TAFS

INTERNATIONAL FORUM FOR TRANSMISSIBLE ANIMAL DISEASES AND FOOD SAFETY
a non-profit Swiss Foundation

(January 2009)

TAFS[1] Position Paper on BSE in small ruminants

The recognition in 2004 that a French goat killed in 2002[(25)] was infected with BSE raised the profile of prion diseases in small ruminants in Europe, especially with respect to the risk that this may represent to consumers. Research into BSE in small ruminants, using experimental infections of sheep with BSE, were expanded and will inevitably add to our understanding of how BSE behaves in sheep and goats if infected. Meanwhile surveillance for prion diseases in small ruminants in Europe has identified scrapie cases in most countries, sometimes only in small numbers, but the evolving process of data analysis and scientific investigation is challenging past assumptions about the recognition and behaviour of prion infections of small ruminants. In particular, there is an intensification of the search for BSE amongst positive cases detected by surveillance, making use of newer discriminatory tools.

This paper summarises recent results, both published or soon to be published, and their implications to both consumers and authorities who are responsible for ensuring the protection of consumers.

The search for BSE in sheep

Why worry about whether BSE occurs in sheep?

- BSE in cattle is thought to have originally arisen from sheep scrapie. Consequently it was theoretically possible that BSE could infect sheep. Experimental studies have subsequently shown that sheep can indeed be infected by mouth, and by inoculation [(9,10,26, 29, 30, 31, 32, 33, 35)].
- Research into scrapie in sheep has shown an association between the genetic makeup of the sheep, referred to as its genotype, and susceptibility to infection or clinical disease[(7, 11, 27, 34)]. The information referred to usually relates to the gene responsible for controlling the production of prion protein in the sheep (called the PrP gene), and normally letters are used to identify the specific genotype. Each set of three letters is carried on one chromosome, and the genotype represents the sequence on both chromosomes. Only sheep with the more common genotypes have been challenged with BSE by mouth, so the amount of data available on susceptibility to BSE is not comprehensive.(see footnote ϕ)[(23,32,35)]

[1] TAFS is an international platform created by a group of scientists, food industry experts, animal health regulators, epidemiologists, diagnosticians, food producers, and consumers. Its purpose is to establish and maintain lines of communication for the dissemination of reliable information to the public that can maintain confidence in the safety of food with regard to Transmissible Animal Diseases (TAD).

ϕ The level of risk to a sheep of contracting scrapie varies according to breed type and the genotype. The sheep PrP gene produces a protein of 256 amino acids, each of which is encoded by three DNA bases (one codon) in the gene. Susceptibility to scrapie has been shown to be linked to the PrP protein genotypes which are defined by variations in the amino acids encoded at codons 136, 154 and 171, and are termed polymophisms. At least five variant alleles have been found with respect to a risk of contracting scrapie which are depicted as ARQ, ARR, VRQ, AHQ and ARH. The codes represent polymorphism of amino acids at each codon i.e. $A_{136}R_{154}Q_{171}$ (ARQ) where A = alanine, R = arginine, Q = glutamine. The other two amino acids are; H = histidine and V = valine. Homozygous and heterozygous pairing of the two alleles inherited from a ram and a ewe therefore

- Initial studies have shown that sheep with the genotypes ARQ/ARQ, AHQ/AHQ and AHQ/ARQ are susceptible to oral infection[9,30,32,33]. Experiments involving the oral challenge of sheep of other genotypes are still in progress. There is however already evidence that the use of three codons only (136, 154 and 171) may be too simplistic. Coding for leucine at codon 168 seems to confer a degree of resistance to experimental challenge with BSE[35].
- Transmission by blood transfusion has also been demonstrated[46].
- There is already evidence that sheep of other genotypes can be infected by direct inoculation into the brain[45], or spleen[60]. It is however still necessary to prove that natural exposure, most probably by mouth, can also succeed in infecting such sheep[2, 10, 45, 60].
- Consequently, **if** sheep became infected with BSE, and, **if** like scrapie it transmitted from sheep to sheep, then BSE-infected sheep could remain alive on farms in Europe. If transmission did not occur from sheep to sheep then BSE would have died out in the national sheep flocks as exposure through the consumption of contaminated feed will have stopped when feed bans were introduced. The number of such sheep (or goats) that remain alive would depend on the longevity of the sheep, and the date at which feed bans became effective in individual countries[39, 51, 52].

The behaviour of BSE in sheep

Can sheep be infected with BSE?

