TAFS

INTERNATIONAL FORUM FOR TRANSMISSIBLE ANIMAL DISEASES AND FOOD SAFETY
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# TAFS[1] Position Paper on Atypical scrapie and Atypical BSE

In recent years there have been a small number of reports in the scientific literature that unusual isolates of BSE have been detected in cattle in various countries around the world. In addition, following the introduction of enhanced surveillance programmes for scrapie in small ruminants in Europe, unusual or unexpected results were also widely reported. In both instances, the shortage of scientific data at the time did not enable scientists to precisely identify what they were dealing with. Because of similarities with the diseases that they were searching for, namely BSE in cattle and scrapie in sheep, the immediate response was to call the isolates "atypical BSE" and "atypical scrapie" for reasons that will be explained below. Some additional local terminology was applied in some countries, but for the moment the term "atypical" is more commonly applied. This paper aims to provide the background to these findings, and explain their signficance.

**Are "atypical BSE" and "atypical scrapie" the same?**

- No. Atypical BSE occurs in cattle. Atypical scrapie occurs in sheep and goats. They differ in their characteristics when examined by both molecular tests and infectivity to laboratory rodents.
- The term "atypical" has only been adopted to indicate that the findings in animals tested were slightly different to what was expected from our understanding of the characteristics of BSE or scrapie. At some point sufficient data will be gathered to rename these isolates, but for the moment they remain grouped under the term "atypical".
- Because they are different, in terms of diagnostic characteristics, species of origin and geographical distribution, they will be dealt with separately below.

**What is the significance of the term "atypical"?**

- The term "atypical" has only been adopted to indicate that the findings in tested animals were slightly different from what was expected. Eventually it should become possible to precisely name such isolates, and to clarify their relationship with BSE and scrapie, but for the moment they remain grouped under the term "atypical" even

[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).

though there is still some variability within each category. In other words, all atypical BSE cases are not identical. Similarly there is variability within the category atypical scrapie.

- Atypical is really only an alternative for "previously unrecognized". This is mainly because the development of scientific tools has allowed the search for prion diseases of cattle and small ruminants to be expanded considerably. Historically the search would have focused on animals that were suffering from clinical disease, but today it can include healthy animals or those found dead or dying from undetermined causes.
- Testing of the additional target populations (healthy or dead) presents additional challenges as positive animals may be detected at an earlier stage of incubation than usual. Testing of brain tissue several days after death when the carcase has started to decompose is an additional confounding factor, as it can influence test results. It is therefore necessary to be cautious because of the potential for the generation of artifacts that have no bearing on the strain of prion actually infecting the animal. This is particularly so where molecular mass of prion protein bands is a critical criterion for categorization as this can be influenced by autolysis.
- At the moment there is no basis for attributing greater or lesser risk to humans to atypical cases than to BSE and scrapie.

## Atypical scrapie

### What does the term atypical scrapie mean?

- Mankind has been aware of scrapie for over 250 years. In the past, infected sheep were usually detected when they became clinically affected, and were subjected to examination of brain tissue after death.
- In the past 15-20 years, through the study of the genetics of sheep clinically affected with scrapie, it appeared that some were susceptible to scrapie, but others were apparently not as they were never represented in the clinical population (see TAFS Position paper on BSE in sheep for more extensive discussion). This absence of sheep of particular genotypes from the population of clinically affected sheep led to a belief that such sheep were at least resistant to clinical disease, and possibly resistant to infection by natural routes.
- The expansion of research programmes due to concerns about BSE has demonstrated that such sheep are not totally resistant as they can be infected by inoculation directly into the brain [(23)].
- Investigations into sheep that were ataxic (walking with difficulty, in an unco-ordinated manner) in Norway identified a form of scrapie that seemed to differ from that seen in other flocks and countries. It occurred in genotypes of sheep that were rarely affected elsewhere. This form of scrapie was termed Nor98 [(5)]. It is the first of the "atypical scrapie" isolates to be recognized.
- When rapid tests were introduced into surveillance programmes in Europe in 2002, many of the sheep being tested were clinically healthy. Test results therefore had to be interpreted in isolation from data concerning clinical signs. Scientists in several countries faced a challenge to confirm positive results obtained by one test in particular, the BioRad Platelia® or TeSeE™ test. Traditional test methods seemed ineffective in confirming the primary results. Equally challenging was the fact that the unconfirmed positive results were in genotypes of sheep that were previously assumed to be resistant. There were doubts about whether or not the fact that the tests had not been evaluated with sheep scrapie was a factor. The results could have been false

positives due to reactions that would not have been recognized or anticipated by their prior evaluation solely on cattle BSE.

