission route (SSC, 2001).

A study of possible horizontal transmission of BSE (Hoinville et al., 1995) revealed that, although there may have been an increased risk of BSE occurring in animals that were born on the same day or between 1 and 3 days after an affected animal had calved, there was no plausible mechanism for this. The use of bulls for artificial insemination was not incriminated in the occurrence of BSE cases (Bradley and Wilesmith, 1993).

Such routes of transmission could not be ruled out unequivocally during the epidemic, but it was widely acknowledged that the feed-borne route dominated the epidemiological picture and could have 'masked' smaller numbers of cases resulting from alternative transmission routes. The SSC Opinion (2001) stated that 'Any other cause than from feed or maternal transmission becomes a potential 'Third Way'. Many are theoretically possible (e.g., environmental contamination after unauthorised burial of carcasses of non-declared BSE cases) but, if existent, unlikely to have significantly contributed to the BSE epidemic. It can be concluded that in the UK most, if not all, cases of BSE can be attributed to feed exposure and the residue is resultant upon some form of imprecisely determined transmission that may not occur at all in the absence of a feed-borne source. Explaining solitary incidents of BSE whilst there is still a risk of feed exposure is unlikely to ever be possible (other than possibly for genetic causes involving the PRNP gene) since exposures would have been distant in the past'. The Philips Inquiry (2000) also supported the view that maternal transmission could account for some cases of BSE, but was uncertain of the role of environmental contamination.

3.2.3. Environmental contamination

There is no unequivocal evidence of indirect transmission of BSE as a consequence of environmental contamination, but there is precedent for environmental contamination being a significant route of transmission in both scrapie in sheep (summarised in EFSA BIOHAZ Panel, 2014) and chronic wasting disease in cervids (summarised in EFSA BIOHAZ Panel, 2017).

The assumption is that any indirect infection would arise through the consumption of infected material (other than proprietary feedstuffs), in particular the risk from grazing contaminated pastures and/or exposure to contaminated fomites within the animal accommodation, as has been demonstrated with scrapie (Hawkins et al., 2015). The amount of infectivity required to achieve oral infection, and any other potentiating host or environmental factors (such as age, general health status, other dietary factors such as mineral content, as previously discussed) that may influence this route of transmission are unknown. It has never been established whether an infectious dose can be successfully ingested through repeated exposure to very low infectivity, or whether a full infectious dose needs to be consumed at one time. It has been reported (Johnson et al., 2011) that the binding of prions to small soil particles can enhance transmission via the oral route relative to unbound prions. A report (Herlin and Andersson, 1996) noted that cattle could consume up to 1 kg of soil per day suggesting a risk might be present should an effective oral dose of the BSE agent be present.

There are several plausible routes via which such contamination might occur. Infectivity has been demonstrated in faeces from sheep and deer, and is thought to be linked to presence of PrP^{Sc} in gut-associated lymphoid tissues (GALT), and its subsequent shedding. GALT involvement has also been demonstrated in cattle with BSE (Terry et al., 2003; Wells et al., 2005; Hoffmann et al., 2011; Stack et al., 2011), but to a lesser extent than in small ruminants or deer. The relatively minor involvement of the GALT, together with the dilution, by the faeces, of any shed PrP^{Sc} would also make it unlikely that feed or forage contaminated with faeces would deliver an infectious oral dose or that this presents a major route for transmission, especially as cattle are not intentionally coprophagic. Such a conclusion is supported by the relatively low within-herd incidence of BSE in herds affected by the disease, below 3% in any 6 months period in the UK epidemic (Bradley and Wilesmith, 1993). Once shed into the environment, TSE agents have been shown to be resistant to degradation over long periods in soil (Genovesi et al., 2007; Wiggins, 2009; Smith et al., 2011). There is also evidence of environmental persistence on farm equipment such as pens and troughs, in addition to pasture (Maddison et al., 2010a).

Gale and Stanfield (2001) made a quantitative risk assessment for BSE in sewage sludge in which the main sources of uncertainty were the degree to which sludge treatment inactivates the BSE agent, whether there is a threshold dose, and the amount of central nervous system (CNS) material that enters the sewage system from abattoirs. Similar types of contamination might also be envisaged in relation to knackeries. They concluded that the dose consumed by grazing cattle is insufficient to sustain the epidemic of BSE in the UK. In another study, abattoir waste water from a facility with one positive case of BSE was estimated to contain less than $0.6–26 \times 10^{-4}$ cattle oral ID(50) per litre as a result of contamination with specified risk material tissue (Maluquer de Motes et al., 2008). However, infectivity has been shown to persist for long periods in waste water, with little reduction in infectivity in the first year, although the ability to detect PrP^{Sc} (as a proxy for infectivity) had been lost (Maluquer de Motes et al., 2012; Requena et al., 2016) or for at least 18 months (Maddison et al., 2010b). Infectivity gradually fell by one to three logs (depending on whether the medium was saline or waste water) but could still be detected after 6 years (Marin-Moreno et al., 2016) This raises the concern that any risk assessments reliant on data relating to PrP^{Sc} detection, but not infectivity, might underestimate the risk of environmental contamination. Similar observations have been made with regard to environmental contamination with scrapie, where infectivity assays proved more sensitive than *in vitro* testing for PrP^{Sc} (Konold et al., 2016).

On-farm burial of fallen cattle was not uncommon in the UK in the 1980s and early 1990s (before any systematic screening of fallen cattle for BSE), and during the foot and mouth epidemic in 2001. It can be assumed that a proportion of these animals will have been incubating BSE. The risks from the burial of BSE infected carcases or materials on farm or in licensed landfill sites are only likely to cause a potential risk of contamination via leachate. A risk assessment published by Det Norske Veritas (DNV, 1997) for the UK Environment Agency, revealed that estimates for the contamination of the water supply by leachate from licensed landfill are below any level that would be considered to be of significance. Similar conclusions were reached when estimating the risk associated with wastewater from carcase-handling facilities (Adkin et al., 2013).

The SSC opinion (2001) considered that mechanical contamination of plant leaves by prions was theoretically possible following the spreading of organic fertilisers, manure, blood, incinerator ash, sewage sludge or rendering condensate. Recent work (Xu et al., 2014) suggests that the composting of waste materials results in a reduction in detectable PrP^{Sc} (BSE) of one log within 28 days, but Pritzkow et al. (2015) have demonstrated that topically applied infectious material resulted in retention of PrP^{Sc} for several weeks in the living plant. They have also demonstrated that plants can take up prions from contaminated soil and transport them to aerial parts of the plant (stem and leaves), thereby efficiently binding prions and acting as potential carriers of infectivity.

3.2.4. Animal-to-animal transmission

Unlike some other animal TSE, in particular scrapie in small ruminants, and chronic wasting disease in cervids, BSE has never been considered to be contagious, although it is possible that a combination of the large numbers of feed-borne cases, the very long incubation period, and the dairy industry practice of segregating calves and dams very quickly, could have concealed a small number of cases resulting from direct or indirect transmission from infected animals.

3.2.5. Iatrogenic transmission

Iatrogenic transmission of scrapie through vaccines prepared from ovine material has been well documented in the past (Gordon, 1946; Bertolini et al., 2012), and CJD in man has also been transmitted via pituitary extracts (Rudge et al., 2015), donated tissues (Molesworth et al., 2014), surgical instruments (Lumley, 2008) and blood (Checchi et al., 2016), but bovine pharmaceuticals are generally prepared from bovine sources (SSC, 2001). Posterior pituitary extract was available and used in veterinary practice at the start of the BSE epidemic, but no association was found between its use and the occurrence of BSE (Wilesmith et al., 1988). The Phillips Inquiry concluded, however, that they 'could not absolutely rule out the transmission of BSE via hormones and veterinary preparations'.

3.2.6. Genetic susceptibility

There are fewer polymorphisms in the bovine *PRNP* gene than in other affected species. Apart from the absence of epidemiological data on a genetic component influencing susceptibility to BSE, there is no evidence from the molecular genetic studies that there is any connection between polymorphisms in the *PRNP* gene of cattle and the occurrence of BSE (Goldmann et al., 1991; McKenzie et al., 1992; Grobet et al., 1994; Hunter et al., 1994), although one study has identified a novel polymorphism (E211K) associated with a case of atypical BSE (Richt and Hall, 2008). In a study reported by Saunders et al. (2007), the *PRNP* gene coding regions from 100 BSE cases (born after the introduction of the reinforced feed ban in August 1996) and 66 matched healthy control cattle were sequenced to investigate whether this would reveal a genetic basis to their origin. The polymorphisms identified were not found to be associated with increased susceptibility to BSE. Modelling studies reported by Hau and Curnow (1996) concluded there was no evidence, molecular or statistical, for genetic variations in susceptibility, but more recent data indicates that there can be significant associations with susceptibility to BSE and various different promoter region indel polymorphisms and SNP (Juling et al., 2006; Kashkevich et al., 2007; Brunelle et al., 2008) and even to related genes (*doppel* and *shadoo)* (Murdoch and Murdoch, 2015).

