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BSE: Diagnosis and control, the consequences for human health and geographical BSE risk

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Diagnosis and control

Prion diseases are slow, lethal transmissible neurodegenerative illnesses that affect humans and many animal species. After the start of the epidemic in the UK in 1986, BSE became in most EU Members States a notifiable disease 1990 and regulations were developed for the epidemiosurveillance for ruminant TSE in a number of EU Member states including the herd slaughter and compensation policy. In this "passive" surveillance system, clinical suspected cases in the farm or the slaughterhouse were usually sent to the National Reference Laboratory for TSE examination of the brain using histopathology, immunohistochemistry and Scrapie Associated Fibril (SAF) examination in electron microscope. In this frame, only a limited number of cases were detected in some till 2000. Because of obvious underreporting in this passive surveillance system, the EU developed since 2000 a method to assess the geographical BSE risk (GBR), defining a GBR level (Table 1) . All EU countries are at present situated in class II till IV, class I being the lowest, meaning that it is considered highly unlikely that one or more cattle are clinically or pre-clinically infected. However, a number of EU countries did not detect any case of BSE before 01/01/2001 despite their rather high GBR level. Therefore, and also to protect consumer health, the EC imposed the application of rapid BSE tests on all cattle when slaughtered for human consumption above 30 months of age, and on risk populations such as emergency slaughtered animals and animals found dead on the farm, sent to the rendering plants ("active" surveillance). A number of rapid tests were therefore validated (EC/2000/764): an indirect ELISA (cELISA), a colorimetric sandwich ELISA (sELISA I), a chemiluminescent sandwich ELISA (sELISA II), a Western blot and an automated conformation-dependent immunoassay (aCDI). At present 4 similar post mortem tests are under validation as well as one live test based on FT-IR (Fourier Transform Infrared Spectrocopy). Other live tests are still under development.

The evaluation of the outcome of the rapid diagnostic tests in the EU indicated that BSE is indeed present in most EU countries and this will contribute to the improved determination of the

GBR level. Moreover, it was now confirmed that BSE became a worldwide problem, mainly due to imports of contaminated MBM and animals in the past.

The number of reported cases of BSE worldwide are as follows (March 2004): UK: 182.567; Ireland: 1.405; France: 912; Portugal: 879; Switzerland: 453; Spain: 404; Belgium: 124; Germany: 312; Italy: 120; Netherlands: 75; Denmark: 13; Liechtenstein: 2; Luxemburg: 2; Greece 1; Austria: 1, Finland: 1; Poland: 13; Slovakia: 14; Slovenia: 4; Czech Republic: 9; Japan 11; Israel: 1; Canada: 2; USA: 1.

In 2002, a total of 10.423.882 bovine animals were tested in the framework of the EU TSE monitoring program of which 2.126 turned out positive. 1.238.617 risk bovine animals were tested by rapid tests and 9.124.887 healthy animals slaughtered for human consumption. 2.658 animals were tested in the framework of passive surveillance. In addition, 57.720 animals were tested in the framework of culling animals with an epidemiological connection to a BSE case. 68% of positive cases were detected by the active monitoring (testing of risk animals, healthy slaughtered and culled cattle) and 32% were detected by passive surveillance. Finding BSE cases is almost 30 times higher in fallen stock, emergency slaughtered cattle and cattle with clinical signs at ante-mortem ("risk animals") than in healthy slaughtered cattle. In culled animals, the prevalence was almost 10 times higher than in healthy slaughtered cattle.

Human health

The transmissibility of the agent of Bovine Spongiform Encephalopathy (BSE) to man, causing variant Creutzfeldt-Jakob disease (vCJD), is now generally accepted but the extent of a possible future epidemic in man can not be defined at present. However, the incidence of vCJD (and also sporadic CJD for which there are recent indications for a link with BSE) in the UK appears to be increasing exponentially. At present 139 cases are confirmed in UK, 6 in France,1 in Ireland, 1 in Hong Kong, 1 in USA and 1 in Canada.