- Verification of the results occurred quickly, but uncertainty as to their meaning led to the adoption of various terms – unclassified, atypical, discordant – to describe the cases, and eventually they collectively became known as "atypical".
- In a report to the European Commission, the Community Reference Laboratory Strain Typing Expert Group advised that it was likely that sheep were susceptible to infection with a spectrum of prion strains, only some of which had previously been detected and associated with what was known as classical scrapie. While it was possible that the atypical scrapie isolates were simply part of that spectrum, and had previously not been recognized because appropriate tests had not been available, there was also a possibility, albeit less likely, that they were a new phenomenon.
- This history is extensively discussed in two EFSA Opinions. One, in October 2005[16], describes attempts to classify isolates in small ruminants to ensure that there is consistency of approach between countries in terms of names applied to isolates. The second, in July 2006[17], describes much of the background especially in relation to the interrelationship with genotype and the likely impact on programmes to breed for resistance.

**What are the differences between classical and atypical scrapie?**

- This is still an incomplete area of science, and therefore difficult to fully explain.
- Both types of scrapie clearly involve the presence of abnormal prion protein, and are recognized as prion diseases.
- Classical scrapie, identified in clinically affected sheep, occurs in certain genotypes of sheep. Historically there was only one recorded incident of a positive case in what was thought to be a fully resistant genotype (ARR/ARR) [24]. There were doubts about the diagnosis in that case. Most classical scrapie cases have traditionally been in sheep carrying the VRQ and ARQ alleles (homozygous or heterozygous), occasionally in combination with ARR and AHQ. While it is still a point of debate as to whether a sheep can be classified as infected with classical scrapie if it is not exhibiting clinical signs, there is no doubt that the prion protein found, and the range of genotypes affected, are identical in healthy, found-dead or clinically affected sheep.
- Atypical scrapie has been found predominantly in sheep carrying the AHQ, ARQ and ARR alleles, either homozygous or heterozygous. Full detail is given in the EFSA Opinions[1, 3, 4, 5, 9, 10, 13, 17, 18, 19, 20, 28, 29, 30, 31, 32, 33, 35].
- The abnormal form of prion protein in atypical cases has been shown to be more susceptible to enzymatic digestion than in classical scrapie. Because most of the rapid diagnostic tests use enzymes to prepare the sample for testing, some at higher concentrations than others, they were unable to detect atypical scrapie while others could. The protein had been totally or partially digested during the processing for some tests, to the point where antibodies used in the tests were no longer able to recognize and bind to target sites on the prion protein. This phenomenon has subsequently been demonstrated more precisely[21].
- Other methods based on ELISA or Western Blot techniques are now available which clearly can detect atypical scrapie, but as demonstrated by the evaluation of rapid tests for small ruminants conducted by EFSA in 2005, they are certainly not equally capable[14, 15].