3.3. Epidemiology of BARB-60 and other BARB cases: risk factors and current surveillance

3.3.1. Risk factors

In theory, once MBM was effectively eliminated from the rations of all farmed animals, the source of the infection for cattle should have been removed. However, cases of BSE continued to occur in animals born after the total feed ban put in place in the UK in July 1996, the reinforced feed ban in Ireland in October of the same year and the total feed ban in the rest of EU in 2001. The emergence of BARB cases in MS other than UK and IE led to renewed questions about the possible origins of disease in these animals. If it is assumed that feed cannot be implicated in their origins, the options listed in Sections 3.1 and 3.2 for the origin of BSE and risk factors are also the options for the origins of the BARB-60 cases (e.g. maternal, genetic mutation, environmental contamination). Alternatively,

feed might still be implicated; the bans may have been difficult to police absolutely, or there might have been residual feed on farm.

It is widely accepted that the initial feed bans, while very effective at reducing numbers of cases, were not as robust as they needed to be, and further reinforced bans were implemented.

There are a few studies which were specifically focused on the epidemiology of BARB cases. They have been carried out in the MS where, despite an early implementation (1996) of a total feed ban, well before the implementation of the Council Decision 2000/766/EC, a number of BSE cases (BARB) was still emerging (Wilesmith et al., 2010; Ortiz-Pelaez et al., 2012; Ryan et al., 2012). The assumption of such studies is that, if the total feed ban has been effectively enforced, these BARB cases must differ in some as yet unidentified way from the cases born before the total feed ban.

Initially, descriptive studies based on case series or on spatial analyses were carried out with the aim of formulating hypotheses on the potential sources of the BARB cases. Case–control studies have also been carried out to test the hypotheses on the role of some of the putative sources and risk factors.

A preliminary epidemiological analysis of the first 11 cases born in Great Britain after the implementation of the EU total feed ban was presented by Burke (2009). The evidence supported the continued hypothesis for a feed-borne source for these cases. This may have been due to a combination of factors including the extremely low dose of material required for oral infection, the persistence of traces of contaminated feed in feed stores and the importation of feed with trace levels of contamination.

In a letter to the Veterinary Record in relation to herds in Great Britain, Gibbens (2005) postulated that the persistence of the BSE agent inside feed bins was a possible source of infection for BARB cases. This was based on an investigation of farms which were associated with multiple BARB cases.

In the UK, Wilesmith et al. (2010) reported a reduction in the risk of infection, by three orders of magnitude, for cattle born after July 31, 1996 compared with that for cattle born earlier, with a statistically significant exponential reduction in the estimated prevalence between successive annual birth cohorts after this date. The study considered 164 BARB cases from 149 herds that were born after July 1996 and detected by the end of 2008. This case-series study used geographical information and data obtained through a questionnaire survey. The main findings were:

- the identification of herds with more than one case (e.g. 2–4) in same herd (in total 26 cases out of 164), despite an extremely low probability of such an occurrence;
- the predominance of affected dairy herds (72% of the total), compared with a general proportion of 31% of dairy herds in the UK cattle population. The same phenomenon had been observed within the BSE epidemic;
- no association with previous BSE cases reported on farm;
- no difference in the age distribution of cases compared with those born before the reinforced ban;
- no cases of BSE reported among dams, which generally (83%) survived more than 12 months after the birth of the case;
- a homogeneous risk (in terms of occurrence at population level) across Great Britain (GB), different from the distribution of the disease in animals born before 1988 and between 1988 and 1996

In their interpretation, the authors excluded environmental contamination not associated with feeding stuffs, maternal transmission, and any genetically based aetiology. An exogenous feed-borne source associated with import was suggested as a plausible explanation.

A case–control study and a spatial analysis were also carried out by Ortiz-Pelaez et al. (2012) on the UK BARB outbreak, including in the analysis 164 BARB cases and 499 controls. The risk of BARB occurrence was associated [odds ratio (OR): 2.56; 95% CI: 1.29–5.07] with the exposure to homemix or a combination of homemix and proprietary feeds; and the BARB cases were less likely to occur in holdings where prereinforced feed ban BSE cases had occurred (OR: 0.59; 95% CI: 0.50–0.69). The authors argued that this effect is biologically plausible as the sources of ingredients for home mixing may not be quality assured as thoroughly as those used for the production of commercial concentrates. Home mixing can result in the use of, and therefore exposure to, larger volumes of individual ingredients in individual diets compared with the composition of proprietary feedstuffs. The spatial analysis allowed the identification of three areas of excess BARB density (north-west and south-west of Wales and north-east of Scotland) supportive of some heterogeneity in the risk of occurrence of BARB cases.

Ryan et al. (2012) focussed on 44 BARB cases from 40 Irish herds: all the animals were born after the enforcement of a reinforced feed ban in October 1996 and detected as BSE cases by July 2010. The analysis of the case-series was combined with a case–control study. In the case-series, the feeding of concentrates was confirmed for all cases for which the information was available (33, 75%). A strong association with dairy herds (OR: 14.5; 95% CI: 5.95–35.5), was found in the case-control study. Finally, the spatial analysis applied to the distribution of the Irish BARB cases detected a 100% clustering within the same geographical hexagon.

The geographical aggregation or clustering of BARB observed in both the Irish and the UK studies, while suggesting a common underlying cause, argues against the likelihood of a spontaneous origin for the disease.

3.3.2. BSE surveillance in the EU

In terms of the absolute number of tests done (for a summary of tests done in cattle by MS and year, see Appendix B), the total number of cattle tested by the 11 BARB MS between 2001 and 2015 was 97,483,791 in order to detect the 60 BARB-60 cases. On average, 1.6 million tests have been conducted in order to detect one BARB-60 case. During the same period, the 17 non-BARB MS tested 16,345,152 cattle, which means that about 10 BARB-60 cases should have been detected by the non-BARB MS if it was assumed that the prevalence of classical BSE was uniform across the European Union. However, this effort is very variable between BARB MS. For example, on average, LU had to test only circa 169.000 cattle to detect one BARB-60 case, whereas DE had to test 11.7 million cattle to detect each of its two BARB-60 cases: the difference is potentially consistent with an heterogeneous national prevalence of BARB cases.

Amongst the non-BARB MS, DK and AT tested approx. 2.5 million cattle each and they did not detect any BARB-60 cases. An even higher testing level was achieved by BE with almost 4 million tests done during the same period but no BARB-60 cases have been ever confirmed. All the other non-BARB MS tested less than 1.6 million cattle during the same period.

The annual detectable design prevalence was estimated for each MS for the period 2001–2015 (Appendix B) using the total number of cattle tested and a binomial distribution, as a measure of the ability of a MS to detect at least one case if the prevalence was greater than the design prevalence (estimated). The design prevalence was very low in the BARB MS (high surveillance sensitivity) except for LU, although sensitivity in general has decreased in the last few years, due to the changes in the mandatory surveillance requirements resulting in a decline in the overall testing effort. Until 2013, most of the MS retained a detectable design prevalence lower than six in 1,000,000. The detectable design prevalence in the non-BARB countries was on average 10-fold higher (lower surveillance sensitivity), until 2013 where the increase in the design prevalence also occurred due to the reduction in the total number of cattle tested: a marked decrease in the surveillance sensitivity except for MS such as BE, AT and DK, that had profiles similar to the BARB MS, confirming the pattern observed in the outputs of the C-TSEMM model and in the descriptive analysis of the raw figures.

Table 4 shows the results for different design prevalence calculations that would be detected by the baseline monitoring regime in place, by MS, with different levels τ of confidence applying the C-TSEMM model. Design prevalence results are shaded where the estimated prevalence detected is less than one in 100,000. As the level of confidence is increased from $\tau = 0.925$ to $\tau = 0.975$, it can be seen from the table that the estimated design prevalence increases, reducing the sensitivity of the surveillance. To avoid the issue of low numbers of tested healthy slaughtered animals in recent years, this value is estimated by fitting an exponential curve through the historical cases.

Table 4: 'Detectable design prevalence (1 in X tested animals), achievable by the each national baseline monitoring system

Country	Detectable prevalence in standing population (1 in X)		
	τ = **0.925**	τ = **0.95**	τ = **0.975**
AT[(a)]	69,511	60,104	48,811
BE	179,634	155,322	126,139
BG[(a)]	5,148	4,451	975
CZ	20,741	17,934	14,564
CY[(a)]	5,273	4,560	3,705
DE	565,384	488,861	397,004
DK	131,628	113,814	92,430
EE	7,356	6,361	5,166
EL[(a)]	N/A	6,966	5,478
ES	185,972	160,802	130,587
FI	65,265	56,432	N/A
FR	1,416,571	1,224,843	N/A
HR	21,589	18,668	15,161
HU	13,300	11,500	9,339
IE	380,990	340,250	267,527
IT	56,354	48,727	N/A
LT	5,465	4,726	3,838
LU[(a)]	12,409	10,730	8,715
LV	4,399	3,804	3,090
MT	N/A	N/A	N/A
NL	N/A	217,912	171,337
PL	60,137	51,998	42,228
PT	N/A	36,167	N/A
RO[(a)]	38,747	33,502	5,396
SE[(a)]	59,964	51,849	42,108
SI	10,626	9,188	7,462
SK	6,172	5,337	4,334
UK	336,298	290,781	236,143

The estimates apply to the standing population in 2015. Estimates refer to τ = 0.925, τ = 0.95 and τ = 0.975 confidence levels'. Blue shading represents those MS with a design prevalence lower than 1/100,000. N/A signifies that the model has failed to find viable value. Codes of the BARB MS are given in red font.
(a): Prevalence proxy of EU-17 or EU-25 used.

