# Opinion of the Scientific Panel on Biological Hazards on classification of atypical Transmissible Spongiform Encephalopathy (TSE) cases in Small Ruminants[1]

**(Question number EFSA-Q-2005-073)**

## Adopted on 26 October 2005


## SUMMARY

Since the introduction of active monitoring in small ruminants in January 2002 (Chapter A.II of Annex III to Regulation (EC) No 999/2001) several Member States (MS) have detected atypical scrapie cases. However there appeared to be differences in the way individual member states were assessing and reporting such cases. To facilitate harmonisation within the EU, the European Commission requested that the European Food Safety Authority (EFSA) assess whether it is possible to propose a definition for atypical scrapie and whether different types of atypical scrapie exist. The Scientific Panel on Biological Hazards further requested that the implications for TSE surveillance also be considered.

They concluded that an operational definition of atypical scrapie in small ruminants is possible (annex 1 of the opinion); this definition is provided in juxtaposition with similar definitions for scrapie and BSE in small ruminants. Sub-categorisation of scrapie and atypical scrapie is premature although this may become possible when more data are available.

The implications of atypical scrapie, as distinct from scrapie, are difficult to quantify in terms of its impact on animal health due to insufficient data. If investigations are to progress to the point where advice can be given on issues such as means of transmission of atypical scrapie within and between flocks, the value of flock slaughter and the implications of atypical scrapie for breeding for TSE resistance programmes, it is essential that statistically valid surveillance should continue in the immediate future.

Surveillance programmes should use appropriate combinations of tests and sampling to ensure that atypical cases continue to be identified. Wherever possible samples of brain tissue should be as large as possible and include brain stem and cerebellum as a minimum. Furthermore, collection of whole carcases should be encouraged. This will enable the biological characterisation of isolates to continue, and facilitate


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transmission experiments in the natural hosts (sheep and goats) and the preparation of reference material for future test evaluations.

**Key words:** TSE, scrapie, atypical, definition, surveillance, sheep.

## BACKGROUND

Since the introduction of active monitoring in small ruminants in January 2002 (Chapter A.II of Annex III to Regulation (EC) No 999/2001) several Member States have detected atypical scrapie cases. These cases seem to differ from classical scrapie in several ways, such as an unusual sensitivity of associated $PrP^{Sc}$ to proteinase K treatment, the pattern of deposition in the brain of the abnormal form of the prion protein, the pathology, the epidemiology and the unusual occurrence in sheep with genotypes previously associated with resistance to scrapie. It was however not clear if all of these cases were really different from classical scrapie, and for those that were, whether there were different groups of atypical cases. Atypical scrapie cases often show similarity to the Norwegian Nor98 cases. Harmonisation between Member States is needed in order to decide which cases should be considered atypical.

An overview of TSE cases considered as atypical in different Member States was provided by the European Commission services in the mandate and is attached. The Scientific Panel on Biological Hazards of the European Food Safety Authority (EFSA) has considered the first cases of atypical scrapie in an earlier opinion (EFSA, 2003). Furthermore, an expert Panel on strain-typing, coordinated by the Community Reference Laboratory (CRL), evaluated the nature and the significance of atypical cases in its report to SANCO (CRL report, 2004) and underlined the heterogeneous nature of atypical cases and finally, several scientific papers have been published on atypical cases. The experts of the (EFSA) are requested to evaluate, in collaboration with the CRL and its expert group on strain-typing, the most recent updated scientific information and data on atypical cases in order to indicate criteria to define atypical cases or to identify certain groups of atypical cases taking into account the statements of the CRL panel of experts.

## TERMS OF REFERENCE

In order to harmonise what is considered as atypical scrapie by different Member States, the EFSA is invited to evaluate the existing information on atypical cases in collaboration with the CRL for TSE and its expert group on strain-typing, and:

- To indicate criteria to define atypical cases, or
- To determine certain groups of atypical cases.

## REPORT

### 1. Pre-amble

The original title of the mandate was given as a “request for an opinion of the European Food Safety Authority on classification of atypical scrapie cases in sheep”. It was considered by the Experts at the Scientific Panel on Biological Hazards that the

mandate should be broadened to include all transmissible spongiform encephalopathy (TSE) cases in small ruminants. With regard to the Terms of Reference, the experts further requested that the implications for TSE surveillance were also considered.

## 2. Background

Natural scrapie in small ruminants, in particular sheep, is a transmissible, progressive neurological disease with a range of clinical signs: weight loss, salivation, pruritus and associated fleece loss and skin abrasions, nervousness and altered behaviour and in-coordination of the hind limbs. In the past, the disease was confirmed by examination of the brain tissue principally for histopathological evidence of grey matter vacuolation, and, more recently, by immunohistochemical (IHC) or biochemical detection of abnormal prion protein ($PrP^{Sc}$), in brain, spleen or other lymphoid tissues (Hope, 1998).

Classical cases of scrapie – the so-called "typical scrapie" (OIE, 2004) are associated with vacuolation, the accumulation of a relatively protease-resistant form of abnormal prion protein consistently in the brainstem at the level of the obex and are usually, but not uniquely, found in animals carrying an ARQ or VRQ PrP allele[2]. In heavily infected flocks, evidence of natural infection has been found as early as two to four months of age in VRQ/VRQ lambs by IHC for abnormal PrP in tonsil biopsy samples (Andreoletti *et al.*, 2000). In general, however, the timing and spread of infection within a flock or individual sheep and the time of onset of clinical disease depends *inter alia* on the age at which infection occurs, the route of exposure, breed and PrP genotype, and the type or strain of infecting agent. The multiplicity of factors which contribute to TSE neuropathology and pathogenesis make case definitions based on these criteria very broad and unhelpful, while their inevitable simplification in guidelines for surveillance may lead to severe underestimation of the extent of infection. This caveat applies to clinical, histochemical or molecular definitions of disease: for example, it may be misleading to define scrapie simply as "pruritus", or simply as "vacuolation at the obex in a sheep showing neurological signs" or simply by evidence of "protease-resistant prion protein in brain or lymphoid tissue by Western blotting or immunohistochemistry". The multiplicity of factors which contribute to the presentation or case definition of classical scrapie in small ruminants is expanded in more detail in recent reviews (OIE, 2004).

## 3. Practical definitions of TSE in small ruminants

The broad range of criteria for a diagnosis of scrapie described in the international literature (OIE, 2004) has presented difficulties for the classification of suspect TSE in

[2] The sheep prion protein gene encodes a protein-coding open reading frame of 254 amino-acid codons. Common polymorphisms at codons 136 (V or A), 154 (H or R) and 171 (R or Q) define alleles (represented by the single letter amino-acid code in the order 136:154:171; eg. ARQ : alanine at codon 136, arginine at codon 154 and glutamine at codon 171) that are linked to survival time of sheep exposed to natural scrapie or experimental scrapie and BSE.

small ruminants in some EU member states especially where clinical cases of scrapie have not previously been recognised in a country, or have rarely been found. In 2004, to clarify this issue, the EU CRL WG on TSE strain typing recommended operational guidelines based on the test criteria used in active and passive surveillance - namely detection of abnormal prion protein by immunoblotting, ELISA, immunohistochemistry, electron microscopy (for scrapie-associated fibrils – aggregates of abnormal prion protein) or any additional PrP-based confirmatory tests approved in future (CRL Report, 2004). Positive tests for abnormal PrP would be indicative of a type of prion protein disorder and such cases should be called "TSE in small ruminants" that conforms to the nomenclature of TSE Regulation 999/2001 even though this may not yet be justified on purely scientific grounds. This operational definition of "TSE in small ruminants" encompasses classical scrapie, atypical scrapie including Nor98 and BSE in sheep and goats and it is now mandatory throughout the EU that such cases are subjected to further testing to discriminate between scrapie and BSE (ref)[3]. The criteria for these TSEs in small ruminants are summarized in the Table "Criteria for the categorisation of TSEs in small ruminants" (Annex 1).