**How was atypical scrapie found?**

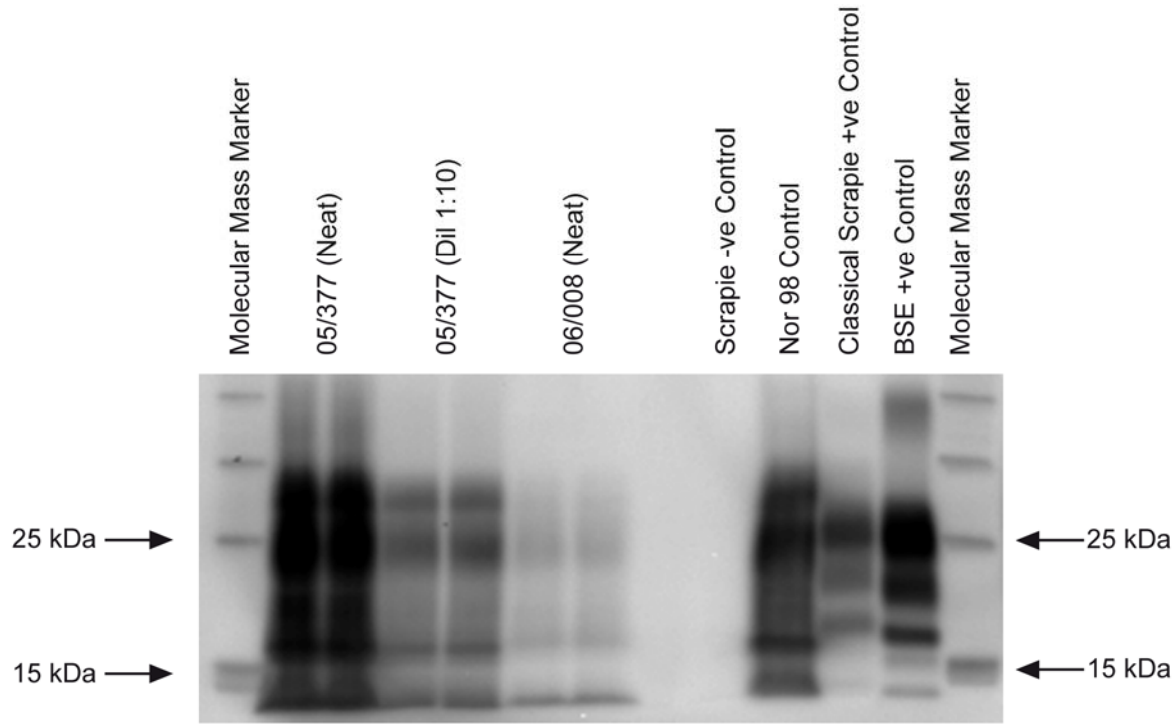
- Nor98 was identified through the investigation of unusual clinical signs in sheep in Norway.
- In the EU it was entirely as a result of the introduction of rapid testing for active surveillance, and initially particularly through the use of one test manufactured by a company called BioRad. Other tests have subsequently demonstrated that they can also detect atypical scrape following the evaluation of tests specifically for use on small ruminants.

**Where has atypical scrapie been found?**

- This has been detailed in the EFSA Opinions described above, but it is clear that apart from Norway, where Nor98 was first identified, atypical scrapie has now been identified in several European and other countries - including the United Kingdom, France, Germany, Belgium, Finland, Iceland, Italy, Ireland, The Netherlands, Portugal, Sweden, Switzerland and the Falkland Islands[1, 3, 4, 5, 9, 10, 13, 17, 18, 19, 20, 28, 29, 30, 31, 32, 33, 35].
- As rapid tests used for active surveillance are not all equally capable of detecting atypical scrapie, this will inevitably influence the apparent geographical distribution. In other words, countries that only use tests that are either unable or less able to detect atypical scrapie are unlikely to have reported cases. Only when the tests evaluated specifically on small ruminant tissues replace those previously evaluated on cattle tissue, will they be able to detect atypical cases with any certainty. This has already occurred in the European Union.

**Is there more than one strain of atypical scrapie?**

- At the moment it is not possible to confirm this until further characterization has been completed, especially by bioassay in laboratory rodents.
- While there is a degree of conformity between western immunoblotting results in most countries, there is also evidence for variability between animals when whole brains are examined by immunohistochemistry. Whether this is determined by the strain or the genotype of the sheep or goat, is not yet known.
- Immunohistochemically the distribution of staining in the brain is quite different to classical scrapie, with only faint, focal, staining of the brain stem at the level of the obex, but more substantial staining of cerebellum of most animals, although in some it is the frontal cortex that is targeted. In some animals focal plaques are also seen in both grey and white matter.
- Many of the cases are similar to Nor98 in terms of diagnostic criteria that have been characterized so far. The western blot below demonstrates how the banding pattern for classical and atypical scrapie differ.