- It is now clear that experiments conducted over the past 15 years have shown that sheep of certain genotypes (see below) can be infected experimentally with BSE. Having said that, it is also clear that sheep are not easily infected by mouth. Most successful infections have followed oral challenge with 5g of infected brain, and even then they do not all become infected. Exposure to lower doses (eg. 0.5g) results in a lower success rate, so in some respects this is reassuring. At this stage it would be premature to over-interpret studies that are still under way. At the end of the day, this may mean that at doses that sheep would have been naturally exposed to, via contaminated feed, infection will not have been inevitable even if the sheep were of susceptible genotype[35].
- It would therefore be wrong to assume that the successful infection of sheep by mouth with 5g of infected brain indicates that sheep will always be infected if exposed, irrespective of dose.
- In addition, although our understanding of the influence of genotype on susceptibility to infection with BSE is limited at the moment, there is also firm evidence that some genotypes appear to be resistant to oral infection.
- For example, at the Veterinary Laboratories Agency in the UK, sheep of ARQ/ARR and ARR/ARR genotype have not succumbed to oral infection with 5g of BSE-infected brain given at age 6 months. Groups of sheep remain healthy 9 years or at termination at 10 years after such exposures (as at March 2009). Other exposed sheep have been killed during this period, and their tissues examined for evidence of infection, but all results have so far been negative. In other words, infectivity has not been detectable[9]. Further studies are under way with tissues from these and other sheep, using more sensitive immunochemical tests, and sensitive genetically modified mice for bioassay, to determine whether infectivity is present, but at extremely low levels.
- Slightly contradictory results have arisen in another study in France in which 10 lambs of ARR/ARR genotype were orally challenged with sheep-BSE [2]. They were given 2.5g by mouth at 24 hours of age, and again at 14 days of age. When three were killed and examined at 10 months of age, abnormal PrP was detected in the spleen of one animal, but not in any other tissue. While studies continue to confirm that this was indicative of

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results in considerable variation of PrP genotype. National breeding schemes to produce flocks with the low susceptibility genotypes (ARR) are underway in several countries.

the presence of infectivity, and other tissues are being assayed as well, the study demonstrates that there may be circumstances under which all lambs may be susceptible, although the conditions of this experiment are severe, and do not reflect farm conditions. There is increasing evidence that age at exposure may be a significant factor in determining susceptibility to infection, and that caution is needed in extrapolating results from sheep exposed at 6 months of age to all sheep.

- Further experiments are under way to examine the influence of genotype, and also breed, on susceptibility. In addition, attempts are being made to produce naturally infected flocks by experimentally infecting some sheep, and allowing them to lamb in an environment that other, unchallenged sheep, will be exposed to. The aim is to maximise opportunities for transmission of infectivity, especially at lambing time,. This will help to quantify whether sheep that may have been infected via feed in the past will have had the capacity to transmit to other sheep and thereby maintained infection in exposed flocks. Preliminary evidence that transmission may occur from ewe to lamb has now been published, but it is too early to say how often it happens[8]. It is however clear, from unpublished data in the same flock in which maternal transmission has been demonstrated, that horizontal transmission between sheep (ie. other than from ewe to lamb) is not easy. This may mean that in reality BSE could not naturally be sustained in an exposed flock.
- Another small study to investigate whether or not BSE will transmit from experimentally infected mothers to their lambs has been in progress for several years. No transmission has occurred so far, but the size of the experiment is relatively small and cannot therefore prove conclusively that such transmission will never occur[29]. Another study involving goats has also been unsuccessful in transmitting BSE by embryo transfer[31].

Does it matter if BSE has infected sheep?