**a) Scrapie**

On the basis of the criteria proposed here, the term "scrapie", both classical and atypical, would be reserved for (i) cases that were positive in confirmatory tests for abnormal PrP[4], and (ii) where a diagnosis of BSE in sheep or goat was excluded. Confirmation of such a case of scrapie has important risk management consequences, such as statutory genotyping or slaughter in the flock of origin. These recommendations are an attempt to align the working and evolving knowledge of these diseases with the terminology used in legislation.

[3] All such cases are required to be submitted to testing by an approved discriminatory western immunoblot specific for the prion protein, initially at the National Reference Laboratory or other approved laboratory. Evidence of a PrPres band 1-1.5 kDa lower than 21 kDa for the unglycosylated band of PrP or poorer staining with monoclonal antibody P4 (or equivalent N-terminal antibody according to the discriminatory blot method adopted) when compared with that using 6H4 (or equivalent core-directed primary antibody), or a poor signal on the blots, would require submission of further material to a ring trial co-ordinated by the CRL. Given the current uncertainty about the interpretation of molecular tools, full agreement of the results of ring trial tests (one WB, ELISA plus IHC) is required for identification of a suspect case of BSE in small ruminants and the case should be confirmed by discriminatory mouse bio-assay.

[4] Or in specific cases identified by passive surveillance where clinical signs are consistent with a scrapie diagnosis and pathognomic vacuolar changes are seen in the brain or brain stem at post-mortem histopathological analysis

### b) Atypical TSEs in small ruminants

In 1998, the molecular and histopathological spectrum of TSEs in sheep was extended by the discovery in Norway of an experimentally-transmissible, PrP-related, neurological disease of sheep that was distinguishable from classical scrapie and was therefore considered to be an "atypical" form of scrapie (Benestad *et al.*, 1999, and 2003). These Nor98 cases, the prototypes of "atypical" TSE, have little or no vacuolation or abnormal PrP at the obex[5], but in most cases exhibit an intense cerebellar $PrP^{Sc}$ deposition/accumulation characterised at a molecular level by a smaller and less stable protease-resistant core of $PrP^{Sc}$. Nor98 and other "atypical" cases subsequently identified are more often but not uniquely, found in animals carrying alleles not usually associated with classical scrapie (Annex 2). For Nor98, this genotype correlation has been further refined recently to implicate another dimorphic codon in the PrP open reading frame, L141F (Moum *et al.*, 2005). Other "atypical" TSE phenotypes , including those that are similar to or the same as Nor98 have now been published or in press/submitted from France (Buschmann *et al.*, 2004b), Germany (Buschmann *et al.*, 2004a; 2004b), Sweden (Gavier-Widen et al. 2004), Ireland (Onnasch *et al.*, 2004), Portugal (Orge *et al.*, 2004), Belgium (De Bosschere *et al.*, 2004) and the UK (Simmons *et al.*, 2005; Everest *et al.*, 2005).

**Table 1: Atypical TSE cases reported to the European Commission**

| Member State | Number of atypical cases | Comment |
|---|---|---|
| France | 69 | 7 ARR/ARR sheep; 6 goats |
| Spain | 17 | 5 cases found on herds with other atypical cases |
| United Kingdom | 87 | 14 ARR/ARR sheep |
| Germany | 64 | 82 % of all cases; 4 ARR/ARR sheep |
| Portugal | 29 | All cases atypical (23 Nor98); 5 ARR/ARR |
| Sweden | 6 | All cases atypical (Nor98 like) |
| Ireland | 4 | Nor98 like, 3x clinical signs |
| Belgium | 2 | 1 ARR/ARR sheep |
| Norway | 45 | Nor98 : 80% of TSE cases; 1 ARR/ARR |
| Finland | 1 | Nor98 |
| Netherlands | 1 | Nor98 |

Table 1 above shows the number of cases reported in these and other EU member states at 30th June 2005. Further data from individual member states are provided in

[5] IHC does now reveal minimal amounts of immunostaining in the nucleus of the spinal tract of the trigeminal nerve at the level of the obex in many "atypical" cases. However, immunostaining of the dorsal motor nucleus of the vagus (which is consistently involved in 'classical' scrapie) would appear to be distinctively not involved in 'atypicals'.

Annex 2. In general, these data are included in reports to the EC for the period of surveillance, 2002-2004, when the designation "scrapie" was based on a positive screening test on brain or spinal cord using one of the EU approved BSE test kits for abnormal prion protein $PrP^{res}$ or $PrP^{Sc}$ (Grassi *et al.*, 2001; Moynagh and Schimmel, 1999) followed by confirmation of diagnosis by PrP immunohistochemistry at the level of the brainstem or $PrP^{res}$ Western blotting of residual brainstem or spinal cord. The PrP genotype was determined and reported for these suspect cases.

In several countries, it was not possible to confirm diagnosis of scrapie by standard IHC and WB confirmatory methods. These "unconfirmed" cases contained a form of abnormal prion protein that is less robust than that usually found in classical scrapie cases and which, as a result, were consequently missed by standard biochemical methods which included a stringent PK digestion step. Some rapid screening tests appear more capable of identifying the abnormal PrP in these atypical forms. This increased sensitivity has recently been confirmed in an EU test evaluation carried out by the EC, Joint Research Centre (JRC-IRMM) (EFSA Report, 2005a, 2005b). These atypical cases seem to predominate in PrP genotypes not usually associated with classical scrapie, including the ARR/ARR genotype in which natural clinical scrapie is exceedingly rare or absent.

## 4. Classification of atypical TSEs

The Table in Annex 1 summarises the various criteria that have been used in Member States to define scrapie and atypical scrapie. It also contains the criteria used for laboratory diagnosis of experimental BSE in sheep. These criteria should be applicable to tissues obtained by both passive and active surveillance. It may not be possible to apply all these criteria if brain tissue is compromised by post-mortem autolysis and so minimum criteria are proposed for each category.