**From Konold et al. ref 23**. Case 05/377 is a British case of atypical scrapie, with duplicate samples tested at each dilution. Nor98 is a positive control sample from Norway, and sits alongside classical scrapie and bovine BSE for comparison.

**Can atypical scrapie cause clinical signs?**

- Yes. In addition to Nor98 cases reported in Norway, a small number of cases have been detected in the UK. In Norway the most common sign was progressive incoordination (ataxia), together with some weight loss. There was no evidence of scratching. Scratching is not universally seen in classical scrapie however, and may depend on the strain of classical scrapie with which the sheep is infected[5].
- In the British sheep the predominant sign was again ataxia, with inconsistent recording of changes in temperament, loss of condition, or circling, and in one case scratching[26, 27]. Video clips of clinically affected sheep can be viewed via reference 24. Similar clinical cases have also been recorded in Ireland[32].

**Is it transmissible?**

- Experimentally, it has been shown that it can be transmitted to genetically modified mice[28], and by intracerebral inoculation to sheep (unpublished work in progress).
- These transmissions do not prove that it will transmit naturally from sheep to sheep, but studies involving oral infection of sheep are under way.
- Although most atypical cases occur singly in flocks, there are some instances where two affected sheep have been identified in flocks. This may indicate that natural transmission may occur, or that the sheep were infected from a common alternative source[22, 29]. Possible indications of an association with the feeding of vitamins and mineral feed supplements were detected in Norway, but remain to be proven[22].

**Does it represent a risk to human health?**

- This is currently unknown, but if atypical scrapie is not a new phenomenon, and has simply been discovered recently, then the lack of epidemiological association between

prion diseases in humans and sheep, or consumption of sheep products, suggest that atypical scrapie does not represent a risk to humans. This is not however demonstration of absolute safety.

**What is the impact of this finding on breeding programmes in sheep flocks?**

- Based on apparent resistance to clinical scrapie that is conferred by particular genotypes, especially those coding for arginine (R) at codon 171 of the PrP gene (see TAFS Position paper on BSE in sheep), many countries have introduced breeding programmes that aim to increase the resistance of their national flocks to scrapie. The driving force for such programmes was the fear that sheep might be infected with BSE, and experimental evidence suggested that such sheep might also be resistant to BSE if exposed to it on farm.
- The detection of atypical scrapie in what were presumed to be resistant genotypes has inevitably undermined confidence in the breeding programmes. Apart from the recognition that such a policy cannot guarantee the elimination of prion infections in sheep, there are also concerns that they may result in the selection of new strains that specifically target the "resistant" genotypes, and that these may even represent a greater risk to animal and human health.
- The EFSA Opinion of July 2006[17] considers all available evidence and concludes that there is still no basis for stopping such breeding programmes, but has recommended that additional investigations are required to fully understand the impact of atypical scrapie on both science and policy.
- It is however entirely possible that reconsideration of future policy will be needed, country by country, farm by farm, depending on the risks faced.
    - For example, if the risk from BSE in sheep is considered to be low, negligible or acceptable, the pressure to produce "low-risk" sheep for slaughter, by breeding for R at codon 171 of the PrP gene will reduce. This is largely a decision to be taken at national, or international levels.
    - If BSE is ruled out as a driver for the breeding programme, responsibility for decision making will almost certainly revert to farmers. Some may continue to breed for resistance because it presents them with a commercial advantage, or because they have experienced clinical scrapie in their flocks, and wish to eliminate clinical disease, and hopefully infection, by maintaining such a breeding policy.
    - It is conceivable that most farmers will, however, abandon any policy to breed for resistance, or at least will give it a lower priority, because in general scrapie does not significantly affect production and profitability in the majority of affected flocks.