The possibility that BSE is present in sheep flocks cannot be excluded. However, to date this has never been shown outside experimental conditions. Additional precautionary measures are presently not justified, but scenarios were developed in case BSE in sheep should be confirmed. This would represent a higher risk to humans as more tissues are infective and would be much more difficult to eradicate as it probably will spread within the flocks by horizontal and maternal transmission, and could maintain in the environment

A number of BSE preventive measures to protect human and animal health were taken at EU level of which the most important are:

27 July 1994: Meat and bonemeal (MBM) ban in the whole EU (94/38/EC)
1 April 1997: MBM production at 133°C/3bar/20min/100% relative humidity (96/449/EC)
1 October 2000: EU SRM ban (97/534/EC, reinforced by 2000/418/EC)

1 January 2001: Compulsory rapid BSE tests on bovines above 30 months (2000/374/EC)
Total MBM ban, including fishmeal (2000/764/EC)
1 April 2001: Vertebral column above 12 months is SRM (2001/233/EC)
1 July 2001: Regulation for prevention, control and eradication of TSE and safety of ruminant
derived material (gelatin, fat, tallow, cosmetics, medicinal products, mechanically recovered
meat, dicalciumphosphate, organic fertilizers, animal waste) (2001/999/EC)

Geographical BSE risk analysis

The EU developed a method to assess the geographical BSE risk (GBR), defining a GBR level, which is based on a qualitative analysis of (1) the likelihood that the BSE agent was introduced into a country and if so, when and to what extent; (2) the potential of it being recycled or eliminated; (3) the likelihood that today one or more cattle could be infected.

All EU countries are situated in class II till IV, class I being the lowest, meaning that it is considered highly unlikely that one or more cattle are clinically or pre-clinically infected. However, a number of EU countries did not detect any case of BSE before 01/01/2001 despite their rather high GBR level. Therefore, and also to protect consumer health, the EC imposed the application of rapid BSE tests on all cattle when slaughtered for human consumption above 30 months of age, and on risk populations such as emergency slaughtered animals and animals found dead on the farm, sent to the rendering plants (“active” surveillance).

The Geographical BSE-Risk (GBR) is a qualitative indicator of the likelihood of the presence of one or more cattle (GBR-C) or small ruminants (GBR-S) being infected with BSE, pre-clinically as well as clinically, at a given point in time, in a country. Where presence is confirmed, the GBR gives an indication of the level of infection.

The SSC-methodology for the assessment of the GBR-C is based on the assumption that BSE arose in the United Kingdom (UK) and was propagated through the recycling of bovine tissues into animal feed. Later the export of infected animals and infected feed provided the means for the spread of the BSE-agent to other countries where it was again recycled and propagated via the feed chain.

To determine the GBR-S, the GBR-C methodology was adapted to small ruminants, including other routes of transmission than infected feed, i.e. vertical and horizontal pathways, and a much more widespread tissue infectivity distribution, assuming BSE in small ruminants behaves similarly to sheep and goat scrapie.

The Geographical BSE risk assessment for BSE in cattle (GBR-C).

The Geographical BSE-Risk (GBR-C) is a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE (Bovine Spongiform Encephalopathy), pre-clinically as well as clinically, at a given point in time, in a country. Where its presence is confirmed, the GBR-C gives an indication of the level of infection.

The Scientific Steering Committee of the European Commission (SSC) has developed a transparent methodology (8), to assess the GBR-C for any country that provides the information required for the assessment. This methodology is limited to bovines and feed based transmission of BSE. It does not take into account any other initial sources of BSE than the import of infected cattle or contaminated feed. It is assumed that the disease first appeared in the UK from a still unknown initial source. An important characteristic of the methodology is that it does not depend on the confirmed incidence of clinical BSE, which is sometimes difficult to assess due to serious intrinsic limitations of surveillance systems. Surveillance should be understood as the process of identifying BSE-cases and animals at risk of being infected.

The qualitative nature of this methodology and its limitations should be understood in the context of present scientific knowledge on BSE and of the availability and quality of data. As they both evolve, and with the advancement of new diagnostic methods, the need may arise for the methodology to be revised and/or its application to particular countries to be repeated.