## 5. Implications of atypical scrapie

*Section 5 in this part of the document is reproduced from the report of the CRL expert group on strains of 16/17 September 2004 (CRL, 2004) addressing the nature of atypical scrapie. Several WG members wished to amend or update this section and their comments have been included as footnotes in the relevant places.*

### a. The nature and significance of cases currently referred to as "atypical scrapie" or "unclassified scrapie"

*The terms "atypical scrapie" and "unclassified scrapie" have evolved through the difficulties presented in confirming scrapie in samples that were positive in the Bio-Rad TeSeE test. As such cases were investigated more fully it became clear that they were not "false positive" products of rapid testing but represent a real component of the spectrum of prion protein disorders of small ruminants.*

*Investigation has frequently been compromised by shortage of test material. Most samples have arisen during active surveillance, and therefore have not usually been associated with clinical signs at the outset, which could simplify post-mortem*

*diagnosis. Sampling and storage arrangements have also potentially compromised subsequent investigations, sometimes by premature freezing of tissues, or failure to select preferred target sites for histopathological examination. Autolysis is another confounding factor and may prevent the conduct of immunohistochemical examination if the brain is totally liquefied.*

*Nevertheless, the accumulated evidence from samples detected in 2002, 2003 and 2004 (see Annex II) indicates that* ***"atypical" scrapie simply represents one or more isolates of scrapie (or more correctly TSEs in small ruminants) that have not been described in the past****. The similarity of many to the phenotype of Nor98, where clinical signs have been described, suggest that what was initially perceived as a local strain in Norway may be distributed much more widely, although the heterogeneous nature of "atypical" cases suggests that attempts to classify them all as Nor98 or Nor98-like would be premature. The lack of detail about the clinical history of cases found through active surveillance can only be rectified by investigation at the farm of origin, but may assist in future case definition.*

*In line with an operational classification of samples (see above), it is now clear that almost all samples previously classified as "atypical" should now be incorporated within the broader operational terms of prion protein disorder, TSE in small ruminants, or scrapie.*

*The widespread distribution of "atypical" scrapie cases in Europe suggests that they represent a previously unrecognised isolate or isolates rather than one that has recently dispersed between countries. There is limited evidence from ongoing studies in France that some atypical isolates including Norwegian and French Nor98 or Nor98-like isolates (among them ARR/ARR isolates), can be transmitted to laboratory transgenic mice over-expressing ovine PrP.*

*However, to date, the presence of infectivity in the brains of ARR/ARR sheep has yet to be seen using these transgenic transmission models. Similar bioassay programmes in other countries have not yet progressed to the point of supporting the French evidence. The consistency of post-mortem diagnostic phenotype in atypical cases across a range of sheep genotypes suggests that atypical scrapie in all genotypes may have a common aetiology. This led the CRL WG to predict that it is likely that atypical scrapie in the brains of ARR/ARR sheep will transmit to transgenic mice.* [6]

*Transmission to transgenic mice does not, of course, prove that atypical scrapie transmits naturally from sheep to sheep, either horizontally or vertically, but there is little evidence to suggest that atypical isolates behave any differently from recognised forms of scrapie. Limited evidence of multiple cases in some flocks suggests that*

[6] There is now clear evidence that ARR/ARR atypical scrapie (3 cases) is also transmitted to tg338 mice in a very similar way to other atypical cases (6 cases including a goat) (Le Dur *et al.*, PNAS in press). In addition, all atypical cases (including ARR/ARR animals) transmit a disease with a very similar phenotype to that observed when samples from Norwegian Nor-98 cases are inoculated.

*horizontal transmission or exposures to common sources must be considered possible (see Annex 2). Spontaneous and/or genetic origins cannot be excluded.*

*Evidence from Norway indicates some sheep infected with Nor98 should present with clinical signs, although the majority of cases have been found through active surveillance in the absence of clinical signs. It is possible that in older sheep such signs will be too indistinct to prompt suspicion of scrapie. The apparent absence of clinical signs in "atypical" scrapie cases may also reflect a combination of age, poor observation, and lack of awareness on the part of farmers and veterinarians. There has been no scope so far to take into account the influence of breed, country or age on the occurrence of "atypical" scrapie.*

**b. The relevance of "atypical" scrapie cases with respect to their relationship to BSE in sheep and to programmes for breeding for resistance for TSEs in sheep.**

***None of the evidence gathered so far suggests that "atypical" cases of scrapie are caused by BSE in sheep. Indeed, available evidence strongly challenges such an association.*** *Experimental BSE in sheep, produced by oral or parenteral infection of sheep, has been thoroughly characterised using current diagnostic methods of histopathology, immunohistochemistry and western immunoblotting. The results of all these methods of investigation are quite different for BSE in sheep, scrapie and "atypical" scrapie sources or cases. Furthermore, some of the "atypical" cases have occurred in sheep carrying the ARR allele, which have hitherto remained resistant to oral challenge with BSE (see below). BSE-challenged sheep show none of the diagnostic characteristics identified in "atypical" scrapie cases.*[7]
*It has not yet been possible to look at the tissue distribution of infectivity or immunostaining throughout the body of sheep affected with "atypical" scrapie cases. If the similarity between Nor98 and "atypical" scrapies was applicable also to pathogenesis, then a comparison of pathogenesis in Nor98 and BSE in sheep should also inform the debate. BSE in ARQ/ARQ sheep produces widespread involvement of lymphoreticular tissues. There is, as yet, no evidence of peripheral involvement of lymphoid tissues in Nor98 cases in a range of PrP genotypes either during oral or ic challenge of ARR/ARR genotypes with BSE. Such interpretation should however be treated with care in view of the limited amount of data available.*

***The detection of "atypical" scrapie in genotypes previously presumed to be resistant to scrapie clearly raises doubts about the extent to which breeding for resistance will eliminate all scrapie from European sheep flocks (Bayliss & McIntyre, 2004)****. In the UK the range of genotypes affected with atypical scrapie is wide, but with the*

[7] Transmissions to tg338 from both Norwegian Nor-98 cases, and atypical scrapie from France, are very different from those observed with BSE (BSE in cattle, experimental BSE in sheep or goat and natural BSE in the CH-636 French goat) transmissions to the same transgenic model (Le Dur *et al.*, PNAS, in press).

*top three groups (1, 2 and 3 in the UK National Scrapie Plan) in terms of resistance to currently recognised clinical forms of scrapie being apparently disproportionately represented. A more susceptible genotype (group 4, specifically one ARQ/VRQ heterozygote) is however represented. Whether or not genotypes are disproportionately represented requires a comparison with an appropriate population of samples from the national flock. Until now such samples are usually biased because of the nature of the populations being genotyped*[8].

