Norwegian Scientific Committee for Food Safety

Comments on:

The opinion of the Agence française de sécurité sanitaire (Afssa) on changes to the control measures for sheep and goat herds in which a case of classical or atypical scrapie has been detected

Norwegian Scientific Committee for Food Safety

Panel on Biological Hazards
&
Panel on Animal Health and Animal Welfare

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Contents

Background

The Agence française de sécurité sanitaire (Afssa) was asked by the Direction général de l'alimentation (DGAI) to comment on various proposals made by the CES ESST (expert committee on TSEs) in the EC TSE Roadmap.

On January 24rd 2007, the Norwegian Scientific Committee for Food Safety (Panel on Biological Hazards and Panel on Animal Health and Animal welfare) received a request from the Norwegian Food Safety Authority for comments on Afssa's opinion. In response, an *ad hoc* Working Group of experts was appointed with the mandate to draft a note regarding this issue.

Terms of reference[1]

The Norwegian Food Safety Authority requests comments to the Afssa report from the Norwegian Scientific Committee for Food Safety, with particular emphasis on the situation concerning atypical scrapie. Additionally, there is interest in whether anything scientifically new emerges from the report. The Norwegian Scientific Committee for Food Safety is also requested to examine in detail the point in which the test that differentiates BSE from other TSEs in sheep and goats is questioned. The situation concerning classical scrapie is obviously also of interest, but due to time restraints this is considered secondarily in this commission.

Atypical scrapie

In 1998, the molecular and histopathological spectrum of TSEs in sheep was extended by the discovery in Norway of an experimentally-transmissible, prion disease disease in sheep that was distinguishable from classical scrapie, and therefore considered to be an "atypical" form of scrapie, designated Nor98 (Benestad et al. 2003). Other "atypical" TSE phenotypes, including those similar to, or indistinguishable from, Nor98, have now been published from several countries in Europe (Arsac et al. 2007;Buschmann et al. 2004;De Bosschere et al. 2004;Everest et al. 2006;Gavier-Widen et al. 2004;Klingeborn et al. 2006;Konold et al. 2006;Onnasch et al. 2004;Orge et al. 2004;Stack et al. 2006), and the Falkland Islands (Epstein et al. 2005).
Atypical scrapie is clearly distinguishable from classical scrapie and BSE, and the diagnostic differences have been published in an EFSA opinion (EFSA 2005). In the atypical cases, essentially due to smaller and less stable protease-resistant cores of PrP^{Sc} the results of the rapid tests, using a stringent proteinase K (pK) digestion, are negative. Tests using a milder pK digestion (Bio-Rad TeSeE or IDEXX ELISA test) give positive results, and the patterns obtained by WB (Bio-Rad WB) are unlike those for BSE and classical scrapie, as there is a recognisable band at less than 15 kDa. The signal is generally stronger in the cerebellar tissue than in the brain stem.

[1] Bestilling:
Mattilsynet ber VKM om å kommentere AFFSA sin rapport, og da i første rekke forholdene rundt atypisk skrapesyke. Det er videre av interesse om det faktisk er noe vitenskapelig nytt som fremkommer i rapporten. Vi ber også VKM se nærmer på punktet hvor de trekker testen som skiller BSE fra andre TSE hos småfe i tvil. Forholdene rundt klassisk skrapesyke er selvsagt også av interesse, men på grunn av kort tidsfrist blir dette sekundært i denne bestillingen.

Additionally, the lesion profile and pattern of PrP^{Sc} immunolabelling in the brain differs between classical and atypical scrapie (EFSA). In sheep with atypical scrapie, PrP^{Sc} has so far been detected only in the brain and cerebellum and occasionally in the spinal cord. Transmission studies in transgenic mice, inoculated with material from peripheral organs from cases of atypical scrapie, are underway to address the question of infectivity in peripheral organs.
Atypical scrapie is generally found in older animals than classical scrapie; in Norway atypical scrapie cases tend to occur in animals of approximately mean 6-7 years, whereas classical scrapie occurs in animals of approximately mean 2-4 years.