- Governments and other agencies are at the moment adopting a precautionary stance with regard to BSE in sheep, and are assuming that if it is present it will represent the same risk to consumers as it would if present in cattle. By the limited criteria used so far to characterise prion strains[13,14], BSE in cattle and sheep are indistinguishable. For example, they both transmit easily to mice, with essentially identical characteristics of incubation period and pathological changes in the mouse brains. Consequently a precautionary approach is sensible given past experiences with BSE in cattle[18, 19, 20. 21].
- One recent study in genetically modified mice (BoPrP-tg100 mice) attempted to compare the susceptibility of the mouse to inoculation with BSE derived from cattle, and from experimentally infected sheep. Because the mice carry a bovine PrP gene, but appeared to be more susceptible to BSE from sheep than from cattle, the authors hypothesised that BSE might become a greater danger to humans if exposed via sheep tissue than if exposed via cattle[26]. Nevertheless, other studies[16] highlight the fact that such conclusions could be premature. Results can vary significantly even though the source inocula appear similar, and other factors must be taken into account before scientists can justifiably extrapolate from such experimental models to risk to consumers.
- Confirmation of BSE in sheep may require the extension of the definition of specified risk tissues that are removed from the human food chain (see TAFS position paper on Specified Risk Material). Confirmation of BSE in a goat (see below) did not however result in such a change. It is possible that the timing of recognition will play a great part in public and official reaction. In other words, whether or not the case occurred in the past and was detected retrospectively, or is found in the present, will influence the perception of risk.
- Nevertheless, the definition of SRM in sheep is much more difficult than in cattle. This is because, in sheep that are fully susceptible to scrapie, infectivity is dispersed widely in the body. In sheep that are susceptible to BSE, infectivity is similarly distributed[8, 32, 47, 65]. It is not yet known whether there are intermediate stages of susceptibility to BSE, and whether a more restricted distribution of infectivity occurs, as happens with scrapie in different genotypes. This means that the SRM list could have to include tissues that are

found within joints of meat (lymph nodes and nerves), and their total removal would be virtually impossible without dismembering the carcase. Furthermore, there are suggestions that the insensitivity of test methods may still be a limiting our full understanding of this distribution. There are indications that in some circumstances even muscle fibres may be marginally positives in scrapie-infected sheep[3]. This is why at the moment the rules focus on removal of only the tissues of greatest risk. These can be removed relatively easily while leaving a marketable carcase behind.

What tests have been used to trace the distribution of BSE infectivity in experimentally infected sheep?

- Immunohistochemistry has allowed a rapid evaluation of tissues once collected. All results have been negative so far except in intestinal, lymphoid and nervous tissues which were predicted to be the likely sites of replication[33, 48].
- Alternative approaches, involving highly sensitive immunoblotting and ELISA methods, are now being applied to tissues collected in several studies.
- Secondly, the traditional approach of bioassay in laboratory rodents has been used, and inevitably takes a long time to come to conclusion. Preliminary results have been published, and, in ARQ/ARQ and AHQ/AHQ sheep, the distribution of infectivity is widespread. In fact it is very similar in distribution to that for scrapie in genotypes that are fully susceptible to scrapie[9,33] .
- In outline the distribution of infectivity detected in studies at the VLA, UK is summarised below[9,33, 48]. Not unexpectedly, lymphoid tissues are found to be infectious first, even within the first few months following infection. By later stages the brain and spinal cord are positive, and close to clinical onset there is widespread distribution of infectivity in the gastro-intestinal tract, involving the stomach, small and large intestine. It is important to note that a key difference between early and late stages of incubation will be the quantity of infectivity present, even if the overall distribution is similar. Clinically affected animals always contain the greatest amount of infectivity. No infectivity has been found so far in muscle of experimentally BSE-challenged sheep.

Genotype of sheep	**Tissues that are infected or positive for abnormal PrP early in the incubation period.**	**Tissues found to be infected or positive for abnormal PrP in clinically infected sheep.**
ARQ/ARQ; AHQ/AHQ	Retropharyngeal lymph node; prescapular lymph node; mesenteric lymph node; spleen; thymus; tonsil; Peyer's patches in the lower small intestine (distal ileum); distal ileum (non lymphoid tissue); liver.	Lymph nodes as in preclinical period; spleen; Peyer's patch; distal ileum; liver; brain; spinal cord; celiac-mesenteric ganglion; vagus.
ARQ/ARR	None	No clinical disease at 9 years post exposure in Suffolk sheep at the time of writing (early 2009), or at termination at 10years in Romneys.
ARR/ARR	None	No clinical disease at 9 years post exposure in Suffolk sheep at the time of writing.(early 2009), or at termination at 10 years in Romneys.

Can goats become infected with BSE?

- Yes. Goats have been experimentally infected by oral exposure with BSE infected brain. Unfortunately few studies have been carried out in goats as research has concentrated primarily on sheep[30, 31].