*Under these circumstances, while progression towards a scrapie-resistant population may eliminate clinical disease in susceptible genotypes, it may not eliminate all scrapie infections. This was of course recognised at the outset as an unknown that could not be guaranteed. Nevertheless, if breeding programmes reduce economic losses by eliminating the side effects of clinical scrapie in younger sheep (higher mortality; lower production figures in terms of lambs produced by each ewe during its lifetime) this may still be beneficial to farmers. It is however too early to comment on whether breeding for resistance as currently practiced is neutral in terms of its potential to select new and/or pathogenic strains of TSEs.*

*On the basis of experimental evidence however, namely the resistance of ARR carrying sheep to oral infection with BSE (based on absence of clinical signs to 7 years post challenge, and negative IHC (46 months) and infectivity (22 months) data in sheep culled during the incubation period), breeding for resistance appears to be the most appropriate strategy at this time. This is of course dependent on risk assessments on the likelihood that BSE has transmitted to sheep, and that the primary route of exposure for sheep is oral.*

## 6. The implications of atypical cases for the EU surveillance programme.

The implications of atypical scrapie are difficult to quantify in terms of its impact on animal health. Its biological characterisation through research is still in the early stages and, although the current surveillance programmes in Member States have demonstrated that the brain regions targeted for deposition of abnormal PrP by the infection and those sampled for diagnostic testing – brainstem and cerebellum - are large enough and overlap sufficiently to allow the detection of some atypical scrapie

[8] This remark comes from analysis of UK genotyping data obtained during the 2002-2003 scrapie surveillance exercise. All sheep sampled during active and passive surveillance for TSE testing during this period were genotyped at the standard three codons of the ovine prion protein gene, 136, 154 and 171. In this sample, high frequencies of gene haplotypes associated with relative resistance to clinical scrapie (ARR, AHQ) were observed. However it was concluded that this might be a consequence of the age structure of this sampled population (most animals > 3 years) rather than reflect the true haplotype distribution in the UK flock. Susceptible genotypes (VRQ carriers) would be more likely to have died of scrapie or have been culled before the age of 3 years.

cases as well as classical scrapie, data on the relative sensitivities of current sampling protocols to detect these different types of scrapie are lacking. Test evaluations conducted on behalf of the European Commission have indicated wide variation in performance between rapid screening tests with respect to their capacity to detect atypical scrapie. A full case definition of the pathogenesis and pathology of these cases, therefore, will not be possible without more detailed examination of whole carcases.

If investigations are to progress to the point where advice can be given on issues such as the means of transmission within and between flocks, the value of flock slaughter it is considered essential that surveillance programmes continue in the immediate future and use appropriate combinations of tests and sampling to ensure that atypical cases continue to be identified. Circumstances permitting, samples of brain tissue should be as large as possible, and include brain stem and cerebellum as a minimum. This will enable the biological characterisation of isolates to continue, facilitate transmission experiments in the natural hosts (sheep and goats) and the preparation of reference material for future test evaluations.

It is noted that the current reporting procedures to the European Commission do not facilitate direct comparison between one isolate and another.

## CONCLUSIONS

The Scientific Panel on Biological Hazards concludes that:

1. An operational definition of atypical scrapie in small ruminants is possible (see Annex 1). This definition is provided in juxtaposition with similar definitions for scrapie and BSE in small ruminants. Sub-categorisation of scrapie and atypical scrapie is premature although this may become possible when more data are available.

2. The implications of atypical scrapie, as distinct from scrapie, are difficult to quantify in terms of its impact on animal health due to insufficient data. If investigations are to progress to the point where advice can be given on issues such as means of transmission within and between flocks, the value of flock slaughter, its implications for breeding for TSE resistance programmes, it is essential that statistically valid surveillance should continue in the immediate future.

3. Surveillance programmes should use appropriate combinations of tests and sampling to ensure that atypical cases continue to be identified. Wherever possible samples of brain tissue should be as large as possible and include brain stem and cerebellum as a minimum. Furthermore, collection of whole carcases should be encouraged. This will enable the biological characterisation of isolates to continue, and facilitate transmission experiments in the natural hosts (sheep and goats) and the preparation of reference material for future test evaluations.

## RECOMMENDATIONS

The Scientific Panel on Biological Hazards recommends:

1. The classification system at Annex 1 is adopted by all MS until further notice. It represents no more than the current state of knowledge, and will require to be updated as scientific knowledge advances.

2. Surveillance programmes, including tests and sampling arrangements, be used so as to enable detection of all forms of TSEs in small ruminants

3. Analysis of data arising from EU surveillance programmes in small ruminants and any investigations specifically established to study the epidemiology of "atypical scrapie" is reviewed in order to ensure that surveillance and test methodologies are appropriate.

## DOCUMENTATION PROVIDED TO EFSA

– Opinion of the Scientific Panel on Biological Hazards of the European Food Safety Authority on the interpretation of results of EU surveillance of transmissible spongiform encephalopathies (TSEs) in ovine and caprine animals, culling strategies for TSEs in small ruminants and the TSE-related safety of certain small ruminant products, adopted on 26 November 2003.

– Report of the CRL expert group on strains of 16/17 September 2004 addressing the nature of atypical scrapie.

– Publication of M. Baylis and K.M. McIntyre, Transmissible spongiform encephalopathies: Scrapie control under new strain, *Nature* **432**, p. 810-811.

– Overview of atypical cases reported to the European Commission and some more detailed information on individual cases when available.

## SCIENTIFIC PANEL MEMBERS

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## ANNEX 1: CRITERIA [1] FOR THE CATEGORISATION OF TSES IN SMALL RUMINANTS

| | Test methods | | | | Comment |
|---|---|---|---|---|---|
| | **1** | **2** [3] | **3** [3] | **4** | |
| **TSE type** | **Approved rapid screening test (EC/EFSA)** [2] | **"Stringent" WB** [4] **(including approved VLA and AFSSA modified SAF-immunoblot)** | **"Mild-PK" WB** [5] **(including approved CEA discriminatory WB, OIE SAF WB +/- NaPTA and , and TeSe Western blot (Biorad))** [6] | **Immunohistochemistry and histopathology** | **Additional criteria to be taken into account in classifying samples.** |
| **Classical Scrapie** | Positive | Three major PrPres bands above 15kDa and of which the di-glycosylated form is dominant and the lowest is non-glycosylated, all reactive with both N terminal and core specific. mAbs; (molecular mass range of 16-30 kDa).Occasionally negative or fewer than 3 bands depending on PrPres concentration. | Three major PrPres bands above 15kDa and of which the di-glycosylated form is dominant and the lowest is non-glycosylated, all reactive with both N terminal and core specific mAbs; (molecular mass range of 16-30 kDa). Analytical sensitivity results in fewer negatives than for "stringent" WB methods, but all three bands not always obvious. | Vacuolation of grey matter present.<br>And/or (MS)<br>Immuno-labelling by IHC in the caudal medulla and always (MS) involving DMNV.<br><br>LRS usually positive with multigranular pattern using 93-106 specific antibodies (JL) | Age at clinical onset usually 2-5 years.<br><br>Epidemiological association with known cases.<br><br>Genotypes are usually of susceptible.types. |