*Methodology for assessing the GBR-C***.** Germany, Italy, Spain, and the Czech Republic and the Slovak Republic all were classified as GBR-C III before they detected their first case. The GBR-C-assessment for Denmark was already in an advanced stage, pointing to GBR-C III, when the first case was confirmed. In addition Japan and Greece have now confirmed first domestic BSE-cases. Also Austria, Finland and Slovenia, all three in GBR-C-II, recently detected a first domestic case of BSE. In all cases active surveillance detected BSE-cases that would have remained undetected by the already existing, passive surveillance, which was targeted at animals with neurological symptoms. The methodology of the GBR-C-assessment, and the model and assumptions it is based on, remains unchanged. Consistency of the past and future assessments is therefore ensured, but the assessment of the external challenge is refined and the process is streamlined since the start in 2000.

Basically the GBR-C–methodology tries to answer two questions:

1. Is there a risk that the BSE-agent was imported into the country under consideration?
2. If the BSE-agent was introduced into a country, would it have been recycled and amplified or was the BSE/cattle system of that country able to eliminate the agent?

The following factors contributing to the incident and propagation risks in a geographical area are to be included in the assessment:

1. Structure and dynamics of the cattle, sheep and goat populations

2. Animal trade
3. Animal feed
4. Meat and bone meal (MBM) bans
5. Specified bovine offal (SBO) and specified risk materials (SRM) bans
6. Surveillance of TSE, with particular reference to BSE and scrapie
7. Rendering and feed processing
8. BSE and scrapie related culling

Basic assumptions. Origin and transmission of BSE: The assessment of the GBR-C continues to be based on the assumption that BSE arose in the United Kingdom (UK) and was propagated through the recycling of bovine tissues into animal feed. Later the export of infected animals and infected feed provided the means for the spread of the BSE-agent to other countries where it was again recycled and propagated via the feed chain.

For all countries other than the UK, import of contaminated feed or infected animals is the only possible initial source of BSE that is taken into account. Other sources such as a spontaneous occurrence of BSE at very low frequency, or the transformation into BSE of other (animal) TSEs (Transmissible Spongiform Encephalopathy) (scrapie, CWD or Chronic Wasting Disease, TME or Transmissible Mink Encephalopathy and FSE or Feline Spongiform Encephalopathy) being present in, or imported into a country are not considered, as they are not scientifically confirmed. In addition surveillance data on other TSEs are generally inadequate for assessing their prevalence. The only transmission vector considered in the model continues to be feed. Blood, semen and embryos/ova are not seen as effective transmission vectors and accordingly, blood meal or embryos/ova and semen are not taken into account. The recent results of large scale BSE-testing in combination with reports on feed controls have further underpinned the opinion of the SSC that any cross contamination of cattle feed with mammalian MBM, even well below 0.5%, represents a risk of transmitting the disease. However, the influence of potential cross-contamination on the GBR-C has to be seen in the light of the risk that the animal protein under consideration could carry BSE-infectivity.

Other transmission routes than feed are debated but they are not scientifically confirmed and anyway their potential impact on the GBR-C is regarded negligible in comparison to contaminated feed. This includes vertical transmission as well as any unknown third mode of transmission of BSE. Also transmission via the environment or the possibility that sheep and goats may have become infected with BSE (5,6) and could be a source of BSE, are not scientifically confirmed. They will be taken into account once scientific evidence of their existence is available allowing assessing their impact on the GBR-C.

Geographical limitation: So far the present GBR-C risk assessments are only addressing entire countries and national herds. This is because of the limited availability of detailed, regionalized data. However the issue of regional differences, for example in the types of animal husbandry,

e.g. dairy or beef, or with regard to feeding or to slaughtering ages is not discounted. If complete data sets could be provided on a regional scale, i.e. clearly relating to a defined geographical area smaller than a country, these could be assessed in the same way as data referring to entire countries.