- It has always been recognised that the consumption of compound feed by milking goats may have put them at risk of infection with BSE in the past, but this has not been reflected by a high incidence of a scrapie-like disease in the goat population in the UK where exposure to BSE would be greatest.
- In countries where goats are more important to the economy, and are present in greater numbers, they do succumb to scrapie, and cases have been recorded in several countries in Europe as a result of active surveillance in the European Union. Greece, Spain and France have the largest goat populations, so it is no surprise that most of the scrapie cases in goats have occurred there. In total, there were only 41 cases in the EU in 2002 out of 54,626 animals tested, 241 in 2003 (6,040 tested), and 398 in 2004 (36,115 tested). In 2005, when surveillance targets were raised, a total of 265,518 goats were tested, with 989 positive. It is worth noting however that 86.5% of all positive cases in goats detected in this period (2002 to 2005 inclusive) were detected in Cyprus (1443/1669), where BSE has never been found, and where there is a serious problem in controlling scrapie outbreaks in goat herds. In contrast, the next highest countries were France with 96 cases (212,638 tested – 0.05%) and Greece with 78 (24,818 tested – 0.31%). France in particular has spectacularly increased the numbers of goats being tested following the detection of BSE in the French goat.
- In 2007, a total of 277,196 goats were tested in the EU, of which 1272 were positive. Cyprus remained the country most affected, with 1158/6781 cases (positive/tested), followed by Greece (53/5880), United Kingdom (26/2732), Spain (20/36,638), France (7/159,721), Italy (6/24,514) and Romania (2/618). The UK situation is unusual in that caprine scrapie has been rarely diagnosed in the past, but a small number of herds have been significantly affected more recently, with multiple cases.
- In contrast to limited analyses on classical scrapie in sheep[17,28], there is no evidence of a declining trend in prevalence in caprine scrapie, but the extent to which Cypriot data distort the overall picture has to be borne in mind. Details of ongoing surveillance statistics in the EU are available at:-
  http://ec.europa.eu/food/food/biosafety/bse/monitoring_en.htm
- In comparison with sheep there has been far less research into the role of goat PrP genetics in determining susceptibility to infection with scrapie. At one time goats were considered to be universally susceptible, but a recent publication identified much greater variability in the caprine PrP gene of British goats than was expected[36]. Some tentative associations between genotype and frequency of appearance of clinical scrapie have been recorded, so it is possible that further research may be able to definitely associate polymorphisms with susceptibility to infection with scrapie[12]. There is little data on the influence of genotype on susceptibility to infection with BSE.

Has BSE been confirmed in a French goat?

- Yes[25]. A goat that was found to test positive in surveillance for scrapie in small ruminants in 2002 was subsequently tested using one of the discriminatory methods mentioned below. It looked similar to BSE by the western immunoblotting and ELISA methods, but this was before these tests were fully evaluated for this purpose. The herd of origin was slaughtered and destroyed in 2002.
- Meanwhile, mice were inoculated with some of the sample. The results of the bioassay studies were evaluated at the end of 2004 by the EU Community Reference Laboratory (CRL) Expert Group on Strains (see below) on behalf of the European Commission.
- Because the brain tissue in this instance was frozen, and not placed in fixative to preserve it, it was not possible to obtain full agreement of immunoblotting, ELISA and immunohistochemical methods. Consequently the analysis of biological data alongside molecular test results was critical to the interpretation. These studies were considered to be compatible with an infection with BSE in the goat.

What is the significance of the confirmation of BSE in the French goat?

- The finding of one infected goat, or indeed one infected sheep, is not necessarily a reason for serious concern. Prior to this finding, the precautionary approach adopted by authorities in Europe has anticipated exposure, and probable infection, but had stopped short of draconian measures until infection with BSE was confirmed. An important factor in determining the scale of response is the number of sheep and goats that are found to be infected with BSE. Despite expansion of the surveillance programme since this finding, no further cases have been identified.
- Consequently, confirmation of BSE in the goat simply confirms that the small ruminant (sheep and goat) population of Europe was exposed to BSE, and potentially infected, but the risk of infection will have varied from country to country. It will have been greatest where there was a significant incidence of BSE in cattle, and a significant goat population that also received feed that was potentially contaminated with BSE.
- Based on the surveillance programme in Europe, there is so far no evidence of an epidemic of scrapie-like disease in small ruminants that is potentially associated with BSE. In addition, the search for BSE in sheep in the United Kingdom, where the risk of exposure should have been highest because it had the largest epidemic of BSE in cattle, has failed to identify a BSE-like infection in sheep or goats[39,51, 52, 62].
- It is therefore unlikely that any country has sheep or goat populations that represent a public health risk on the scale that BSE in cattle did historically. The numbers of potentially infected flocks and herds are expected to be low (see "The interpretation of test results in the United Kingdom"). In addition, the precautionary removal of some specified risk materials from the human food chain will have reduced the probability of exposure of consumers to potentially infectious material.
- The list of SRMs in small ruminants was not modified as a result of this finding. Although much reliance was originally placed on historical research on scrapie in goats for the purposes of risk assessment[41, 42, 43, 57, 58, 59] data from ongoing studies on BSE in sheep are also now taken into account.
- In order to quantify the prevalence of infection more precisely, the European Food Standards Authority recommended and the European Commission and changed surveillance procedures, with increased numbers of goats being targeted for surveillance purposes. As no further cases were detected, surveillance targets have again reduced.
- Although stating the obvious, it is important to remember that the carcases of all animals in which BSE or scrapie are diagnosed (bovine, ovine or caprine) are destroyed and prevented from entering the food chain.