DK and BE were the only non-BARB MS with a design prevalence (at the τ = 0.95 confidence level) in the standing population lower than 1/100,000 in 2015. Six out of the 11 BARB MS (DE, ES, FR, IE, the NL and the UK) achieved in 2015 a design prevalence in the standing population lower than 1/100,000, with the other five (the CZ, IT, LU, PL and PT) not reaching that level. Being a BARB MS is significantly associated to detectable design prevalence lower than 1 in 100,000 in 2015 (Fisher's exact test. P = 0.005). The odds of being a BARB MS is 17.9 times higher if the MS had a surveillance with a detectable prevalence lower than 1 in 100,000 in 2015 (95% CI: 1.6–984.4).

Limiting the analysis to classical BSE and those early untyped cases which are most likely to be classical cases, the power of surveillance in the EU-28 is 1 in 3,435,589 at the 95th level of confidence when considering MS as separate epidemiological units. Dividing the estimate into the two groups of interest, the power of surveillance in the 17 non-BARB-60 MS to detect BSE cases is much lower (1 in 546,584) than that in the 11 BARB-60 MS (1 in 2,889,005), and corresponds to 15.9% of the total power of surveillance for EU-28, as compared to the 84.1% of the power held by the 11 BARB-60 MS.

The different number of tests necessary to detect such a case suggests different levels of occurrence of residual BSE infections at national level after 2001.

Such a heterogeneous occurrence could be accounted for by the existence of geographically associated risk factors with a heterogeneous distribution within Europe. The lack of detection in

countries where the surveillance sensitivity was particularly high argues against the presence of sporadic disease (similar to sporadic CJD in man, or atypical BSE or scrapie in cattle and sheep) where a more consistent disease prevalence would be expected across MS.

3.4. Description of the BSE BARB-60 cases. The questionnaire survey

The sixty BSE BARB-60 cases included in the mandate were reported by 11 MS (see list of cases in Section 1.1).

In general, the incidence of the BARB-60 cases seems to follow a similar pattern to the overall BSE epidemic: the number of cases detected per year and the number of MS reporting cases have decreased over time. The first year in which BARB-60 cases were reported was 2005, with 11 cases in seven MS. The subsequent evolution of cases and involved MS is shown in Figures 2 and 3.

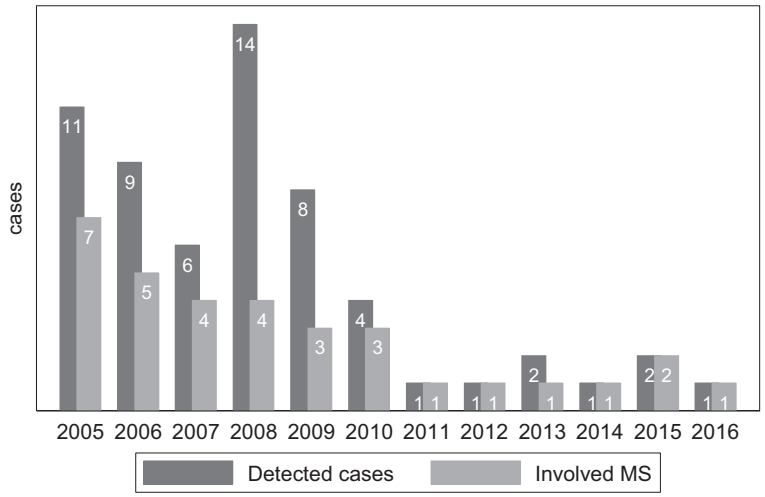


Figure 2: Number of BARB-60 cases and number of involved MS per year of detection

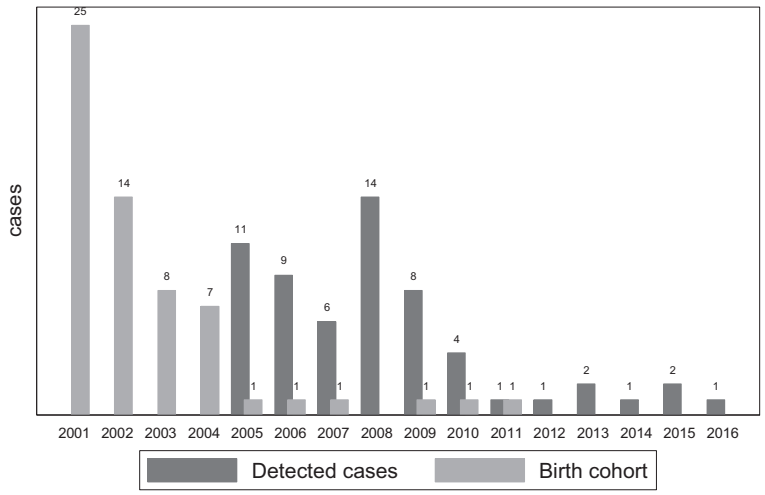


Figure 3: Number of BARB-60 cases per year of detection and year of birth

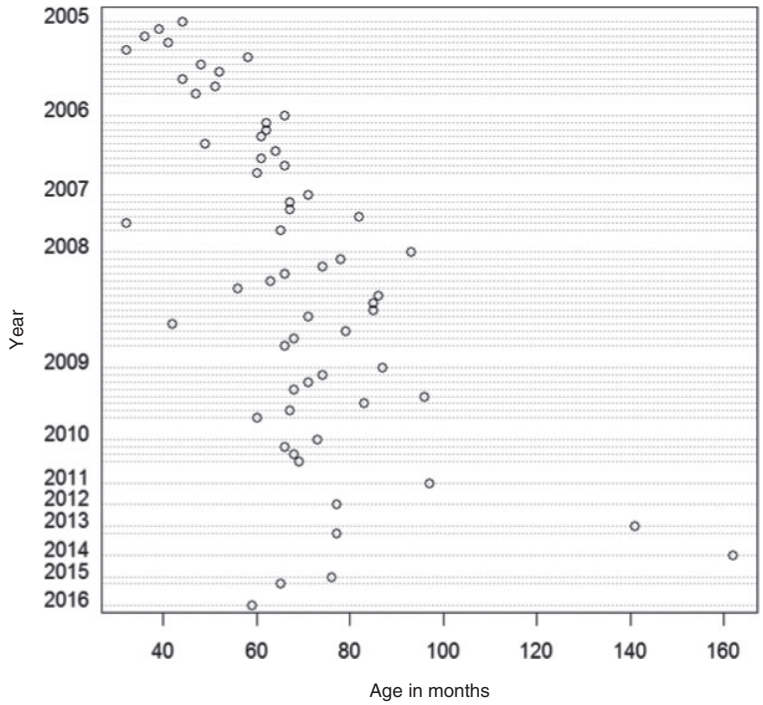


Figure 4: Distribution of BSE BARB-60 cases by year of detection and age in months

A total of 25 cases (41.6%) were born in 2001, the first year after the entry into force of the total feed ban in most of the EU MS. A further 14 cases were born in 2002 (23.3%), eight in 2003 (13.3%), seven in 2004 (11.6%), and the remaining six cases were born between 2005 and 2011 (Figure 3).

In terms of surveillance stream, 36 cases were detected within the 'fallen stock' population (60%), followed by the 'healthy slaughtered' with 11 cases (18.3%), five cases in the 'suspects subject to laboratory examination' category, four in the 'eradication measures' category, and two each in the 'emergency slaughter' and the 'clinical signs at ante-mortem inspection' routes

The youngest BARB-60 cases were two animals confirmed with BSE at the age of 32 months (2.6 years), in PT and PL, respectively. The oldest was an animal confirmed in the UK in 2014 at the age of 162 months (13.5 years). The median age of the BSE BARB-60 cases was 66 months (5.5 years) (Figure 4).

All 11 BARB MS participated in the survey, covering all the BARB-60 cases. Six MS sent additional data in the form of case reports, investigation forms or national risk assessments for a total of 44 cases (73.3%), as follows: three cases from FR, 10 cases from IE, one case from IT, one case from LU, one case from NL and 28 cases from the UK.

All participating MS collected epidemiological data on the cases and tried to ascertain the origin of the exposure to the BSE agent. Ten of the 11 MS have a standard protocol including field visits to farm/s and/or other premises as part of the epidemiological investigation of the cases. In seven of the MS, the outputs of the investigations were collected through a standard form for internal use only, with no formal report produced. In the other four MS, they are confidential reports with restricted access.

It is important to emphasise the intrinsic difficulties of conducting epidemiological investigations of anamnestic data such as that of the BARB-60 cases. First, it is impossible in many cases to identify and question herd managers on feeding and waste management practices implemented 10 or more years before the case had been confirmed (Ortiz-Pelaez et al., 2012). Secondly, recall bias calls into question the accuracy and reliability of the data and information gathered. Thirdly, missing data are common in this type of investigations. This difficulty is reflected in the number of indicators of potential source of infection for which the category 'insufficient data to take a decision' (c) was assigned in the analysis of the questionnaires and case reports. It is also difficult to validate the data collected, except for the records of tested cattle for BSE in the herd of interest.