The external challenge assessment

The term **"external challenge"** is referring to both the likelihood and the amount of the BSE agent entering into a defined geographical area in a given time period through infected cattle or MBM . The following basic guidelines for assessing the external challenge are used (8,14):

1. The external challenge is regarded independent from the size of the challenged BSE/cattle system and in particular the size and structure of the cattle population.
2. The assumed challenge resulting from imports from the UK during the peak of the BSE-epidemic in the UK is the point of reference.
3. The challenge resulting from imports during other periods and from other BSE-affected countries is established in relation to this baseline.

In the light of new scientific knowledge and data it is necessary when assessing the external challenge to take account of imports from all countries with a BSE risk. This includes all countries with one or more confirmed domestic cases or being classified in GBR-C III while not having identified any domestic cases (Table 2,3).

Regular updating of GBR-C assessments.

From new scientific knowledge and data a need might arise to update the GBR-C-methodology and to re-apply this to countries that are already assessed (8,14). The BSE-cases, confirmed some years ago in Austria, Finland and Slovenia that were classified as GBR-C II, underlines the appropriateness of this statement. One of the possible explanations for these cases could be that imports into these countries from GBR-C-III countries were not regarded as external challenge when the GBR-C of these countries was assessed.

It is therefore appropriate to verify for all countries, classified so far as GBR-C I or II (Table3) , if external challenges can now be identified that were not previously been taken into account in the GBR-C assessment. If necessary the GBR-C-report/opinion is updated (Table 4)

The Geographical BSE risk assessment for BSE in small ruminants (GBR-S).

Because it has clearly been demonstrated that BSE can be orally transmitted to certain genotypes of small ruminants, and because it is likely that potentially BSE-contaminated MBM has been fed to some sheep and goats, one has to assume that BSE could have been introduced into the sheep and goat population. (5,6). Therefore it cannot be excluded that the risk could persist it, even after an effective implementation of a ruminant feed ban.

The most likely way of introduction has been through infected MBM. It is possible that the BSE-agent has been maintained, propagated and/or recycled by horizontal and vertical transmission in sheep and goats if the agent behaves like scrapie in these species (11).

The same factors contributing to the incident and propagation risks in a geographical area for the GBR-C are to be included in the GBR-S (18).
The GBR-S is based on the exploitation of the GBR-C, with some additional information elements such as:

- Routes of infection: not only through the contamination of the feed chain but also horizontally through direct or contact or by contaminating the pasture.
- Susceptibility of different small ruminants strains: it is known that this varies substantially depending on the breed and on the presence of specific genotypes (7,16). Kao *et al.* (2002)(4) have presented a rationale for considering the speed of spread of BSE infectivity within heterozygous semi-resistant sheep to be slower than in the fully susceptible animals. The incubation period for infectivity is assumed to be approximately 3 years longer than the susceptible group.
- Dose-response: Estimations using the limited data on the proportion of susceptible sheep succumbing to BSE after consuming 0.5g of infected cattle brain, do not allow any threshold dose to be shown to exist.(4)
- Infectivity distribution and total infectivity load: It appears that the main difference between cattle and small ruminants is that lymphoreticular tissues in BSE in sheep and goats, and possibly their blood (2,3,10,12,17,19), should be considered comparable in their level of infectivity with central nervous system tissues. However the amount of infectivity detected in blood is very likely much smaller than that detected in most lymphoreticular and nervous tissues.
- Prevalence of BSE in TSE affected sheep population: there are indications that BSE is likely to be at a prevalence significantly below that of scrapie, and figures based on the failure to detect BSE in samples of TSE affected brains provided an upper bound for BSE-prevalence of about 0.83% till 2%.(1,13,16).

Prevalence of scrapie in small ruminants: on the basis of the TSE testing results in the frame of the EU program started in April 2002, the TSE positives are 0.5% of risk sheep and 0.055% of healthy adult sheep

Prevalence of BSE in small ruminants: on the basis of a maximum TSE infection prevalence of 1.0% in the sheep population, with no more than 1.0% of the scrapie sheep possibly being in reality BSE, the worst case hypothesis is 1 BSE animal in 10 000 small ruminants (0.01%).