Has BSE been detected in British goats?

- No. The authorities in the United Kingdom did announce that they had evidence that one goat that died of a scrapie-like disease in 1990 may have had BSE.
- Because of the findings in the French goat, scientists at the Veterinary Laboratories Agency attempted to confirm that one of the discriminatory methods described below for sheep (immunohistochemistry, IHC) worked equally well in goats. They examined the brains of goats experimentally infected with BSE or scrapie, and compared them with some natural cases of goat scrapie submitted historically. Using the IHC method, one of these brains appeared to be indistinguishable from BSE in goats, but, as indicated below, this is insufficient on its own to confirm the presence of BSE.
- Further investigations involving bioassay of the goat brain remain inconclusive at the end of 2008, and require further sub-passages to assist interpretation of results.

The search for BSE in sheep

The problems of identifying strains of prions

- Many techniques have been perfected which allow precise comparison of microorganisms in order to determine whether they are closely or distantly related. These techniques

involve assays to examine the nucleic acids (DNA or RNA) present in the organism, or to detect the presence of proteins (antigens). Such assays have become increasingly sophisticated and precise with time.

- Unfortunately, prions do not appear to require or carry nucleic acids. Prions consist of protein that is produced by the host. The only difference between the normal and infected animal is that the protein in the infected animal is folded abnormally. This makes it difficult for the body to eliminate, and eventually leads to disease.
- This means that the host does not produce antibodies which can be used to characterise the prion. At face value, when routine diagnostic tests are used to confirm the presence of infection, they cannot yet distinguish between strains of prion.

Is it possible to distinguish between scrapie and BSE in sheep?

- During the course of the BSE epidemic, initial research to characterise BSE, and to compare it with scrapie, involved the use of a biological method of characterising prion strains. This involved the inoculation of in-bred strains of mice, the measurement of incubation periods in the mice (time between inoculation and death or the onset of clinical disease), and the examination of the pattern of damage caused in the brain (lesion profiling)[13, 14].
- This technique takes time - potentially five or more years. Individual scrapie isolates eventually generate consistent incubations and lesion profiles in mice that are considered to be characteristics of "the strain". This usually requires brain tissue from infected mice to be inoculated into further generations of mice (sub-passage).
- When BSE was investigated by the same technique, it also generated incubation periods and lesion profiles that were characteristic of BSE and no other strain. In addition, it appeared that BSE could be distinguished from scrapie at the stage of primary inoculation (i.e. from cow to mouse). Nevertheless, this still takes 2-3 years, and is therefore not ideal for routine screening of animals. It is also expensive.
- A range of molecular methods were however being developed as research tools, and were shown to have some potential to distinguish between BSE and scrapie[5, 6, 37, 38, 40, 47, 49, 50, 53, 54, 56, 61, 63, 64]. As these were based on methods that were also being introduced for routine testing of cattle and sheep for surveillance purposes, they presented opportunities for faster testing of larger numbers of samples. They also potentially reduced the need for animal experimentation.

What is discriminatory testing?

- In the context of this paper, discriminatory testing means the application of molecular tests to distinguish between BSE and scrapie in sheep. Although it may become possible to distinguish between individual strains of prions in future, this is not yet possible.

How long does discriminatory testing take?

- In principle discriminatory testing should take only a matter of days. In Europe, National Reference Laboratories conduct the first stage of discriminatory testing, using a western immunoblot approach. If the samples appear to deviate from the normal in particular ways they will then be subjected to closer scrutiny.
- The Veterinary Laboratories Agency, which is the Community Reference Laboratory (CRL) for the EU, will then co-ordinate further investigation with a group of experts, and using the methods already evaluated. The results will then be interpreted by the experts and reported to authorities in the country of origin.
- Because of the need to send samples from laboratory to laboratory this will inevitably introduce a slight delay in the evaluation, but the outcome will be known much more rapidly than if relying on characterisation by bioassay. Approximately 5 such evaluations arise each year for the whole of the EU. None have so far been categorised as BSE.