Based on the data available, i.e. the results of the investigation by the MS, the answers to the questionnaire and the additional information provided in the case reports and additional documentation, which are in effect a case-series without a control group, it was not possible to draw

conclusions on the indicators of potential source of infection for individual cases. The role of any specific indicator of potential source of origin investigated by the MS could not be epidemiologically/ statistically tested as no information for suitable controls was available.

Based on the decision rules set out in Section 2.2.2, the results of the assessment of the data available from the questionnaires are presented in Appendix C and described in the following subsections. The summary of the results of the application of the decision rules on the 60 BARB-60 cases included in the mandate are shown in Table 5.

3.4.1. Feeding

A lack of exposure to proprietary concentrates (i.e. 'the presence of the risk factor was not supported by the data') has been reported only in one case. It was a suckler cow born in 2002 in the UK and detected in 2009 within the 'emergency slaughter' surveillance stream (National case number: 000900030). According to the data available, this animal had not been fed with proprietary concentrates, milk replacer or feed intended for other species, but only with home-grown feedstuffs in the two holdings of residence during the first year of life. However, for the reasons above mentioned, the uncertainty associated to this information cannot be underestimated.

In all other cases, the exposure to proprietary concentrates as a source of infection 'cannot be excluded'. Thirteen cases were born within 2 years of the enforcement of the total feed ban (nine in the first year and four in the second one). The only available information for the final case is that it was born three years after the total feed ban, from a cow that subsequently tested negative for BSE.

The use of milk replacer 'cannot be excluded' in only seven cases (born between 2001 and 2004). Data were not available for 32 cases whereas for 21 cases, the presence of the risk factor was 'not supported by the data'. The possibility of exposure to contaminated feed intended for other species was 'not supported by the data' in more than half of the cases (32, 53.5%); it 'cannot be excluded' for five cases and in the remaining cases no data were available.

3.4.2. Maternal transmission

Based on the decision rules, a potential for maternal transmission is 'not supported by the data' in 26 cases (43.3%), whereas it 'cannot be excluded' for six cases (10%) and data were not available, or were considered insufficient for 28 cases (46.7%).

3.4.3. Environmental contamination

With regard to the existence of previous BSE cases on the same farm, explicit data were made available for 45 BARB-60 cases: this risk factor is 'not supported by the data' in 32 cases and 'cannot be excluded' in 13 cases, respectively.

For some of the other hypotheses considered in the review, such as the environmental contamination due to waste materials from abattoir, the investigation did not reveal any relevant data that would prevent/consider them to be ruled out or not. They were mainly categorised for all cases as 'not enough data available': this holds, e.g. for farmyard application of manure or sewage sludge (no data in 58 cases) or carcass disposal on farm (no data in 49 cases).

Other potential sources of environmental contamination were not identified, apart from evidence of the proximity of a knackery near the farms where three cases were confirmed in IE.

The results of the application of the decision rules to each of the BARB-60 are displayed in Table 5.

Table 5: Summary of the results of the application of the decision rules on the BARB-60 cases included in the mandate

Indicators of source	(a) Presence of risk factor not supported by data	(b) Presence of risk factor cannot be excluded	(c) insufficient data to take a decision	Total
FEEDING (Milk replacer)	21	7	32	60
FEEDING (Proprietary concentrates)	1	45	14	60
FEEDING (Other species on the farm)	32	5	23	60
MATERNAL TRANSMISSION	26	6	28	60
ENVIRONMENT (BSE on the farm before)	32	13	15	60
ENVIRONMENT (Disposal of carcasses on the farm)	5	6	49	60
ENVIRONMENT (Farmyard application of manure/sewage)	2	0	58	60
ENVIRONMENT (Other potential sources of infection in the vicinity of the farm)	0	3	57	60

3.4.4. Other potential sources

Genotype data was available for 15 of the 28 UK BARB-60 cases. These represented seven breeds (including cross breeds), with both dairy and beef breeds represented. In these cases, data were provided on the open reading frame (ORF), and also the PrP promoter indel (23 bp) and the intron 1 indel (12 bp).

Twelve animals had an octapeptide repeat profile of 6:6, two were 6:5 and a single animal was 6:7. ORF polymorphism was present at codon 78 (seven animals were heterozygous and one was homozygous for Q), codon 113, where one animal was heterozygous for P, and codon 192, where four animals were heterozygous for N. At the 12 bp promoter indel, four animals were homozygous for the deletion, and one for the insertion, while the remaining nine animals were heterozygous, and there was a single animal for which data was unobtainable. At the 23 bp indel, a similar spread of outcomes was observed, with six animals homozygous for the deletion, one for the insertion, and the remaining nine animals were heterozygous. Individual animal data are shown in Table 6. In summary, there was no indication of any consistent genetic feature in these animals that could be linked to the presence of disease.

Table 6: Individual genetic data for 15 UK BARB cases

Year of birth	Breed	ORF					23 bp Promoter indel	12 bp Intron 1 indel
		Octa-peptide repeat	23	78	113	192		
2001	Not available	6:6				N	–/–	–/–
2001	Friesian	6:6		Q		N	+/–	Fail
2002	Limousin ×	6:6					–/–	–/–
2002	Belgian Blue ×	6:6		Q		N	+/–	+/–
2002	Not available	6:6		Q			+/–	+/–
2002	Hereford ×	6:5					–/–	+/–
2002	Charolais ×	6:7		Q			–/–	–/–
2003	South Devon	6:6				N	+/–	+/–
2003	Hereford ×	6:6		Q			+/–	+/–
2003	Limousin	6:6		Q			+/–	+/–
2004	Friesian × Holstein	6:6		QQ			+/+	+/+
2004	Aberdeen Angus ×	6:6			P		–/–	–/–

Year of birth	Breed	ORF					23 bp Promoter indel	12 bp Intron 1 indel
		Octa-peptide repeat	23	78	113	192		
2006	Hereford	6:5		Q			–/+	–/+
2007	Hereford ×	6:5					–/–	+/–
2009	Holstein ×	6:6					+/–	+/–

ORF: open reading frame.

With regard to the possibility of an iatrogenic origin all cases were categorised as 'insufficient data to take a decision', since there were no specific records based on, for example, surgical interference or medicine usage on those particular animals.

3.5. Traceability of ingredients used in the production/mixing of livestock feed

According to the 2015/2016 annual report by the European Feed Manufacturers' Federation (FEFAC)[5], 480 million tonnes of livestock feed were sourced in the EU-28 (FEFAC – DG Agriculture). Of this, 59% comprised forages and home-grown cereals, 10% are purchased feed materials and 30% are industrial compound feed. In terms of production, DE is the leading compound feed producer with 23.3 million tonnes of industrial compound feed equating to 15% of the total 158 million tonnes produced in the EU-28, followed very closely by ES (15% of EU-28 total), FR (13%), UK (10%) and NL (9%). Together with PL and IT, these five MS are the countries with the largest cattle population in the EU, and all of them are BARB MS. Direct imports from third countries of industrial compound feed are limited in the EU-28 to 100,000–200,000 tonnes per year; all species combined. However, a variety of feeding stuffs, destined as animal feed ingredients, are imported each year from third countries, totalling approximately 32.3 million tonnes in 2015 imported into the EU-28. This does not include the volume of as cereals or dairy products materials imported from third countries, as it is difficult to differentiate food from feed destinations in commodity tracking systems.

According to the original piece of legislation related to the total feed ban, the Commission Decision 2001/9/EC,[6] 'the Member States shall carry out documentary checks and tests on feed materials and compound feeding stuffs throughout the production and distribution chain to ensure compliance with the provisions of this Decision and Decision 2000/766/EC. These checks and tests shall be carried out, inter alia, in farms in which ruminants are kept with other animal species'.

According to point 2.c Section B, Chapter III, Annex IV of the Regulation (EC) No 999/2001, 'regular sampling and analysis of the compound feed intended for ruminants must be carried out in order to verify the absence of unauthorised constituents of animal origin using the methods of analysis for the determination of constituents of animal origin for the control of feed set out in Annex VI to Commission Regulation (EC) No 152/2009; the frequency of sampling and analysis shall be determined on the basis of a risk assessment carried out by the operator as part of its procedures based on hazard analysis and critical control points (HACCP) principles; the results of such sampling and analysis shall be kept available to the competent authority for a period of at least five years'.

The Commission Recommendation 2005/925/EC[7] lays down rules for the implementation of the monitoring programme for the total feed ban. According to Annex III, in order to ensure that the ban on feeding processed animal protein to certain animals, as laid down in Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council (2), is effectively applied, Member States should implement a specific control programme based on targeted controls. In accordance with Article 3 of Regulation (EC) No 882/2004[8], that control programme 'should be based on a risk-based strategy where all stages of production and all types of premises where feed is produced, handled and administered are included. Member States should pay special attention to the definition of criteria that

[5] http://www.fefac.eu/files/69455.pdf
[6] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (OJ L 147, 31.5.2001, p.1).
[7] Commission Recommendation of 14 December 2005 on the coordinated inspection programme in the field of animal nutrition for the year 2006 inaccordance with Council Directive 95/53/EC.
[8] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

can be related to a risk. The weighting given to each criterion should be proportional to the risk. The inspection frequency and the number of samples analysed in the premises should be in correlation to the sum of weightings allocated to those premises'.