The implementation of the GBR-S methodology is recommended only in the case BSE has been confirmed under natural conditions in at least one small ruminant (9,15) and should be done in two steps:

Step one : Countries GBR-C levels III and IV

These countries should be classified into GBR-S level III unless data can be provided showing that, since 1980, significant levels of potentially–infected MBM very unlikely or unlikely reached small ruminant through the feed chain. Important elements are:

- -Feeding practices for different types of sheep flocks, preferably including the relative amounts of MBM fed to sheep as compared to cattle
- -Tons of compound feed sold annually for sheep and goats
- -Inclusion rate of MBM in these feeds
- -Price charts for MBM and alternative protein sources
- -Cross contamination
- -Regulatory situation re-use of MBM for sheep
- -Mixed farming practices

Countries in GBR-C level I and II

Should the challenge through the feed chain due to live small ruminants be found negligible throughout, the GBR-S classification would remain identical to the GBR-C one. Otherwise the combined external challenge should assessed and a stability analysis would be necessary for the sheep feeding system since 1980 and a higher GBR-S level would be likely.

Step two: Countries GBR-S level I and II

For countries that remain classified as GBR-S level I and II at the end of step one, it would be necessary to estimate whether BSE might have entered the country through live small ruminants and transmitted through horizontal or vertical routes.

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11. SSC Opinion on *Pre-emptive risk assessment should BSE in small ruminants be found under domestic conditions.* Adopted on 8-9 February 2001

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17. SSC Opinion on *The implications of the recent papers on transmission of BSE by blood transfusion in sheep (Houston et al, 2000, Hunter et al, 2002).* Adopted on 12-13 September 2002

18. SSC Opinion on *The geographical BSE risk for sheep and goats (GBR-S): adaptation of the cattle GBR methodology to small ruminants, in case BSE in small ruminants would become probable or evident under field conditions.* Adopted on 7-8 November 2002

19. SSC Opinion on *TSE infectivity distribution in ruminant tissues.* Adopted on 10 and 11 January 2002 and amended on 7-8 November 2002

Table 1 - Definition of GBR-C and its levels

GBR-C level	Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a geographical region/country
I	Highly unlikely
II	Unlikely but not excluded
III	Likely but not confirmed or confirmed, at a lower level
IV	Confirmed, at a higher level

Table 2: Definition of BSE-challenge levels

EXTERNAl CHALLENGE	Cattle (n° of heads) imports			MBM[1] (tons) imports		
	1988 - 93 from UK	UK-imports before 88 and 94-97: *10; after 97: *100	Imports from other countries with a BSE risk: R1*1000, R2* 100	1986 - 90 from UK	UK-imports before 86 & 91-93: * 10, after 93 *100	Imports from other countries with a BSE-risk: R1*100, R2* 10
Extremely High	> 10.000			> 10.000		
Very High	1.000 - < 10.000			1.000 - < 10.000		
High	100 - < 1.000			100 - < 1.000		
Moderate	20 - < 100			20 - < 100		
Low	10 - < 20			10 - < 20		
Very low	5 - < 10			5 - < 10		
Negligible	0 - < 5			0 - < 5		

[1] "MBM" refers to MBM, MMBM, BM, or Greaves but not to composite feed that could contain it.

From the GBR-C assessments so far available it can be seen that the first occurrence of an internal challenge is rather variable. Therefore in all cases where this information is available, only exports after a first internal challenge could possibly have been present in the exporting country shall be regarded as an external challenge to importing countries.

Table 3: Examples of GBR-C levels and R1 and R2 challenges

Country Name	GBR-C	R1	R2
Albania	III	No data	1988
Austria	III (6)	1988	1990
Belgium	III	1983	1987
Cyprus	III	1980	1990
Czech Republic	III	No data	1988 (1)
Denmark	III	1985	1990
Estonia	III	1987 (2)	1988 (2)
Finland	III (6)	1980	1990
France	III	1979	1980
Germany	III	1980 (3)	19883
Hungary	III	1981	1982
Ireland (Eire)	III	1980	1980
Italy	III	1983	1990
Lithuania	III	No data	1994 (2)
Luxembourg	III	1983	1987
Netherlands	III	1985	1987
Poland	III	1980	1987
Portugal	IV	1979	1987
Romania	III	No data	1981
Slovak Republic	III	No data	1988 (1)
Slovenia	III (6)	1981 (4)	1991 (4)
Spain	III	1985	1987
Switzerland	III	1979	1980
Greece (5)	III	1985 (5)	1990 (5)
Japan (5)	III	1985 (5)	1990 (5)