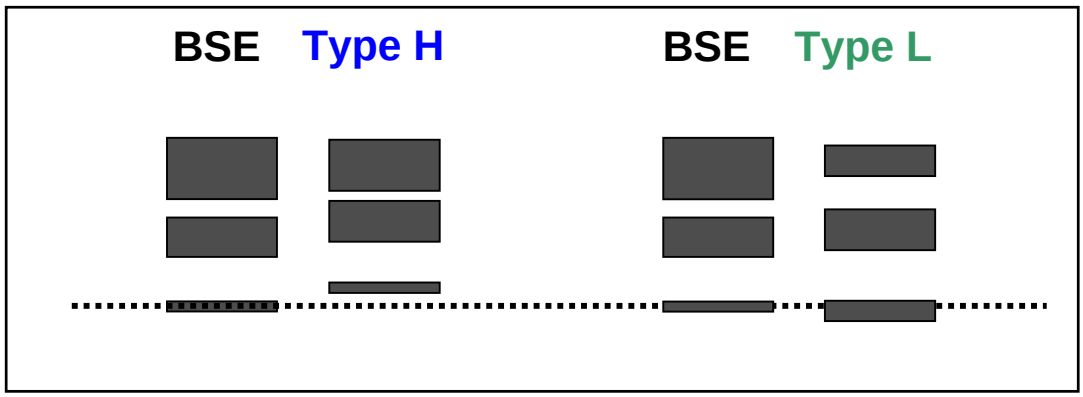
## Atypical BSE

**What does the term atypical BSE mean?**

- During the course of the BSE epidemic in the United Kingdom, the examination of BSE cases, and research investigations, led scientists to believe that the disease was caused by a single strain of prion. It has to be remembered that this characterization of the strain was not based on direct examination of the prion. It was based on an examination of the cattle that were affected with BSE – the distribution of vacuolation in their brains and the consistency of clinical signs. Within both of these parameters

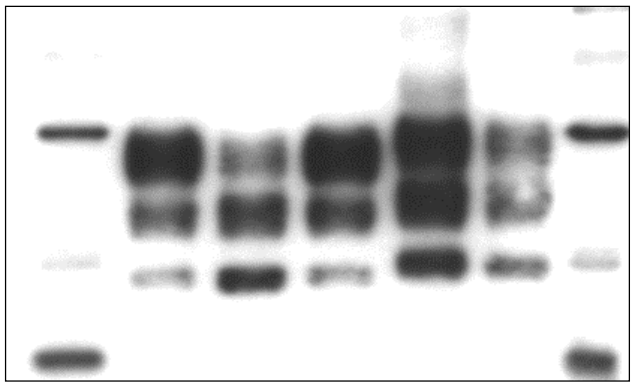
there was some variation, but also consistency of core features that led to the belief that only one strain was involved.

- The first alarm bells rang in Europe and Japan almost simultaneously. In Europe, using tests that were not available for the examination of most of the historical British cases, investigators identified some new features. Western blotting was increasingly used to confirm cases of BSE detected by other methods of testing.




Courtesy of Dr Thierry Baron, AFSSA, France. Please see below for a description.

- The figure above is a schematic representation of the banding pattern normally seen in cases of BSE and in H and L-type atypical cases based on data from France.
- In typical BSE the distribution of bands of digested prion protein always has a dominant upper band, called the diglycosylated band (it has two sugar molecules attached), with the middle band (monoglycosylated – single sugar molecule) of intermediate intensity, and the lower (unglycosylated – no sugars) band much fainter and narrower. The bands are organized by molecular weight, with highest at the top and lowest at the bottom.
- In the French atypical cases, two variations were seen. In the H type, the H refers to the fact that the molecular weight of the lowest band is higher than the corresponding BSE pattern. For the L type, the same band is now lower than for BSE. In addition, the ratios of band intensities differ in all three types (BSE, H and L atypical). An example of actual results by western blotting is provided below[7, 8].