According to the Commission Recommendation, 'as an alternative to these indicative premises and criteria, Member States may forward their own risk assessment to the Commission before 31 March 2006. Sampling should be targeted on batches or events where cross-contamination with prohibited processed proteins is most likely (first batch after the transport of feeding stuffs containing animal protein prohibited in this batch, technical problems or changes in production lines, changes in storage bunkers or silos for bulk material). Controls could also be extended to the analysis of dust in vehicles, manufacturing equipment and storage areas'.

The Commission Recommendation states that the minimum number of inspections per year in a MS should be 10 per 100,000 tonnes of compound feed produced and the minimum number of official samples per year in a MS should be 20 per 100,000 tonnes of compound feed produced.

The hypothesis of the feed source for the BARB-60 cases has been articulated into two possible routes of introduction into the feed chain: the persistence of traces of residual feed contaminated with the BSE agent in feed stores from the pre-feed ban time, for example inside feed bins (Gibbens, 2005; Burke, 2009) and the importation of compound feed or feed ingredients with trace levels of contamination (Wilesmith, 2002). In either of the two proposed scenarios, the amount of infected material expected to be present in any feed source or feed compound would be minimal, but sufficient to cause infection, since the extremely low dose of material required for oral infection is well documented (Konold et al., 2012). The infectivity, if present, is likely to be concentrated in small amount/s of feedstuff in the form of lumps of feedstuff containing protein of animal origin for the former and in traces due to cross-contamination during mixing, storage or transport in the case of the latter.

Despite the large number of safeguards for the manufacturing of feed, the persistence of infectivity in pockets of residual contamination, particularly chunks where historically contaminated material was stored and handled cannot be excluded. The nature of the feed commodities, the mixing, the transportation of batches and the time order intrinsic to manufacturing, processing and production processes will inevitably result in significant focal aggregation of contaminants ('clumpiness') that is likely to be missed in sampling programmes (Paoletti and Esbensen, 2015).

If these scenarios are plausible, some reflections on the monitoring system are pertinent. The importance of the role of sampling to ensure representativeness in food and feed materials collected for testing in hazard identification has been acknowledged elsewhere (Kuiper and Paoletti, 2015). The Commission Recommendation 2005/925/EC includes a thorough set of criteria to define a control programme for the monitoring of the ban on feeding processed animal protein to certain animals. It should be based on a risk-based strategy where all stages of production and all types of premises where feed is produced, handled and administered are included. MS should define the number of samples weighted according to the risk assessed in each criterion and type of premises: feed mills, border inspection posts, farms, dealers, mobile mixers and means of transportation.

Since 2001, the EU legislation on production, storage and use of processed animal protein/s has changed over time, based on the increase in knowledge about TSEs and on the epidemiological situation within EU. During the first FVO missions, when mentioning processed animal proteins, the FVO inspection teams referred to a definition based on Commission Decision 2000/766/EC.[9] This Decision defines 'processed animal proteins' as meat-and-bone meal, meat meal, bone meal, blood meal, dried plasma and other blood products, hydrolysed proteins, hoof meal, horn meal, poultry offal meal, feather meal, dry greaves, fishmeal, dicalcium phosphate, gelatine and any other similar products including mixtures, feeding stuffs, feed additives and premixtures, containing these products.

When the Animal by-products Regulation[10] entered into force in 2002, processed animal protein was defined as feed material entirely produced from category 3 animal by-products, i.e. low risk animal by-products such as those that are produced at an abattoir and are fit for human consumption but are not used for human consumption for commercial or other reasons.

[9] Council Decision of 4 December 2000 concerning certain protection measures with regard to transmissible spongiform encephalopathies and the feeding of animal protein (2000/766/EC).

[10] Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002. Regulation (EC) No 1069/2009 was implemented by Regulation (EC) No 142/2011.

Given the size of the sampling frame (480 million tonnes of livestock feed) from which samples are to be taken, the heterogeneity of the distribution of the processed animal protein in sampling material can be expected to be very large for the reasons above explained. Despite targeting sampling in high-risk instances (previous history, or suspicion of non-compliance, use of derogated processed animal protein, high throughput, etc.) of indicative premises, the representativeness of the material properties in the samples taken with the current protocols for food and feed materials cannot be ensured.

A large number of feed samples are tested in the EU (approx. 100,000). However, in the context of the huge amount of ingredients used for the production of livestock feed and despite the high analytical sensitivity of the laboratory methods in place (not so high in the early years. See Section 3.6), the sensitivity of the control programme in place for the monitoring of the ban on feeding processed animal protein to certain animals is expected to be limited. There is no centralised data collection with regard to feed testing, so information on the actual number of samples tested and the proportions that have been found positive is not available. However, the feed testing EURL still receives test-positive samples for diagnostic second opinion each year, as summarised in their annual reports.[11]

Another factor to consider is that during the period of concern, after January 2001, there were deficiencies in the control programmes of testing of feed materials, as the FVO teams highlighted in multiple audit reports (see Section 3.6). Examples include the lack of supporting documentation with evidence of the tracing, recall and safe disposal of all contaminated feed with processed animal proteins from the last positive sample of ruminant feed identified, the lack of targeting high-risk steps in the feed chain, insufficient coverage of the feed chain or limited implementation of the sampling programme.

The issues described above are not exclusive to the detection of processed animal proteins in feed samples for the monitoring of the feed ban, but are symptomatic of a more general problem that affects the reliability of sampling procedures for agricultural food and feed commodities in general, including the monitoring of the presence of food/feed contaminants, additives, naturally occurring toxins/anti-nutrients, or contaminating microorganisms, and whole foods/feed derived from GM plants/animals (Paoletti, 2017).

3.6. Compliance with the feed ban. The FVO reports

The conclusions of the review of the FVO audit reports regarding the BARB-60 MS showed that, in general, there was an overall effort to comply with the TSE legislation with regard to the enforcement of the total feed ban, in particular to Commission Implementing Decision 2001/9/EC and Council Decision 2000/766/EC. More specifically the implementation in the eleven BARB-60 MS was in general satisfactory. The deficiencies observed by the FVO teams in the early years of the implementation were progressively overcome by measures applied by MS. Some MS had already applied a total feed ban prior to January 2001 (see Section 1.2) which allowed them to have a system in place at the time the total feed ban was enforced.

However, it is important to note that during the first years of implementation there were a larger number of deficiencies reported that could have compromised the feed ban, and that increased the risk that contaminated processed animal proteins (PAP) could have entered the feed chain.

After the first year of the implementation of the total feed ban, progress was observed with respect to deficiencies detected in the 2001 inspections that involved the majority of the BARB MS, with the exception of countries I[12] and M. There was only one exception, in which multiple deficiencies were also identified in the following years. In fact, the national control programmes of this MS were not yet able to demonstrate a fully satisfactory level of compliance with the total feed ban due to a limited official inspection and sampling programme for the feed chain up to 2008.

Nevertheless, deficiencies in other MS continued to be identified by FVO auditors in subsequent years that could have led to the risk of contaminated processed animal protein entering the feed chain not being completely eliminated, as described below.

Some of these deficiencies were related to the logistics and arrangements by the CA required to implement the ban, which was a demanding operation requiring interaction and collaboration between multiple contractors and stakeholders. A lack of coordination between authorities and different departments was reported for different MS and years (F) as well as the unequal implementation of the

[11] http://eurl.craw.eu/index.php?page=162
[12] Names of the entire or parts of the BARB MS subject to the FVO inspections have been replaced by letters

ban among the different regions within the same MS (C, G, H). Significant delays in the transposition of certain aspects of the legislation like the controls in importation of processed animal proteins, the withdrawal of processed animal proteins produced before 1 January 2001 (G and F), and the authorisation and registration of establishments using fishmeal (E) were highlighted in some audit reports.

The monitoring programme for the feed ban has, as a strong component, the design and implementation of an appropriate risk-based sampling programme for the monitoring of the presence of processed animal proteins along the feed chain. The lack of targeting of high-risk steps in the feed chain (feed mills and intermediate contractors) (G), insufficient coverage of the feed chain (I), limited implementation of the sampling programme (F) and deficiencies in the documentation, protocols, information technology (IT) systems, harmonisation and co-operation among responsible services (G) were all highlighted in early audits.

Other important aspects that hampered the monitoring of compliance with the feed ban in the early years were the issues related to the analysis of the feed samples. The FVO teams highlighted shortcomings in the proficiency of official laboratories (G), the verification of the laboratory network (H), the use of an insufficiently sensitive test, with a detection limit not allowed by the legislation (E, F), the delay in reporting the results (J) of up to 6 weeks (B), leading to the fact that sampled feedstuffs would have been consumed before inconclusive or positive results were obtained (H) preventing any follow up action in case of detection of a positive sample (B). In subsequent years, there were shortcomings in the performance and supervision of laboratories (H, 2004), and a lack of audits in the diagnostic laboratories (D, 2005), despite the significant progress in the laboratory testing performed within the framework of the feed ban controls.