| | Test methods | | | | Comment |
|---|---|---|---|---|---|
| | **1** | **2** [3] | **3** [3] | **4** | |
| **TSE type** | **Approved rapid screening test (EC/EFSA)** [2] | **"Stringent" WB** [4] **(including approved VLA and AFSSA modified SAF-immunoblot)** | **"Mild-PK" WB** [5] **(including approved CEA discriminatory WB, OIE SAF WB +/- NaPTA and , and TeSe Western blot (Biorad))** [6] | **Immunohistochemistry and histopathology** | **Additional criteria to be taken into account in classifying samples.** |
| **BSE in small ruminant** | Positive | Three major PrPres bands with di-glycosylated dominant with mAb directed at core epitope; absent or less intense staining with mAb directed at N terminal epitope; molecular mass of unglycosylated band < than that in classified scrapie of controls.<br><br>(See CRL Handbook of methods for discriminatory testing for interpretation). | Three major PrPres bands with diglycosylated band dominant with mAb directed at core epitope; absent or less intense staining with mAb directed at N terminal epitope (eg, P4).<br><br>Molecular mass of unglycosylated band < than for classified scrapie controls.<br><br>( See CRL Handbook of methods for discriminatory testing for interpretation (except OIE SAF WB)) | Vacuolation usually (MS) present in grey matter.<br><br>Immunolabelling by IHC in the caudal medulla, and usually involving DMNV.<br><br>Discriminatory IHC detects generalised and markedly reduced intraneuronal and intraglial immuno-labelling with antibodies recognising epitopes within aminoacids 92-99 of the sheep PrP protein.<br>LRS usually positive with unigranular pattern using 93-106 specific antibodies. | Age not definable, but experimentally clinical onset within the range seen for classical scrapie.<br><br>Possible epidemiological association with exposure to BSE<br><br>Genotypes – only described in ARQ/ARQ sheep following oral exposure, but genotype alone cannot yet be used to exclude the likely presence of BSE. |

| | Test methods | | | | Comment |
|---|---|---|---|---|---|
| | **1** | **2** [3] | **3** [3] | **4** | |
| **TSE type** | **Approved rapid screening test (EC/EFSA)** [2] | **"Stringent" WB** [4] **(including approved VLA and AFSSA modified SAF-immunoblot)** | **"Mild-PK" WB** [5] **(including approved CEA discriminatory WB, OIE SAF WB +/- NaPTA and , and TeSe Western blot (Biorad))** [6] | **Immunohistochemistry and histopathology** | **Additional criteria to be taken into account in classifying samples.** |
| **Atypical scrapie (including Nor98)** | Positive | Negative, or banding pattern not consistent with diagnosis of classical scrapie or BSE.<br><br>Molecular mass range (35kDa-10kDa) : the banding pattern will depend on mAbs used. | Positive with [N terminal or core], with variable banding pattern (number of bands and molecular mass), unlike BSE and classical scrapie, but with a recognisable band at < 15 kDa.<br><br>Signal stronger if cerebellar tissue used, but pattern still consistent with testing of brain stem. | Neither vacuolation nor immunolabelling invariably present. at the obex, although to date, immuno-labelling has been consistently greater in the cerebellum than the brainstem.<br><br>Where available, the cerebellum displays conspicuous immuno-labelling which may be accompanied by vacuolation.<br><br>In caudal medulla immuno-labelling absent from DMNV but commonly present (although restricted, and frequently minimal) in the nucleus of the spinal tract of the trigeminal nerve. LRS negative with all PrP-specific antibodies. | Collection of cerebellum considered essential for full characterisation of Nor98 cases. (plus any other atypicals/nor98-like etc that have been described to date).<br><br>Mixed hind brain and cerebellum samples may be screened to facilitate rapid test detection of atypicals routinely.<br><br>Suspects usually > 3.5 years when detected, but clinical signs only rarely recorded. Most cases detected by active surveillance.<br><br>Genotypes commonly include ones generally considered resistant to typical scrapie, or one AF141RQ allele.<br><br>Epidemiology – little known of association between cases, but evidence of flocks with more than one case. |

## NOTES TO TABLE 1: CRITERIA FOR THE CATEGORISATION OF TSES IN SMALL RUMINANTS

**1.** To facilitate this definition, some operational parameters are discussed below and examples of western blots included in Annex 3. The application of these criteria in MS National Reference Laboratories will need more detail than can be provided here, and would need to be subject to standard proficiency monitoring and control by the CRL. In practice, this will require a ring trial of various tests in designated laboratories using a standard panel of atypical and scrapie samples prior to going "live" in MS surveillance schemes.

**2.** ***Criterion 1.*** Rapid tests approved for surveillance for TSEs in small ruminants. See Commission Regulation 999/2001 as amended and EFSA` Opinions and Reports on the evaluations of small ruminant rapid tests" (EFSA, 2005a and 2005b). In general, but not uniquely, scrapie and "BSE in sheep" will give ELISA OD values > 1.0 with a mild PK ELISA test (eg. Bio-Rad TeSeE) while atypical samples will in general, but not uniquely give ELISA values < 1.0. This is further expanded in the CRL manual on "TSE strain characterisation in small ruminants – A technical handbook for national reference laboratories in the EU."

**3.** ***Criteria 2 and 3***. To better illustrate these criteria, examples are provided in Annex 3 of Western blots of classical and atypical scrapie cases from France, Norway and Germany.

**4.** ***"Stringent PK"***: This is short-hand for stringent conditions for PK digestion (there should come clear descriptions of the PK conditions at a defined sample protein level, not simply the low concentration of proteinase K. Temperature, presence of detergents, chaotropic agents, pH, also need to be defined; additionally, for the case of "stringent PK" WB the precise banding pattern will inevitably depend on the detection mAb. However, without prejudice or favouring a particular commercial test, we can give as an example of these as : I) the Prionics Check WB, and II) the VLA and AFSSA approved discriminatory WBs as described in the CRL Manual of discriminatory methods, III) the discriminatory WB methods of FRCDVA and CIDC-Lelystad currently approved for use in Germany and Netherlands respectively.

**5.** ***"Mild PK"***: This is short-hand for mild conditions for PK digestion (there should come clear descriptions of the PK conditions) at a defined sample protein level, not simply the low concentration of proteinase K. Temperature, presence of detergents, chaotropic agents, pH, also need to be defined. The precise banding pattern will inevitably depend on the detection mAb. However, without prejudice or favouring a particular commercial test, we can give examples of these as : a) the Bio-Rad TeSeE sheep and goat, b) the OIE SAF immunoblot, with or without precipitation with NaPTA. Note that the OIE SAF immunoblot is not an approved test for discrimination between BSE and scrapie.

**6.** ***WB systems*** can be further defined (both for "mild and stringent PK" conditions as detailed above) by inclusion of a concentration step before or after proteinase

treatment and SDS-PAGE analytical system. Examples of this are the OIE SAF WB (which uses ultracentrifugation) or the combined SAF/NaPTA methods. Note PK treatment after NaPTA treatment influences the mobility of PrPres bands and therefore becomes unreliable for discriminatory purposes.