Courtesy of Dr Thierry Baron, AFSSA, France



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- Cases with a low molecular weight for the bottom band were actually first identified in Italy. In the case of two Italian cows scientists had the advantage of being able to examine whole brain rather than just brain stem. They were therefore able to carry out extensive examinations by immunohistochemistry. This allowed a more detailed comparison of the patterns of staining throughout the brain with those obtained for BSE, and although there were similarities, there were also differences. In particular there was less staining in the brain stem than with BSE, and a greater intensity of staining in the cerebellum. Plaques, focal clumps of staining seen in some other prion diseases but not with BSE, were also found[12]. The Italian scientists coined the term BASE (Bovine Amyloidotic Spongiform Encephalopathy) to describe their case.
- In Japan, concern was raised about one young case, detected through the testing of cattle at slaughter. The animal had tested positive by rapid ELISA test, and faintly also by a sensitive western blot, but confirmation had not been possible using other methods. The young age of the animal (23 months) and the differences in molecular weights of the bands relative to BSE suggested that it might be atypical, but unfortunately very little material remained for further investigations[36].

**How were the atypical BSE cases found?**

- The first findings, in France, Italy and Japan were primarily due to variations in WB pattern, although in Italy this was extended through IHC examination of whole brain. In most countries IHC examination has been impossible, and the primary criteria for classification as "atypical" are the banding patterns described above. As a result, they are increasingly being referred to as H-type BSE and L-type BSE, which may turn out to be more accurate than "atypical"
- All cases were identified in active surveillance programmes, through the testing of healthy animals or what are termed "risk animals". Risk animals are recognized as being at a higher risk of suffering from BSE, and are particularly targeted for surveillance purposes. (See TAFS Position paper on the testing of cattle).

**Where has atypical BSE been found?**

- Although the greatest number of cases is in France(12), increasing numbers of cases have now been identified in other countries – Canada (1), Germany (2), Italy (2), Japan (2), Netherlands (4), Poland (7), Sweden (1), Switzerland (1), UK (1), and USA (2). In Sweden and the USA the atypical cases represent the only indigenous cases detected. In other words – typical BSE has not been detected in native cattle in these two countries [34].
- In France, Poland, Netherlands and Germany both H and L forms of atypical BSE have been reported[25].

**Is there anything else unusual about the cases?**

- Yes. With the exception of the first Japanese case, others have generally occurred in old cows – 8 to 18 years reported in France, 11 and 15 in Italy. Most BSE cases occur in animals between the age of four and six, although very young and very old animals can be affected too.
- In one case, still unpublished, a mutation of the PrP gene has been detected, similar to one found in one form of CJD in humans.It has to be stressed that this has not been identified in every case of atypical BSE, although not all have been analysed in this way.

**Is there more than one strain of atypical BSE?**

- At this stage it is too early to say, but there are early indications that this may be so. Caution is needed because there is a need to be certain that the variations in results are not artifacts, either generated by differences in test methods between countries, or due to degradation of samples before they are tested. This has been shown to generate variations in blotting patterns, but is unlikely to have produced the extensive variations seen in the Italian cases or the H form detected in France and elsewhere.
- So the key to confirming whether or not H and L isolates actually represent different strains will be further characterization following transmission to laboratory rodents and/or cattle. These are the methods normally used to characterize prion strains comprehensively.
- This will also help to confirm the extent to which the atypical BSE cases differ from BSE. In the meantime, especially if it proves possible to transmit isolates to other animals, additional biochemical methods can be used to investigate other aspects of prion protein biology of the different isolates.
- Two publications have already highlighted the difficulties of interpreting data on biological transmissibility. One demonstrates that BSE and "H-type" BSE are different, based upon their behaviour in genetically modified mice, examination of fixed and unfixed brain tissue, and comparison of incubation periods[6]. The other, studying "L-type" BSE (Italian BASE), and using different mouse models, acknowledges apparent differences between it and BSE when first inoculated into mice, but claims that further transmission from mouse to mouse by inoculation produces a strain indistinguishable from BSE (by the limited criteria used in the study) [11].
- These findings suggest that it may prove possible to understand the relationship between BSE and atypical BSE isolates, and between the criteria used to classify them at present, and the actual strain of prion that infects the animal.

**Is atypical BSE transmissible?**

- Investigations are under way in France, Italy, Germany and Japan. Experimental transmissibility to cattle and primates has now been demonstrated for L-type BSE, and to mice for both H and L types [3, 6, 11]. Some of this work remains incomplete and unpublished at the time of writing.
- This does not prove that atypical BSE transmits from animal to animal naturally.