Table 3: Countries in GBR-C III and IV and the year since when it is regarded possible (R1) or likely (R2) that exports of life bovine or MBM could have represented an external challenge to the importing country.
UK is not listed in this table as it is used as reference case.
(1) Part of CSSR, (2) part of Soviet Union, (3) only FRG – incl. GDR only after 1988, (4) former Republic of Yugoslavia, (5) pending a GBR-C assessment the dates for R1 and R2 are preliminary estimates, (6) Austria, Finland and Slovenia were earlier classified as GBR-C II but due to confirmed presence of one or more cattle clinically or pre-clinically infected with the BSE agent they now fall into GBR-C III.
Table 3 provides for some of the already assessed countries, and Greece and Japan, the year since when it is regarded possible (R1) or likely (R2) that exports represented an external challenge to the importing country. To assess the level of this external challenge the following factors shall be used when working with table 2:
R1 = factor 1000 for live cattle and factor 100 for MBM
R2 = factor 100 for live cattle and factor 10 for MBM.
The dates in the table were derived from the available GBR-C-reports and relate to the time when an internal challenge became possible (R1) or likely (R2) in the respective country. The factors are the same as previously used, only for the periods R1 another order of magnitude was added to reflect the lower but not negligible risk. Greece and Japan are countries with confirmed BSE. Based on the outcome of the GBR-C-assessment it is assumed that Greece and Japan posed a potential risk (R2) since 1990, i.e. about two incubation periods before the confirmation of the first case. It is also assumed that a lower risk existed already one incubation period before (R1 for the period from 1985-1989).

Table 4. Overview of all countries with a GBR-C classification

N°	Country	Dossier in	GBR-C
1	Albania	19/10/00	III
2	Argentina	1/03/99	I
3	Australia	1/03/99	I
4	Austria	1/10/98	III
5	Belgium	1/10/98	III
6	Botswana	31/10/00	I
7	Brazil	17/09/00	I
8	Canada	1/03/99	II (case revision pending)
9	Chile	1/03/99	I
10	Colombia	13/11/00	II
11	Costa Rica	21/03/01	I
12	Cyprus	3/11/00	III
13	Czech Republic	1/03/00	III
14	Denmark	1/12/98	III
15	El Salvador	8/11/00	I
16	Estonia	7/11/00	III
17	Finland	1/12/98	III
18	France	1/12/1998	III
19	Germany	1/11/98	III
20	Greece	1/8/01	III
21	Hungary	3/11/00	III
22	India	1/06/99	II
23	Ireland (Rep.)	1/1/99	III
24	Italy	1/3/99	III
25	Japan	1/11/99	III
26	Kenya	29/11/00	II
27	Lithuania	31/10/00	III
28	Luxembourg	1/1/99	III
29	Mauritius	20/11/00	II
30	Namibia	3/11/00	I
31	Netherlands	1/2/99	III
32	New Zealand	1/12/98	I
33	Nicaragua	30/10/00	I
34	Nigeria	31/10/00	II
35	Norway	1/12/98	I (case revision pending)
36	Pakistan	1/07/00	II
37	Panama	17/04/01	I
38	Paraguay	1/03/99	I
39	Poland	3/11/00	III
40	Portugal	3/3/99	IV
41	Romania	1/03/01	III
42	Singapore	17/11/00	I
43	Slovak Republic	3/11/00	III
44	Slovenia	21/02/01	III

45	Spain	1/4/99	III
46	Swaziland	24/11/00	I
47	Sweden	1/12/98	II (case revision pending)
48	Switzerland	1/03/99	III
49	United Kingdom	1/10/98	IV
50	Uruguay	1/07/00	I
51	USA	1/12/98	II (case revision pending)