# ANNEX 2: REPORTED CASES OF ATYPICAL SCRAPIE IN EU MEMBER STATES

## France

| Case | Specie | Genotype | Reason for considering atypical |
|---|---|---|---|
| **1** | Sheep | ARH/AHQ | **TeSeE sheep/goat Western Blot** |
| **2** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **3** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **4** | Sheep | ARR/ARQ | **Discordance Elisa Biorad / AFSSA Modified SAF Ib** |
| **5** | Sheep | Unknown | **TeSeE sheep/goat Western Blot** |
| **6** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **7** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **8** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **9** | Sheep | Unknown | **Discordance Elisa Biorad / AFSSA Modified SAF Ib** |
| **10** | Goat | AHQ/AHQ | **TeSeE sheep/goat Western Blot** |
| **11** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **12** | Sheep | AHQ/AHQ | **TeSeE sheep/goat Western Blot** |
| **13** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **14** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **15** | Sheep | ARH/ARH | **TeSeE sheep/goat Western Blot** |
| **16** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **17** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **18** | Sheep | ARR/AHQ | **TeSeE sheep/goat Western Blot** |
| **19** | Sheep | ARR/ARR | **TeSeE sheep/goat Western Blot** |
| **20** | Sheep | ARR/ARR | **TeSeE sheep/goat Western Blot** |
| **21** | Sheep | ARQ/VRQ | **TeSeE sheep/goat Western Blot** |
| **22** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **23** | Sheep | Unknown | **TeSeE sheep/goat Western Blot** |
| **24** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **25** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **26** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **27** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **28** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **29** | Sheep | ARR/ARR | **TeSeE sheep/goat Western Blot** |
| **30** | Sheep | ARR/ARR | **Discordance Elisa Biorad / AFSSA Modified SAF Ib** |
| **31** | Sheep | ARQ/VRQ | **TeSeE sheep/goat Western Blot** |
| **32** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **33** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **34** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **35** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **36** | Sheep | ARR/AHQ | **TeSeE sheep/goat Western Blot** |
| **37** | Sheep | ARR/ARR | **TeSeE sheep/goat Western Blot** |
| **38** | Sheep | ARR/ARR | **TeSeE sheep/goat Western Blot** |
| **39** | Sheep | ARR/ARR | **TeSeE sheep/goat Western Blot** |
| **40** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **41** | Sheep | Unknown | **Discordance Elisa Biorad / AFSSA Modified SAF Ib** |
| **42** | Sheep | ARQ/VRQ | **TeSeE sheep/goat Western Blot** |
| **43** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |

| Case | Specie | Genotype | Reason for considering atypical |
|---|---|---|---|
| **44** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **45** | Goat | ARQ/AHQ | **TeSeE sheep/goat Western Blot** |
| **46** | Goat | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **47** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **48** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **49** | Goat | ARQ/ARQ | **Discordance Elisa Biorad / AFSSA Modified SAF Ib** |
| **50** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **51** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **52** | Sheep | ARQ/ARQ | **TeSeE sheep/goat Western Blot** |
| **53** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **54** | Sheep | AHQ/AHQ | **TeSeE sheep/goat Western Blot** |
| **55** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **56** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **57** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **58** | Sheep | ARR/ARQ | **TeSeE sheep/goat Western Blot** |
| **59** | Sheep | ARQ/AHQ | **TeSeE sheep/goat Western Blot** |
| **60** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **61** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **62** | Goat | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **63** | Goat | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **64** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **65** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **66** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **67** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **68** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |
| **69** | Sheep | Under inverstigation | **TeSeE sheep/goat Western Blot** |

- ELISA optical density readings were lower in atypical than in normal positives. With regard to the IHC protocol it is not possible to confirm the localization of immunostaining at any particular target site.
- The atypical cases were all found by active monitoring, equally in slaughterhouses and rendering plants.
- Of the 60 positive cases found by culling of the cohort animals only 1 case was atypical (a cohort of index case 714).

**Spain**

- In 2003 and 2004 respectively 15 and 2 atypical cases were detected in a total of 12 flocks.
- All cases were considered atypical because they were positive in the ELISA test but negative in the Western blot.
- 14 sheep had the ARQ/ARQ genotype, 2 were ARQ/AHQ and 1 was ARR/AHQ.

- 3 TSE cases were considered atypical by Spain because the sheep had the ARR/ARQ genotypes. These cases are not considered atypical by the Commission and not included in the above figures.

## United Kingdom

- Only detected by active surveillance
- Initially detected by TeSeE Biorad, but not confirmed by traditional WB and IHC
- Various degrees of discreet immunostaining at the spinal tract nucleus of the trigeminal nerve using antibody 2G11; in 5 instances cerebellar tissue was available and strongly stained; confirmed by IHC with mAB R145
- Genotypes:

| Genotype | % in scrapie cases detected by active monitoring in the UK since 2002 | | % in the UK population in 2002 |
|---|---|---|---|
| | Classical (108 cases) | Atypical (86 cases) | |
| ARR/ARR ("resistant") | 0 | 16 | 20 |
| ARR/AHQ ("semi-resistant") | 0 | 22 | 9 |
| ARR/ARQ, ARR/ARH ("semi-resistant") | 0 | 12 | 32 |
| AHQ/AHQ ("semi-resistant" | 1 | 14 | 2 |
| AHQ/ARQ, AHQ/ARH | 2 | 23 | 9 |
| ARQ/ARQ | 10 | 12 | 14 |
| ARQ/ARH, ARH/ARH | 1 | 0 | 1 |
| ARR/VRQ | 22 | 0 | 6 |
| AHQ/VRQ, ARQ/VRQ, ARH/VRQ | 55 | 1 | 6 |
| VRQ/VRQ | 9 | 0 | 1 |

## Germany

- WB banding patterns not consistent but some attempts were being made to classify the isolates into operational groups. Some clearly stained heavily in the cerebellum.
- 70% of the cases appeared to fit a Nor98 phenotype, the remainder appeared different
- Six pairs of atypical cases have been identified in cohorts
- Among the genotypes represented there were a few ARQ/ARQ, but more ARR or AHQ alleles.

**Portugal**

| Case | Target | months of age | Rapid test | Genotype | Strain typing |
|---|---|---|---|---|---|
| 2003/1 | Healthy slaughter | 22 | TeSeE Biorad | ARQ/AHQ | PrPres immunostaining only in neuropil of spinal tract nucleus of the trigeminal nerve at the obex (=A) |
| 2004/1 | | > 18 | | ARR/ARR | |
| 2004/2 | | > 18 | | ARQ/ARQ | |
| 2004/3 | | > 18 | | ARR/ARQ | |
| 2004/4 | | 124 | | ARQ/ARQ | A + solitary tract nucleus |
| 2004/5 | | > 18 | | ARQ/AHQ | A |
| 2004/6 | | > 18 | | ARQ/ARQ | A + Nor98 type in WB (=B) |
| 2004/7 | | > 18 | | ARR/ARQ | |
| 2004/8 | | > 18 | | ARR/ARR | |
| 2004/9 | | > 18 | | ARQ/AHQ | |
| 2004/10 | | > 18 | | ARQ/ARQ | |
| 2004/11 | | > 18 | | ARR/ARR | B |
| 2004/12 | | > 18 | | ARR/ARQ | A + B |
| 2004/13 | | > 18 | | ARR/ARR | B |
| 2004/14 | | > 18 | | ARQ/ARQ | A + B |
| 2004/15 | | > 18 | | ARR/ARQ | A + B |
| 2004/16 | | > 18 | | ARR/ARQ | A + cerebellum +B |
| 2004/17 | | > 18 | | ARR/ARQ | PrPres I.S.only in cerebell. + B |
| 2004/18 | | > 18 | | Pending | A + B |
| 2004/19 | | 85 | | ARQ/ARQ | B |
| 2004/20 | | > 18 | | ARQ/ARQ | B |
| 2004/21 | | 121 | | Pending | B |
| 2004/22 | | > 18 | | ARR/ARR | B |
| 2004/23 | | > 18 | | Pending | B |
| 2004/24 | | > 18 | | Pending | B |
| 2004/25 | | | | Pending | B |
| 2004/26 | | 51 | | Pending | B |
| 2004/27 | Fallen stock | | | Pending | B |
| 2004/28 | | 77 | | Pending | B |

233 Cohort animals were negative in the rapid TSE test.

**Sweden**

- 4 cases in 2003, and 2 cases in 2004 (100% of TSE cases detected)
- Detected with Biorad TeSeE ELISA, confirmed by immuno- histochemistry and WB, all considered Nor98
- Genotypes: 2 ARR/AHQ, 2 ARQ/ARQ, 1 ARR/ARQ, 1 ARR/ARH
- Different breeds affected, all above 6-7 yrs of age
- No known contact between affected herds
- 5/6 herds culled and examined no more positive cases detected.