Some breaches directly posed a risk of introduction of contaminated processed animal proteins into the feed chain. The most immediate one was the delay in the implementation of the feed ban (F, K) allowing the introduction of processed animal proteins in non-ruminant feed without any conditions, and increasing the risk of cross-contamination. For example, animal protein (including MBM) was still being found relatively frequently (approximately 4% of samples) in 2001 in G. In 2002, there was still a high proportion of cross-contamination and feedstuffs, displaying a persistent source of contamination (J). The degree of contamination of ruminant feeds with prohibited processed animal proteins was unclear in 2002 in A. In cases of positive feed samples for MBM, the delay in delivering laboratory results continued in 2002 (J, H), and the lack of proper action after confirmation of a positive result was also reported in 2002 (L), or insufficient efforts to fully avoid cross-contamination of ruminant feed with processed animal proteins (B, 2003). Difficulty in tracing the origin of contamination detected in feedstuffs was still observed in 2006 (G).

The separation of production lines for processed animal protein (ruminant feed) and fishmeal (mixed) was not always ensured or assessed in the early years (C, D, L). Equally the methods for the transition of plants that were using MBM before the ban were unclear. In several MS at that time the risk-based sampling programme for monitoring the presence of processed animal protein was not clearly implemented or was poorly designed, not covering the whole feed chain. A lack of supporting documentation with evidence of the tracing, recall and safe disposal of all contaminated feed with processed animal protein from the last positive sample of ruminant feed identified the previous year was also observed (G).

Deficiencies related to the transport of processed animal protein and other products were also observed in later years as well, for example, the lack of protocols for the cleaning and disinfection of vehicles used for the transportation of feeding stuffs and containing fishmeal and other products (D). Moreover, the limited attention to the measures implemented by the business operators, in particular in relation to the sourcing of ABP/processed animal protein and their transport was also reported. These limited the capability of the CA to ensure that processed animal protein destined for aquaculture did not contain proteins of ruminant origin (C, 2014), or to effectively monitor the risk of cross-contamination during transport (G, 2015). Accidental cross-species feeding, using fishmeal, in premises keeping ruminants and non-ruminants was also identified as a potential risk (L, H).

During the first years of the feed ban, and according to Annex IV of the TSE Regulation, certain processed animal protein was authorised to be fed to non-ruminant farmed animals, by way of derogations. For example, fishmeal could be fed to monogastric animals under certain conditions. This made it difficult for fishmeal to be tracked to authorised feed mills or stores, with full documentation, thereby posing an increasing risk of cross-contamination of ruminant feed with fishmeal. The inspection at ports on checks of importation of processed animal protein from third countries was not always fully operational when the ban was first implemented (G and C). Deficiencies and

non-compliances with the implementation of the derogations continued during the subsequent years (G), with the importation of fishmeal (C, A), the discrimination of samples from ruminants and monogastric species, and in the correct separation in livestock farms keeping ruminant and non-ruminants and using fishmeal (E).

Similar findings were reported in MS joining the EU in later years. For example, a lack of systematic supervision of the implementation of the planned and additional controls for the feed ban, and delays in taking action in the event that non-authorised processed animal protein was found in feed, were noted one year after accession in I. The number of samples where breaches of the feed ban were detected demonstrates that official controls could not always ensure compliance with the feed ban (I, 2011) The low sensitivity of the analytical method applied by the laboratories and the unequal implementation of the feed ban in regions of M were conclusions of the first audit conducted in this MS in 2007.

In later years, the objectives of the FVO audit missions shifted following changes in the legislation and new identified risks: for instance, the use of animal proteins authorised for the use in organic fertilisers and soil improvers (OF/SI) became an important component of the audits from 2005 onwards. These materials of animal origin include organic manure, non-mineralised guano, digestive tract content, compost and digestion residues. According to Commission Regulation (EU) 142/2011, where OF/SI have been manufactured from MBM derived from Category 2 material or material derived from Category 3 processed animal protein, a mixing component, such as an inorganic or an indigestible substance, should be added in order to prevent their direct or accidental use for feeding purposes. The misuse of OF/SI containing processed animal protein could have resulted in cattle having direct access to the BSE agent. In this regard, MBM was frequently used, as such, as OF/SI without being marked or adequately transformed to reduce its palatability to animals and there was limited awareness amongst competent authorities of the numerous farms using OF/SI: two findings in 2008 and 2011 (F). OF/SI should be prevented from being fed by livestock in the case of arable farms associated to a livestock operation (A, 2009). The inclusion of samples of OF/SI in the control programme for the monitoring of the feed ban was a recommendation to M and G in 2010. In fact, between 2006 and 2009 in Country M, MBM was detected in fishmeal (seven findings), in pig feed (one finding) due to the contamination by OF/SI used on the farm, and in a consignment of dried poultry blood (one finding).

3.7. The rate of the BSE epidemic decay and the spontaneous hypothesis

Historically, using the exponential distribution to model the trend in BSE cases by birth cohort has been an appropriate choice at the tail end of the epidemic. While other distributions could be fitted, analysis of alternative distributions within the C-TSEMM indicated that an exponential decay of prevalence was still appropriate for the majority of European data at the MS and aggregated EU-25 level for BSE cases classified as classical and unknown (Adkin et al., 2016).

More recently, separate trend analysis of the observed decrease in BARB classical cases in the UK, Ireland, and other EU countries also suggested that the choice of an exponential model fit was appropriate (Arnold et al., 2017). In this analysis, MS with fewer than 10 cases were grouped together as 'Other EU' and consisted of DE, ES, FR, IT, LU, the NL and PT. Based on the birth cohorts, the trend analysis covered the time period from 1 January 1997 for the UK and IE, and 1 January 2001 for the Other-EU, up to 31 March 2016. PL and the CZ, which had BARB cases, were not included in the analysis as they did not implement the EU wide surveillance and control measures until joining the EU in May 2004.

Back-calculation models were fitted to the infection prevalence by birth cohort for each of the three groups (the UK, IE and Other EU). The declining exponential trend was compared between groups using a likelihood ratio test, which showed that there was no significant difference in the rate of decline of BSE prevalence between the groups with a P-value of 0.12 (where a P-value of less than 0.05 was considered the threshold of a significant difference). The rate of decline was estimated to be 33.9% per annum (95% CI: 30.9–37%) in successive annual birth cohorts, across the EU. The authors suggest that this demonstrates that the control measures have been equally effective across the EU. This analysis also indicated, based on a large subset of the UK and Irish BARB cases (from 1 January 1997) but restricted subset of Other-EU (excluded Polish and Czech cases), that there was no significant improvement in model fit when adding in a constant element (mimicking the existence of a constant rate of sporadic cases within the cattle population) to the trend equation fitted. That means

that the best fitting model was one where the birth cohort prevalence was ultimately declining to zero for all the MS groups.

The exponential decay is consistent with a progressive decay of the exposure to relevant risk factors, and argues against a spontaneous origin of BARB cases.

3.8. Uncertainty

In the course of the development of the risk assessment, the main sources of uncertainty identified were:

a) the origin of the first case of BSE;
b) the global distribution of BSE;
c) the presence of infectivity (BSE agent) in the feed chain in the EU;
d) the quality and accuracy of data collected during investigations of BARB-60 cases;
e) the role of other potential transmission sources (maternal, environmental, etc.) in the BSE epidemic;
f) the persistence of infectivity (BSE agent) in the environment;
g) the possibility that a disease can be truly spontaneous.

Due to the nature of the questions included in the mandate and the difficulty to provide conclusive scientific evidence to exclude any potential source of BARB-60 cases. It has been clearly stated in the answers to the ToRs whenever uncertainty has affected the assessment. Neither overall assessment of uncertainty nor specific quantification has been performed.

4. Answers to the ToRs

4.1. Answer to ToR1

Using the limited qualitative data provided for the individual BARB-60 cases by the CA, and all other gathered evidence, a number of biologically plausible potential sources of infections (feed, maternal, environmental, genetic, iatrogenic) have been considered.

The source of infection cannot be ascertained at individual level for any BSE case, including these BARB-60 cases. Thus, the uncertainty remains high about the origin of disease in each of these animals.

However, compared with the other potential sources of infection, feed-borne exposure (i.e. associated to proprietary concentrates, milk replacers, or cross-contamination with feedstuffs intended for other species on the farm) is the most likely source of infection. This conclusion has a low[13] uncertainty and is supported by the following:

- For all but one of the BARB-60 cases feed could not be excluded as a potential source of disease.
- Previous epidemiological investigations at population level, looking at a larger set of BARB cases confirmed in the UK and in IE that included most of the BARB-60 cases of these two MS, concluded that feed was a significant risk factor. Some evidence within these two MS of a geographically associated risk or a spatial correlation/clustering of BARB cases was also identified. This supports a common source for at least some of these cases, consistent with a feed source.
- The heterogeneous occurrence of BARB-60 cases between MS could be due to the differences in the exposure to as yet unidentified geographically associated risk factors, whether they are feed-related or not, or affected by differences in the sensitivity of surveillance.
- TSE agents are known to remain biologically active for many years. If undetected, the persistence of infectivity in pockets of residual contamination, where historically contaminated material was stored and handled, and the importation of compound feed or feed ingredients with trace levels of contamination could result in ongoing exposure.
- Despite the large number of feed samples tested in the EU and the high analytical sensitivity of the tests in place, in the context of the huge volumes of ingredients used for the production of

[13] The qualification of low uncertainty was considered equivalent to a 'likely' probability of feed being the source of infection (66–90%), according to the scale proposed by this Guidance for harmonised use in EFSA to express the probability of uncertain outcomes (EFSA Scientific Committee, 2016. Revised draft Guidance on uncertainty in EFSA scientific assessment. DRAFT. Available online: https://www.efsa.europa.eu/sites/default/files/160321DraftGDUncertaintyInScientificAssessment.pdf

livestock feed, the feed surveillance system has limited sensitivity for the detection of low levels of contaminated material. Infectivity, if present, is likely to be concentrated in small amounts in the form of lumps of infected material.