**Does it represent a risk to human health?**

- It is too early to tell whether or not it represents a risk to humans. For the moment it is assumed to be a danger, and is treated like BSE. Results of experimental transmission to primates remain unpublished. Some scientists suggest that similarities between the molecular features of H-type BSE and some prion diseases of humans may indicate that they are related. Care must be exercised in interpreting such preliminary data[8] specifically with regard to suggestions of a cause and effect.
- Transmissibility to cattle has been confirmed, but remains currently unpublished as the study is incomplete. It may therefore be possible to investigate further, by oral challenge, whether or not the infectious agent is distributed around the body in a different way from BSE, possibly infecting tissues that are not considered-infectious in BSE. This may have implications for risk management and public health.
- It is however important to remember that so far only small numbers of atypical BSE cases have been detected compared to the many thousands of BSE cases

- Depending on how atypical BSE cases arise, they may represent a long term problem when BSE has been eradicated, or they may disappear along with BSE because the controls are equally effective in preventing spread of infection. As transmissibility to cattle has now been partially demonstrated, it can be presumed that atypical BSE may transmit to cattle orally through feed, in which case rendering and feed controls should prevent further transmission by that route.

**What is the impact of this finding on measures to control BSE, and to protect consumers?**

- At the moment all measures in place to protect cattle and humans from becoming infected with BSE are considered adequate to protect against atypical BSE. Tests used to detect BSE in cattle have detected the atypical cases too, and on brain tissue, which is already defined as SRM in countries where controls are in place.
- Care will be needed in relaxing such controls, especially if atypical BSE proves to be transmissible directly between cattle, or to humans via tissues that are not currently defined as SRM.
- Similarly, if atypical BSE is demonstrated to arise spontaneously, rare sporadic cases may be expected to occur in all countries with significant cattle populations. This in itself will challenge expectations of total eradication as a result of controls. Nevertheless, the existence of sporadic cases would indicate potential to give rise to further large epidemics if some protective measures are not maintained indefinitely. These may involve prohibitions on the use of certain proteins in animal feed, more rigorous rendering processes, and possibly continued removal and destruction of certain SRM from human food and animal feed chains.

**How did atypical BSE arise?**

- The origin of atypical BSE cases remains undetermined at the moment. The possibilities discussed below are hypothetical, but are possibilities that are the subject of debate within the scientific community.
- It is possible that atypical BSE may be a form of BSE that occurs in older animals possibly after infection early or late in life. Some suggest that BSE may indeed have arisen from atypical BSE cases[(11)], based upon such a transformation identified on transmission from mouse to mouse. Surprisingly the authors do not consider the alternative -- that atypical BSE arose from BSE. It has already been demonstrated with BSE that such inoculations can lead to variability in the nature of the prion eventually isolated[(2)], and this is a recognized feature in the development of laboratory models of prion diseases.
- It is possible that there may be several, unrelated, strains of prion disease in cattle some of which have longer incubation periods than BSE. Clearly, if so, the evidence so far suggests that these occur only rarely.
- Atypical BSE may arise spontaneously in a small proportion of cattle. The existence of sporadic CJD in humans has led to postulation that disease could arise spontaneously in any animal, but this is still not proven to happen. Despite the small numbers of atypical BSE detected so far, in some countries the numbers are too great to suggest that they all arise spontaneously, if it were assumed that such a phenomenon occurred at the same frequency as sporadic CJD in humans.
- It may simply reflect a natural process of maturation or ageing of cows. In other words, prion protein may aggregate naturally, but extremely slowly, to the point where it becomes recognizable as “abnormal” prion protein, and is capable of causing clinical disease in the animal. If this process is so slow that the majority of cattle will

be dead before it occurs, it won't have been recognized in the past. Only the introduction of extensive surveillance in which old cows have been tested would such animals be found..

- It may be that cattle have become infected with prion diseases arising from other species, such as scrapie from small ruminants, although the evidence so far suggests that cattle are not easy to infect with scrapie by mouth.

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