How are molecular tests evaluated?

- The tests used for routine testing of cattle and sheep are based on testing methods that are commonly used for other infections, involving ELISA or immunoblotting techniques. When used to test for BSE or scrapie most tests involve some digestion of the prion protein with an enzyme. BSE and scrapie are digested at different rates, leaving behind parts of the prion protein that are more or less accessible to the antibodies that are used to detect them in the tests. The differences in the ability of antibodies to bind to the prion protein can therefore be used as an indicator of which prion protein is present – BSE or scrapie.
- This led to publication of preliminary data based upon a comparison of natural scrapie and characterised sheep scrapie isolates, with BSE from naturally infected cattle or experimentally infected sheep[38, 40, 53, 61].
- In particular, when detected using western immunoblotting methods three bands are normally detected from digested prions (see figure below). The molecular weight of the lower band differs between BSE and scrapie (lower for BSE). In addition, although both BSE and scrapie are detected in the routine test, the introduction of a second antibody, which appeared to detect scrapie but not BSE, offered potential to distinguish between the two when testing samples from sheep[56, 60, 63].
- Other laboratories have developed similar approaches, using western immunoblotting, and have confirmed the validity of the approach. Similar principles underpin the use of ELISA test techniques – namely reliance on the differential sensitivity of BSE and scrapie to proteinase digestion, and the detection of different signals from the residual molecules[38].
- In April 2002 the Scientific Steering Committee issued advice to the European Commission on the evaluation of molecular methods, and their introduction into a surveillance programme[20]. In the opinion it recommended that tests that were claimed to have discriminatory power should be compared by means of a ring trial. Ring trials involve the provision of samples of brain tissue to test developers, in parallel, with all samples being tested blind. In other words, the testing laboratory does not know if the samples are from scrapie or BSE-infected animals. By identifying one or more tests that correctly identify the sources, they can then be used for future evaluation of field isolates.
- Such a ring trial was conducted in 2003 and 2004 under the control of the Veterinary Laboratories Agency (acting as CRL). Samples of bovine and ovine BSE (ovine BSE was brain material from sheep experimentally infected with bovine BSE), and sheep scrapie were tested. The bovine BSE and ovine scrapie were from naturally infected animals.
- Three western immunoblot methods, one ELISA and one immunohistochemical method were compared. All were in total agreement on the relatively limited range of samples that were available. Full details of the ring trial results will be published in due course. Further methods are currently being evaluated. The Expert Group that undertook the task of evaluating the data recommended that no diagnosis of BSE in sheep should be made on the basis of molecular tests alone unless there was full agreement of western immunoblot, ELISA and immunohistochemical results.
- The immunohistochemical approach is a variant of techniques already in use for confirmatory testing, and uses a range of antibodies to target prion protein in fixed (preserved) sections of brain tissue[22,49,50,64]. This method mimics the western immunoblotting approach because it also detects evidence of differences in cleavage of prion protein in the cell, depending on whether the sheep is infected with BSE or scrapie.
- These methods have now been introduced into the programme of surveillance for scrapie in Europe in 2005, so that any isolate that looks slightly unusual, and it not categorised as "atypical scrapie" will be subjected to further testing to look for BSE[1].
- Details of how these surveillance tools are used to discriminate between scrapie, BSE and "atypical" scrapie are described in an EFSA Opinion on "the Classification of atypical TSE cases in Small Ruminants[22].

How accurate are the discriminatory tests?

- At the moment it is too early to confidently claim that they will always detect BSE in sheep. Only a small number of experimental infections in sheep have been established, using a limited range of genotypes (ARQ/ARQ and AHQ/AHQ – orally; ARQ/ARQ; VRQ/VRQ; VRQ/ARQ; ARR/ARR; AHQ/AHQ; ARQ/AHQ by inoculation) but so far BSE has behaved identically, and been recognisable, in all studies[2, 8, 9, 16, 22, 49, 54, 56, 61, 62, 63, 64].
- Nevertheless, it has to be recognised that there is a theoretical possibility that BSE will change if it is capable of transmitting naturally from sheep to sheep. After a few generations it may no longer be recognisable as BSE[4, 15, 60]. One study involving inoculation of resistant sheep directly into the spleen did produce test results that were more indicative of scrapie rather than BSE[10,60], which does indicate a need for caution in interpreting the results of surveillance. In other words, claims for failure to detect BSE cannot be interpreted as total absence of BSE in the population of animals tested[23].
- Because of this, investigations are underway to test whether transmission from sheep to sheep does actually lead to such a change. This is being done by oral infection of sheep with BSE derived from other orally infected sheep. The aim is to replicate what may be the natural route of exposure if such transmissions really do take place in life.