- There was an overall effort to comply with the TSE legislation with regard to the enforcement of the total feed ban in MS. Over time, the deficiencies observed by the FVO teams in the early years of the implementation were progressively overcome by measures applied by MS. However, contaminated material was still present in the EU after the total feed ban, as documented in the FVO audit reports. Within the national feed audits, animal protein has been detected in feed samples after 2001.
- The exponential decay of the BARB cases, as indicated by the applied mathematical model, is consistent with a EU-wide single epidemic declining to zero.

4.2. Answer to ToR2

- In the investigation of the CA, feeding with material contaminated with the BSE agent was excluded for one case, reported by the UK in 2009. If the farmers' recall and the questionnaire are accurate, and this contains all possible information about feed in the two premises in which this animal resided, only home-grown feed was reported to be used during the first three years of its life. No supporting evidence was identified for any of the other potential sources of infection that could be investigated retrospectively.
- There is considerable uncertainty associated with the data collected through the investigation of the BARB-60 cases. This is due to factors such as the time span of several years between the potential exposure of the animal and the confirmation of disease, recall difficulty, and the general paucity of documented objective evidence available in the farms at the time of the investigation.
- Given this uncertainty, feeding with material contaminated with the BSE agent cannot be excluded as the origin of any of the BARB-60 cases. However, this does not mean that feeding can be definitely attributed as the cause of any of the BARB-60 cases.
- Spontaneous cases, interpreted as occurring without an apparent cause, are not predictable and may not be detectable either. The classification of a case as spontaneous is circumstantial and may change over time subject to additional information. It does not infer that there is no external cause; just that it could not be ascertained. A case is classified as spontaneous by a process of elimination, excluding all other definable possibilities.
- Therefore, it is not possible to conclude that any of the BARB-60 cases had a spontaneous origin.

5. Recommendations

- To maintain the EU-wide current surveillance system in order to: (1) monitor the evolution of the tail of the BSE epidemic; (2) detect a potential re-emergence of BSE and (3) detect a new BSE form in cattle, should it appear.
- To periodically evaluate the new BSE data using epidemiological transmission models, in order to detect deviations from the expected exponential decay.
- To run the C-TSEMM model on an annual basis with updated data in order to monitor the ability of the current surveillance system to detect BSE at both MS and EU-28 levels.
- To collect strain and genotype data for any new BSE case, to facilitate comparison with previous BARB and non-BARB cases.
- To collect high-resolution geographical data for the detection at local level of spatial clustering of BSE cases.
- To create 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.
- To create an EU-level reporting system of the existing feed testing for the monitoring of the feed ban that would allow the collection, collation and analysis of MS feed testing data.

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Glossary and Abbreviations

Ante mortem	Bovine animals sent for normal slaughter but the slaughter of which was deferred because they were: (a) suspected of suffering from a disease which is communicable to humans and to animals or showing symptoms or being in a general condition indicating that such a disease may occur; (b) showing symptoms of a disease or of a disorder of their general condition which is likely to make their meat unfit for human consumption
BARB-60	Sixty BSE cases of classical or unknown type born after the date of entry into force of EU total feed ban
Clinical suspects	Clinical suspects. Bovine animals reported as TSE suspects (clinically suspected cases), as defined in Article 3 of the TSE Regulation, and subject to the measures described in Articles 12 and 13. Clinically suspected animals are normally obtained via passive surveillance
Emergency slaughter	Bovine animals subject to 'special emergency slaughtering' as described in the EU legislation
EU-8	Estonia, Hungary, Latvia, Lithuania, Malta, the Czech Republic, Poland and Slovakia
EU-17	Austria, Belgium, Cyprus, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Slovenia, Spain, Sweden and the United Kingdom
EU-25	All MS as on 1 July 2013, except Bulgaria, Croatia and Romania
EU-28	All MS as on 1 January 2017
Fallen Stock	Bovine animals that have died or been killed on the farm or in transport, but not slaughtered for human consumption nor killed in the framework of an epidemic
Healthy Slaughter	Healthy bovine animals submitted to regular slaughtering for human consumption or slaughtered in the context of a disease eradication campaign other than BSE, without clinical signs of disease
MBM	Meat-and-bone meal (MBM). Product of the rendering industry with high protein content produced from category 1 and 2 animal by-products, which are not fit for human consumption
non-BARB-60	Non BARB-60 cases
PAP	Processed animal proteins (PAP). Meat-and-bone meal (MBM), meat meal, bone meal, blood meal, dried plasma and other blood products, hydrolysed proteins, hoof meal, horn meal, poultry offal meal, feather meal, dry greaves, fishmeal, dicalcium phosphate, gelatine and any other similar products including mixtures, feeding stuffs, feed additives and premixtures, containing these products

AT	Austria
BARB	Born after reinforced feed ban
BE	Belgium
BG	Bulgaria
BIOHAZ	EFSA Panel on Biological Hazards
BSE	bovine spongiform encephalopathy
CI	confidence interval
CA	Competent Authorities
C-BSE	Classical BSE
CCA	Central Competent Authority
CJD	Creutzfeldt-Jakob disease
CNS	central nervous system
C-TSEMM	Cattle TSE Monitoring Model
CY	Cyprus
CZ	Czech Republic
DE	Germany
DEFRA	Department for Environment Food & Rural Affairs
DG	Directorate General
DK	Denmark
EE	Estonia
EL	Greece

ES	Spain
Eurostat	Official EU Statistical data
FEFAC	European Feed Manufacturers' Federation
FI	Finland
FR	France
FVO	Food and Veterinary Office
GALT	Gut-associated lymphoid tissues
GB	Great Britain
HACCP	Hazard analysis and critical control points
HR	Croatia
HU	Hungary
ID(50)	infectious dose at 50%
IE	Ireland
IT	Italy
LT	Lithuania
LU	Luxemburg
LV	Latvia
MBM	meat and bone meal
MS	Member State
MT	Malta
NI	Northern Ireland
NL	Netherlands
non-BARB	non-BARB cases
NUTS	Classification of Territorial Units for Statistics
OF/SI	organic fertilisers/soil improvers
OR	odds ratio
ORF	open reading frame
PAP	processed animal protein
PL	Poland
PRNP gene	prion protein gene
PrP^{C}	normal cellular protein
PrP^{Sc}	abnormal protease resistant isoform or prion protein
PT	Portugal
RO	Romania
SE	Sweden
SI	Slovenia
SK	Slovakia
SNP	single-nucleotide polymorphism/s
SSC	Scientific Steering Committee
ssDNA	single-stranded deoxyribonucleic acid
ToR	Term of reference
TSE	transmissible spongiform encephalopathies
WG	Working Group

Appendix A – Questionnaire on BSE BARB-60 cases

The European Food Safety Authority (EFSA) is currently conducting a risk assessment on the BSE cases born after the total feed ban (1 January 2001 for 'old Member States', and after 1 May 2004 for the Central and Eastern European countries which joined the EU on that date). In the context of this mandate submitted by the European Commission, and in order to address the terms of reference, EFSA has been kindly invited to contact at least the concerned Member States (MS) (countries in which cases of BSE born after the total feed ban have been confirmed) in order to obtain all necessary information for this assessment, in particular information gathered during the epidemiological investigations on these cases.

Furthermore, in accordance with Article 22(7) of Regulation (EC) No 178/2002, EFSA has been kindly invited to closely cooperate for this mandate with the competent bodies carrying out similar tasks to those of EFSA in at least the concerned MS, in particular with a view to discuss the risk assessment methodology of the EFSA scientific opinion.

In the case of **COUNTRY NAME**, this questionnaire refers to the following case/s:

Animal birth year	Animal birth month	Target group	Sampling period year	Sampling period month	Age	Case type	National case number

Please provide answers to the following questions (if the answers are different for each of the three cases confirmed in your country, please fill as many questionnaires as necessary):

1) Have you conducted epidemiological investigations on your BSE BARB-60 case/s at individual level?

☐ YES
☐ NO

If the answer to **Q1 is YES**, go to **Q2** and answer Q2 to Q6
If the answer to **Q1 is NO**, go to **Q7** and answer Q7 to Q8

2) What were the objectives of such investigations (tick all that apply):

☐ Collect epidemiological data on the case
☐ Try to ascertain the origin of the exposure to the BSE agent
☐ Establish the link with other cases
☐ Evaluate the control and preventive measures applied in the country
☐ Any other (please explain):

3) What was the format of such investigations (tick all that apply):

☐ Standard protocol including field visit to farm/s and/or other premises
☐ Standard protocol including the administration of a questionnaire to farmer
☐ Informal telephone/email communication to farmer/s and/or other premises
☐ Office-based report by veterinary officer without field visit/s
☐ Any other (please explain):

4) What were the outputs of such investigations (tick all that apply):

☐ Standard form only for internal use with no formal report produced
☐ Public report available in website in national language
☐ Public report available in website in English
☐ Non-public report, but available upon request
☐ Confidential report (restricted access)
☐ Any other (please explain):

5) With regards to the origin of the exposure to the BSE agent, what were the main conclusions of the investigations?