**Ireland**

- 4 of the 480 TSE cases in sheep detected between 2002 and 2004 have been considered atypical
- Detected by Enfer, confirmed by immunoblot (Nor98 like) and IHC, most pronounced lesions in the cerebellum
- 2 of the 4 cases were clinical suspects; two cases were from 1 flock.
- Genotype clinical case: ARQ/AHQ

**Belgium**

- 2 of the 38 TSE cases in sheep detected between 2002 and 2004 have been considered atypical.
- An atypical case with an ARQ/ARQ genotype was detected in 2002 by testing a healthy slaughtered sheep with the Platelia Bio-Rad rapid test but could not be confirmed by histopathology, SAF, immunohistochemistry and Western blotting with a non-commercial kit using 12F10 and SAF60. Western blotting with commercial kit using 12F10 and SAF60 (bovine), and BAR226 and SAF60(ovine) were both positive. $PrP^{sc}$ glycoprofile with a strong lower band at approximately 12kDa was demonstrated and confirmed by the National Veterinary Institute of Norway. 97 Cohort sheep tested negative.
- 3 years of age, ARR/ARR genotype detected by Bio Rad TeSeE ELISA and confirmed by immunohistochemistry in a fallen stock sheep. SAF and Western blotting were negative. 42 Cohort sheep tested negative.

## Finland

The only atypical case was reported in May 2004, being an 8 year old fallen stock Finnsheep with a ARQ/ARQ genotype. It was detected with the Bio Rad TeSeE ELISA and considered as of the type Nor98.

## Norway

- Nor98 cases: 2 cases in 1998, 1 in 1999, 3 in 2000, 2 in 2001, 9 in 2002, 14 in 2003 and 14 in 2004; classical scrapie: total of 9 in same period
- Nor 98 identified by WB glycoprofile with a 12kDa band, confirmatory test: Bio-Rad WB (antibody Sha31 or P4)
- One single Nor98 case detected in 44 of 45 flocks, probably 2 Nor98 cases in 1 flock (only 1 verified)
- Contact data from 36 flocks: 3 Nor98 flocks have purchased sheep from other Nor98 flocks (<> 50% in classical scrapie flocks), 1 from a flock with classical scrapie

**Genotypes - Table from S. Benestad (October 2005)**

| Genotype | Nor98 cases | |
|---|---|---|
| AHQ/AHQ | 10 | 21.7% |
| AHQ/$AF_{141}$RQ | 7 | 15.2% |
| AHQ/AXX | 28 | 60.8% |
| $AF_{141}$RQ /$AF_{141}$RQ | 6 | 13% |
| $AF_{141}$RQ /AXX | 22 | 47.8% |
| AXX/AXX* | 46 | 100% |
| VRQ/XXX | 0 | 0 |

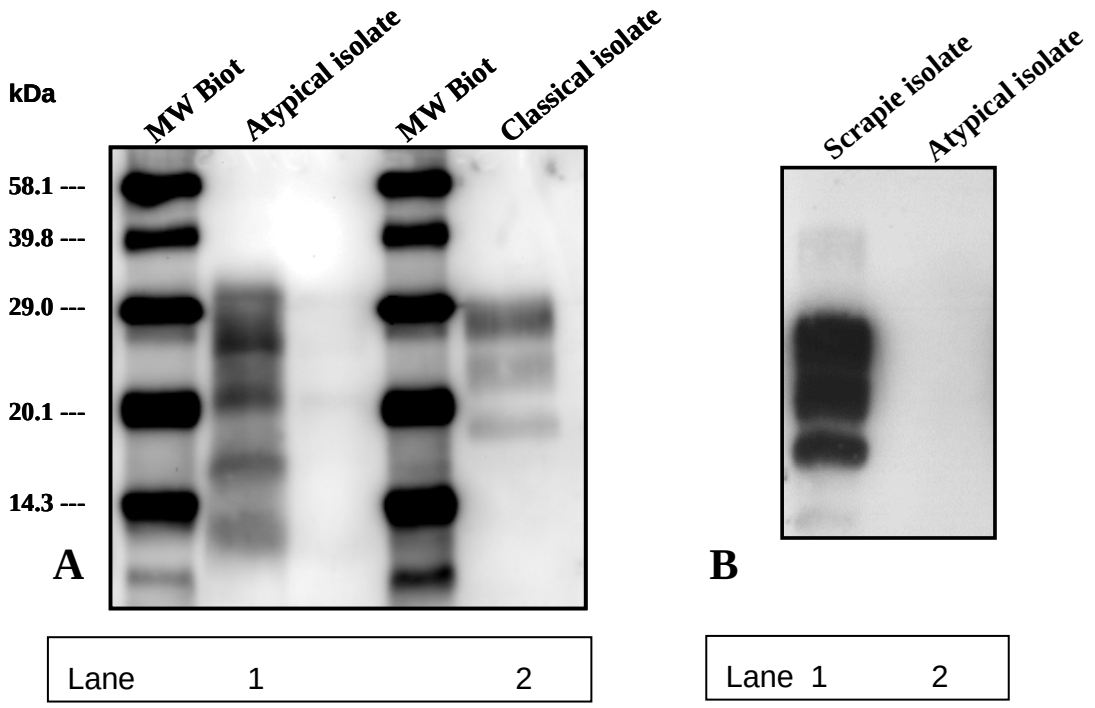
*1 ARR/ARR, 2 ARQ/ARQ

## The Netherlands

- One Nor98 case was discovered in a sheep in fallen stock. Parallel testing in Prionics Check Western blot where homogenates were divided for standard procedure of the EU approved BSE test and for the same procedure with two modifications where digestion with PK was performed at 37 $^{o}$C for 30 min and detection with N-terminus specific monoclonal antibody 9A2 (0.5 µg/ml). This case was negative under the approved test conditions, but positive in the modified procedure. Genotype of the animal: AHQ/AHQ. Age approximately 5 yr. IHC correlated with the Nor98 characteristics described by Dr Benestad.
- Other animals (n=24) on the farm were euthanized except 2 ARR/ARRs, and were negative.