How many sheep have been tested with discriminatory tests?

- In the United Kingdom all scrapie cases reported since November 2001 have been tested by a western immunoblot method developed at the Veterinary Laboratories Agency. In addition, since that time, available samples collected since January 1998 have been retrieved and tested by the same technique.
- In addition, where necessary to resolve any equivocal results, some samples were also tested by immunohistochemistry.
- Preliminary analyses combined the data from the prospective and retrospective testing, and involved a total of 2367 samples from positive cases. A total of 2316 were positive by western blot and unequivocal on the basis of the first retest. None were considered compatible with a diagnosis of BSE in sheep(see update below).

The interpretation of test results in the United Kingdom

- The first analysis of the results have now been published[62]. At face value, the fact that no BSE-like results were identified for the 2147 cases that could be traced to their farm of origin implied that at most 0.14% of positive diagnoses in sheep could be due to the presence of BSE.
- Unfortunately the results were derived from cases that were reported to the authorities as suspect clinical cases. Relatively few farmers are thought to report such cases, so inevitably the population of samples that were tested was not selected at random. Consequently the interpretation of the data required more complex analyses. In addition, instead of focusing only on the number of cases tested, it was possible to use the number of source flocks as the basis for calculation. In this situation, it was estimated that up to 0.66% of diagnoses in sheep could be due to the presence of BSE.
- By taking into account these results, and the outcome of surveillance programmes in the United Kingdom in 2002 and 2003, scientists estimated that around 800 BSE-infected sheep could have existed in the UK in each year, in 19 or 20 flocks. Approximately half the sheep were estimated to be adults, and the remainder lambs.
- This method has continued to be used for surveillance in the UK, in accordance with EU law. To October 2006 the total of scrapie-positive cases tested with BSE–negative result has reached 2839. As a result further unpublished calculations suggest that the most likely number of BSE-infected flocks in the UK is zero, with an upper 95% confidence limit of 17 flocks. To the end of December 2008, the total of positive sheep tested has reached 3057, but this will only have a marginal effect on the predicted number of infected flocks.

- Other epidemiological studies have emphasised the difficulties of predicting the number of sheep and flocks infected with BSE, and of determining trends in infection levels[39, 51, 52].

Could BSE in small ruminants have been missed, or mis-diagnosed in the past?

- Yes. There is no doubt that with the benefit of hindsight and the availability of current test methods one might have been able to diagnose BSE in sheep in the past. Nevertheless, most infected sheep would have been expected to develop clinical signs very similar to scrapie. In addition, the parts of the brain affected by BSE are those affected in typical cases of scrapie. Consequently, with the tools available at the time, such animals would have been dealt with as if they were affected by scrapie.
- Additionally, it is important to remember that no matter how good current surveillance programmes are, they are not perfect, and cannot detect every infected sheep. Historical surveillance will have been less effective, because of the general reliance solely on the submission of clinically affected sheep. Consequently such surveillance will only have had access to a fraction of infected animals.

Do atypical scrapie cases represent BSE infection?

- During the course of surveillance for scrapie in Europe, using rapid tests, it was noticeable that one test in particular was identifying apparent positive sheep that were difficult to confirm using traditional diagnostic methods. These became commonly known as "atypical" or "unconfirmed" scrapie. (see also TAFS position paper on "atypical scrapie" and "atypical BSE")
- None of them resemble BSE in sheep. BSE in experimentally infected sheep has been characterised quite well using molecular tools, and this is used for the discriminatory testing referred to above. None of the atypical samples identified so far resemble BSE in sheep by such methods. Indeed some samples have also been inoculated into laboratory rodents to confirm that they are infectious. The results also differ significantly from BSE in sheep.
- In addition, atypical or unconfirmed scrapie occurs in genotypes of sheep that have already been shown to be resistant to infection by mouth with BSE.

In that case, what are atypical scrapie cases?

- For clarification of issues relating to "atypical scrapie", please refer to the TAFS Position paper on Atypical Scrapie and Atypical BSE.

Do prion strains other than BSE represent a human health risk?