6) **Can these questionnaires/forms/reports/results of the investigation of individual cases be made available to EFSA for their review as part of the current EFSA mandate?**

☐ YES
☐ NO
☐ Under certain conditions (please explain):

If the answer to **Q6 is YES**, please send them to EFSA together with this questionnaire.

Thank you for your participation
Please send this questionnaire to:
biocontam@efsa.europa.eu

7) **What is/are the hypothesis/hypotheses that your authorities consider most plausible to explain the occurrence of BSE BARB cases in your country?**

8) **What is the evidence to support that/those explanation/s (if they are not included in case report/s?**

Thank you for your participation
Please send this questionnaire to:
biocontam@efsa.europa.eu

Appendix B – Number of cattle tested in all surveillance streams in the period 2001–2015 by MS

Table B.1: Number of cattle tested in all surveillance streams in the period 2001–2015 by MS

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
AT	211,591	228,643	222,650	205,658	201,642	223,211	219,257	224,475	191,495	206,332	159,214	120,483	44,645	16,922	21,008	2,497,226
BE	377,909	450,419	392,465	393,869	367,281	364,795	359,852	367,393	225,979	256,103	207,204	127,579	24,311	24,480	24,725	3,964,364
BU		1,670	9,686	8,349	10,468	10,676	18,604	17,500	13,854	13,554	13,693	20,792	19,749	15,046	16,094	189,735
CY			7,726	7,351	9,093	8,238	7,961	8,757	8,037	5,802	4,852	2,985	1,952	1,497	949	75,200
CZ			210,184	200,717	170,823	174,467	160,420	157,392	156,472	146,455	97,848	54,794	36,057	18,293	20,095	1,604,017
DE	2,856,337	3,030,542	2,589,073	2,532,060	2,073,257	1,892,842	1,651,234	1,724,563	1,199,905	1,195,891	1,010,528	647,938	496,382	363,291	218,900	23,482,743
DK	276,965	293,341	289,702	284,234	254,962	241,031	232,627	233,162	159,003	169,794	122,020	75,800	23,477	21,559	19,514	2,697,191
EE			3,983	27,031	31,109	33,748	35,316	33,613	32,815	28,926	18,081	11,071	4,736	4,225	3,633	268,287
EL	17,113	23,735	26,533	28,806	31,684	32,694	30,446	33,782	25,809	23,260	22,321	14,611	14,864	14,495	13,037	353,190
ES	386,262	546,053	567,864	578,125	621,818	536,192	466,826	524,557	468,168	424,943	401,254	318,186	191,307	57,094	60,442	6,149,091
FI	27,876	137,008	131,430	126,085	117,046	124,579	119,338	110,094	71,942	73,457	55,928	38,718	15,911	10,778	11,576	1,171,766
FR	2,527,700	3,183,997	3,205,963	2,891,772	2,593,594	2,514,361	2,356,252	2,414,004	1,828,684	1,831,003	1,636,356	1,251,459	1,076,598	1,059,374	268,480	30,639,597
HR												37,176	36,244	34,045	29,445	136,910
HU			97,488	96,081	83,553	83,893	85,167	86,044	88,639	86,552	59,749	39,494	20,923	11,016	12,440	851,039
IE	675,115	707,544	700,339	701,840	775,840	845,187	846,393	787,871	385,540	392,245	337,584	297,756	108,109	49,975	51,446	7,662,784
IT	445,128	731,585	786,506	785,807	690,993	655,941	629,994	678,319	486,645	482,753	392,797	307,685	167,397	51,118	54,989	7,347,657
LT			9,746	50,503	86,195	87,406	101,214	93,473	88,457	80,760	63,992	50,433	48,620	5,526	3,704	770,029
LU	20,886	18,398	17,714	16,700	14,748	14,562	13,560	13,858	8,977	9,410	7,771	5,498	2,597	2,050	2,205	168,934
LV			6,126	29,576	36,963	39,395	46,545	47,600	44,770	40,854	32,806	22,998	5,612	4,230	3,300	360,775
MT			1,199	2,384	2,843	2,752	2,396	2,423	2,843	2,956	2,007	2,291	1,835	275	200	26,404
NL	501,640	558,429	506,325	533,880	517,203	486,247	465,336	476,361	405,316	376,034	308,483	233,156	62,009	48,399	50,399	5,529,217
PL			455,413	481,116	515,976	607,992	603,810	611,566	638,073	637,273	475,906	326,280	318,849	207,503	222,821	6,102,578
PT	38,755	82,227	109,399	115,017	113,332	100,512	88,836	98,079	97,124	91,958	80,643	67,739	59,796	27,262	21,561	1,192,240
RO						73,444	132,688	131,217	73,310	79,190	72,220	76,504	79,503	76,943	126,463	921,482
SE	28,101	37,497	34,580	36,111	35,277	132,232	189,471	181,952	124,576	120,699	83,107	60,484	20,180	9,447	10,079	1,103,793
SI			66,167	45,666	36,784	32,667	31,384	31,114	30,044	25,914	22,879	19,613	12,656	8,352	8,952	372,192
SK			87,010	82,939	69,222	66,312	61,888	55,200	49,712	42,816	27,293	14,166	13,581	7,461	7,969	585,569
UK	96,296	394,461	460,752	599,540	662,174	728,538	771,458	907,366	622,673	662,038	645,056	550,000	228,050	137,129	139,402	7,604,933

Appendix C – Summary of the results of the questionnaire survey

Table C.1: Summary of the results of the questionnaire survey

# of case (table mandate)	FEEDING Milk replacer	FEEDING Proprietary concentrates	FEEDING Other species on the farm	MATERNAL TRANSMISSION	BSE on the farm before	ENVIRONMENT Farmyard disposal of carcasses	ENVIRONMENT Farmyard application of manure / sewage	OTHER
1	c	b	a	c	a	c	c	
2	c	b	c	c	a	a	c	
3	b	b	a	a	a	a	c	
4	a	b	a	a	a	a	c	
5	c	c	c	c	c	c	c	
6	c	c	c	c	c	c	c	
7	b	b	a	c	a	c	c	Large knackery in close proximity
8	a	b	a	a	a	c	a	
9	b	b	a	c	a	c	c	
10	b	b	a	c	b	c	c	Two cases in same herd
11	c	c	c	c	c	c	c	
12	a	b	a	a	a	c	c	
13	b	b	c	c	a	a	c	
14	a	b	a	a	a	c	c	
15	a	b	a	a	a	c	c	
16	b	b	a	a	a	c	a	Knackery very close
17	a	b	c	b	a	c	c	
18	a	b	a	a	b	c	c	Knackery plant at 5 km
19	b	b	b	a	a	c	c	
20	a	b	a	a	a	c	c	
21	c	b	b	c	a	c	c	
22	c	c	c	c	c	c	c	
23	c	c	c	c	c	c	c	
25	c	c	c	c	c	c	c	
26	c	c	c	c	c	c	c	
27	c	c	c	c	c	c	c	
28	c	c	c	c	c	c	c	
29	c	c	c	c	c	c	c	
30	c	c	c	c	c	c	c	
31	c	c	c	c	c	c	c	
32	c	c	c	c	c	c	c	
33	c	c	c	c	c	c	c	
34	c	b	a	c	b	c	c	
35	a	b	a	a	a	c	c	
36	a	b	a	a	a	a	c	

# of case (table mandate)	FEEDING Milk replacer	FEEDING Proprietary concentrates	FEEDING Other species on the farm	MATERNAL TRANSMISSION	BSE on the farm before	ENVIRONMENT Farmyard disposal of carcasses	ENVIRONMENT Farmyard application of manure / sewage	OTHER
37	c	b	a	a	b	b	c	
38	c	b	c	c	c	c	c	
39	c	b	a	a	b	c	c	
40	a	b	c	a	b	b	c	
41	c	b	a	c	b	c	c	
42	c	b	a	b	a	c	c	
43	a	b	a	a	a	c	c	
44	a	b	a	a	b	b	c	
45	a	b	a	b	a	c	c	
46	a	b	c	b	b	c	c	
47	c	b	a	b	a	c	c	
48	a	b	b	a	a	b	c	
49	a	a	a	a	a	c	c	
50	a	b	b	a	a	c	c	
51	c	b	a	a	b	c	c	
52	a	b	a	c	a	c	c	
53	c	b	c	c	a	c	c	
54	c	b	c	c	a	c	c	
55	c	b	c	c	a	c	c	
56	c	b	a	a	b	c	c	
57	c	b	a	a	b	c	c	
58	c	b	a	a	a	c	c	
59	c	b	b	b	a	c	c	
60	a	b	a	a	b	b	c	
61	a	b	a	a	a	b	c	

a: Presence of risk factor not supported by data.
b: Presence of risk factor cannot be excluded.
c: Not enough data to choose an option.
Case 24 removed. Re-classified as atypical.