## ANNEX 3: EXAMPLES OF WESTERN BLOTS OF CLASSICAL AND ATYPICAL SCRAPIE CASES

**Figure 1. (Contributed by Dr Thierry Baron, AFFSA, Lyon, France)**




A) TeSeE Western blot (Bio-Rad) analysis of samples prepared from the brain stem, with detection using the SHa 31 monoclonal antibody [raised against YEDRYYRE (145-152)] according to the manufacturer's instructions.

Lane 1 Atypical scrapie showing a complex multi-bands pattern between 13 and 31 kDa.

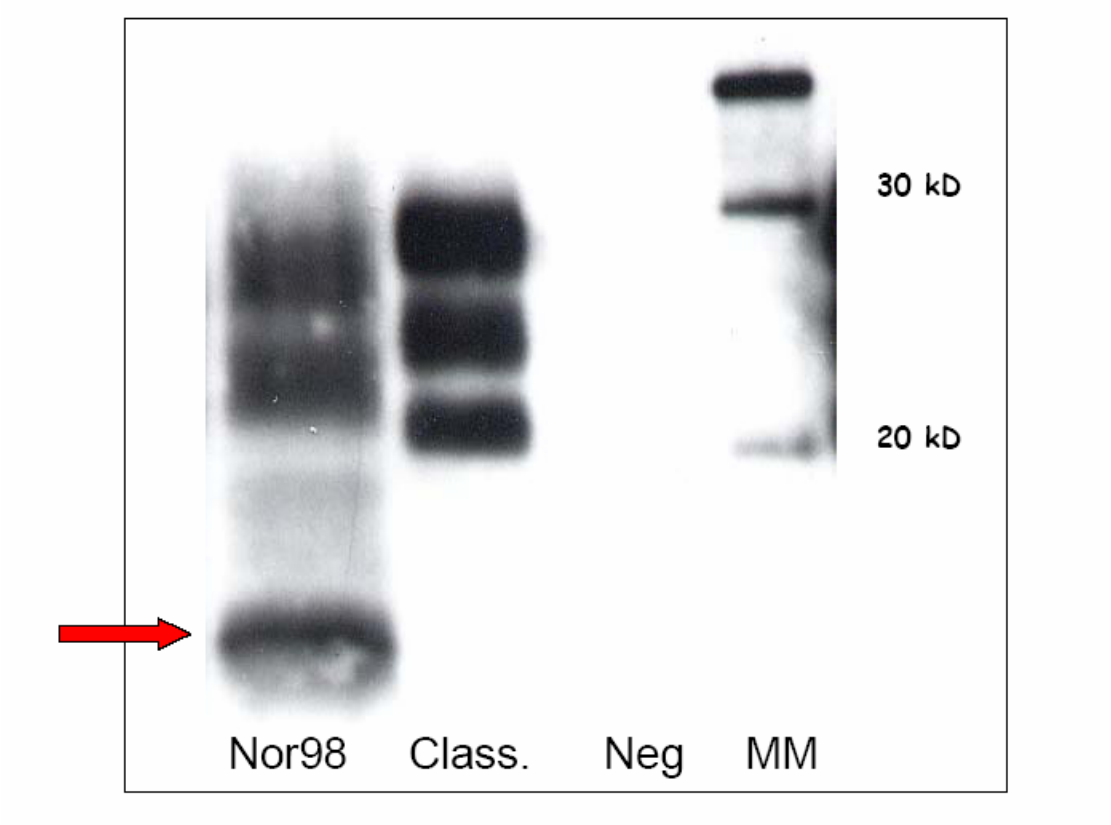
Lane 2 Typical scrapie showing the three bands pattern between 18 and 30 kDa

B) "Stringent" modified SAF-immunoblot blot with proteinase K digestion (10 µg / 100 mg brain stem tissue 1h at 37°C) followed by ultracentrifugation (100 000 rpm, 2h) and detection with SAF 84 monoclonal antibody (1/5000) (Buschmann, A. *et al.*, 2004a).

Lane 1 Typical scrapie showing the three bands pattern between 18 and 30 kDa

Lane 2 Atypical scrapie showing no detectable signal. A typical scrapie case corresponding to the same level of PrPres as detected by the initial rapid test would show the typical 3 bands pattern.

**Figure 2. (Contributed by Dr Sylvie Benestad, NVI, Norway)**

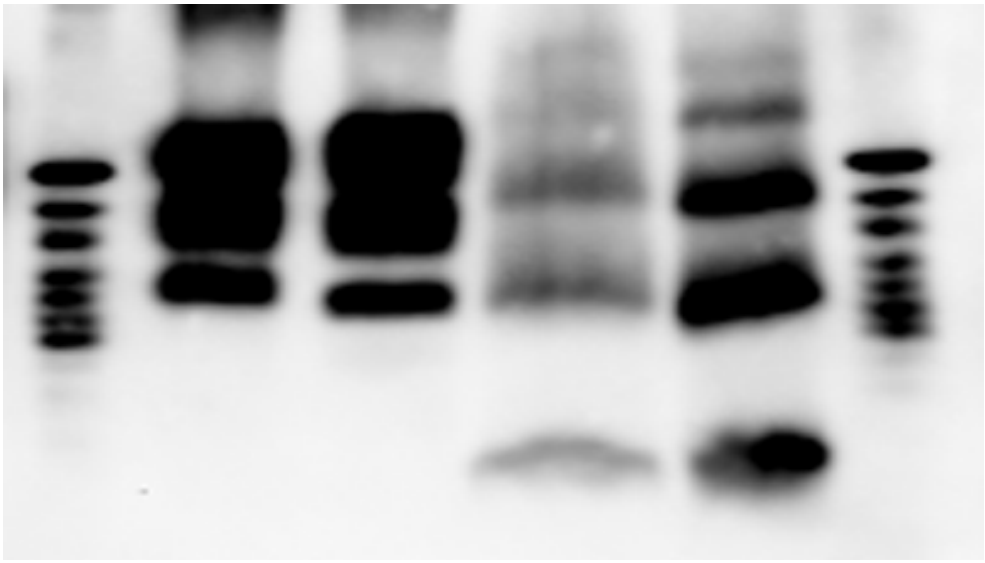



Western Blot (TeSeE™ Sheep/Goat Western Blot, Bio-Rad). The protocol was performed following the producers recommendations. Briefly, 20% homogenates were prepared as for TeSeE™ ELISA, digested for 10 min at 37°C with 500 µl of a Proteinase K solution containing tensio-actif components. $PrP^{Sc}$ was concentrated by centrifugation and 15 µl of each sample were separated on electrophoresis SDS gels. Proteins were transferred onto a PVDF membrane which was processed using the monoclonal anti-PrP antibody SHa 31 raised against the sequence YEDRYYRE (145-152).

Nor98, cerebral cortex AHQ/ARQ

Classical, cerebral cortex VRQ/VRQ

**Figure 3. (Contributed by Dr Anne Buschmann, FLI, Germany)**



| Lanes | M | 1 | 2 | 3 | 4 | M |
|---|---|---|---|---|---|---|

M : Molecular weight markers

1 : Classical Ovine scrapie

2 : BSE in cattle

3 : Nor-98-like scrapie

4 : Nor-98-like scrapie

M : Molecular weight markers