- It is not possible to totally rule out a potential human health risk from prion strains other than BSE found in small ruminants. Nevertheless, although some may argue that precautionary measures should be implemented on the assumption that they represent a risk, consideration of available evidence by the BioHazards Panel of EFSA has not provided a basis for such action.
- While it is accepted that the absence of a clear association between prion diseases of humans and those of small ruminants is based on rather limited evidence, the distribution of these diseases in human and ruminant populations around the world do not match. Furthermore, the historical evidence of association is absent even in countries where scrapie has been known for over 200 years, and where scrapie-infected sheep would have been regularly consumed.
- Results from continuing research inevitably extend the knowledge base, but frequently without sufficient clarity to facilitate changes to risk management measures. There is uncertainty about the relevance to humans of some results derived from studies in genetically modified rodents, or from modifications of molecular test methods.
- EFSA has published two opinions on this issue[23,24], prompted in part by concerns raised particularly in France, where there was pressure for a more precautionary approach. The EFSA position, which is based in part on a review of all historical evidence and

interpretations by the Scientific Steering committee as well as more recent scientific results, remains that a combination of factors – low prevalence of infection, low likelihood of exposure, combined with a species barrier between humans and small ruminants – makes it impossible to definitively claim that prion strains other than BSE represent a risk to human health. BSE remains the only strain defined as zoonotic.

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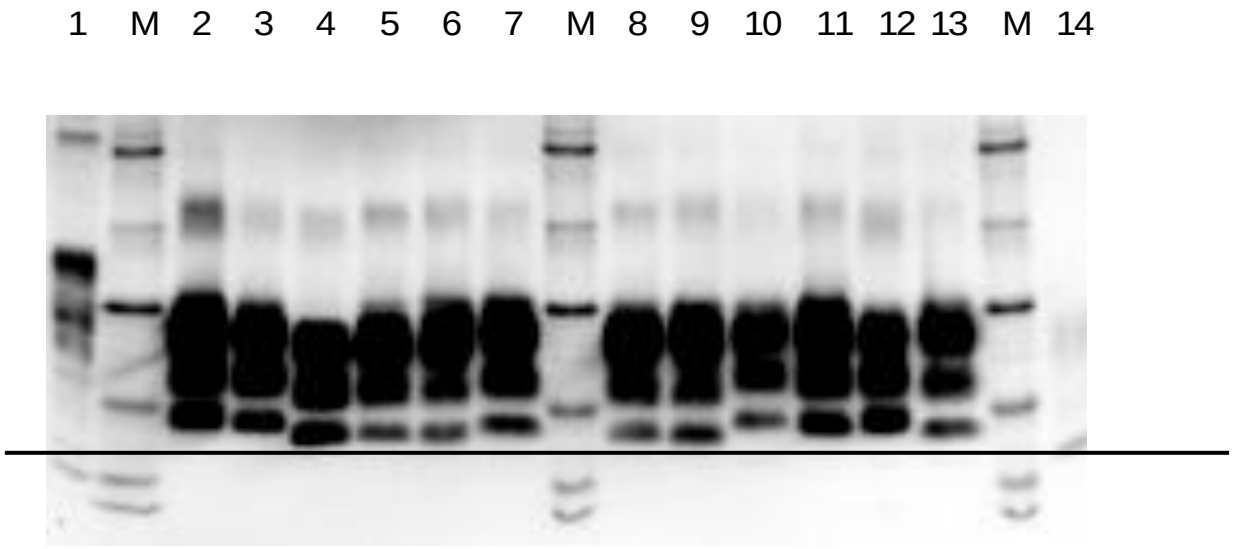
The VLA discriminatory Western blot for BSE/scrapie



BSE (cattle) in lanes 7, 13
BSE in sheep in lanes 5, 6, 8, 9
Scrapie in lanes, 2, 3, 10, 11, 12
Negative bovine control in lane 14

<u>Top photo</u> - gel stained with monoclonal antibody 6H4, which detects all samples.

<u>Bottom photo</u> – gel stained with monoclonal antibody P4 which detects scrapie, but BSE, and BSE in sheep, do not stain, or stain porly.

In lane 4, strain CH1641 is an experimental scrapie strain, maintained in sheep, but which has some, but definitely not all, of the characteristics of BSE. It also stains poorly with antibody P4.
The horizontal line across the top gel gives an indication of the molecular weight of the lower stained band for scrapie. For BSE and BSE in sheep the equivalent band is lower than for scrapie.

Photos and data courtesy of M Stack, Veterinary Laboratories Agency, UK