The molecular genetics of atypical scrapie

Atypical scrapie is often found in sheep with other PrP-genotypes than those associated with classical scrapie (Benestad et al. 2003;Luhken et al. 2007a;Moum et al. 2005), with a predominance of atypical cases carrying the $A_{136}H_{154}Q_{171}$ (AHQ) and $A_{136}F_{141}R_{154}Q_{171}$ (phenylalanine at position 141, AFRQ) alleles. Furthermore, a significant proportion of atypical scrapie cases have occurred in animals carrying the ARR allele, which is associated with resistance towards classical scrapie. Preliminary data (Luhken et al. 2007b) indicate that there may be associations between atypical scrapie and other genetic elements in sheep, however, further investigation is needed. Characterization of proteinase K-resistant fragments in Nor98 atypical scrapie has indicated similarities to the human genetic prion disease, Gerstmann-Straussler-Scheinker (Klingeborn et al. 2006).

The aetiology and epidemiology of atypical scrapie

As atypical scrapie occurs predominantly in older animals, and the PrP^{Sc} appears restricted to the CNS it has been postulated that atypical scrapie might resemble sporadic CJD in humans. The absence of PrP^{Sc} deposits in the dorsal motor nucleus of the nervus vagus in atypical scrapie, in contrast to that found for classical scrapie, might indicate another route of infection. Analytical epidemiological studies in Norway have not demonstrated any association of transmission of atypical scrapie between sheep flocks, and this suggests that atypical scrapie might not be contagious, or is much less contagious than classical scrapie (Hopp et al. 2006).
The finding of individual cases of atypical scrapie in flocks also indicates an absence of, or a low level of, transmission between animals under natural conditions. However flocks with more than one case of atypical scrapie have been detected (Luhken et al. 2007a), but as these flocks were of more than 500 sheep, a non-contagious aetiology is still possible. Nevertheless, the incidence of atypical scrapie is much higher than that of sporadic CJD in humans. Long-term studies on the occurrence and genetics of atypical scrapie, combined with the development of more sensitive methods for detection of infectivity, are needed to elucidate these aspects.

Atypical scrapie is not confined to sheep, as it has also been diagnosed in goats in a few European countries including Norway (Benestad et al. 2006). Currently the molecular characteristics of atypical scrapie in goats and sheep are indistinguishable (Le Dur et al. 2005). However, only a few cases have been found in goats to date, so any epidemiological link between the diseases in these two species is presently unknown.

Studies of the atypical scrapie strain Nor98 in transgenic mice carrying the ovine VRQ allele have shown that this strain retains its characteristic biochemical features (Le Dur et al. 2005). Interestingly, no significant reduction in mean incubation time was observed upon second-passage, indicating that the “species barrier” is relatively low, with an efficient replication upon first-passage. Transmission studies in sheep are currently under way in Norway. Data from these trials are not yet available.

The zoonotic potential of atypical scrapie

While no link between classical scrapie in sheep and goats and human TSEs has been identified (Will et al. 1998), this remains to be ascertained for atypical scrapie. To investigate this, transmission studies in transgenic mice carrying the human PrP-gene, and in primates such as macaque, have been initiated, but data are not yet available. Previous studies concerning the zoonotic potential of classical scrapie might also be valid for atypical scrapie.

The limitations of the discriminatory tests (BSE/scrapie)

The BSE/scrapie discriminatory tests have been evaluated on a small number of samples from experimental BSE in sheep and natural scrapie cases in a blinded ring trial organised by the EU TSE Community Reference Laboratory. Whilst initial data are promising, further trials with larger sample sizes are warranted. Special cases, such as co-infection with scrapie and BSE also remain to be investigated. Through the EC NoE (Network of Excellence, Prion Diag), 13 European laboratories are presently investigating new technologies and methods for differentiation of TSE variants in small ruminants, with particular focus on BSE in sheep, So far no reliable discriminatory tests have been developed.

Answers to the mandate

Surveillance for a 5-year period
Atypical scrapie occurs in older animals than classical scrapie. A two-year surveillance period may therefore be too short to detect secondary cases in a scenario where atypical scrapie is contagious under natural conditions. The Norwegian Scientific Committee for Food Safety, Panel on Biological Hazards and panel on Animal Health and Animal Welfare, therefore support the suggested 5-year duration of surveillance of flocks with atypical scrapie.

PrP-genotyping of the whole flock at four codons (136, 141, 154, 171)
The currently available knowledge regarding sheep genotype and occurrence of atypical scrapie, indicates a more complex situation than that for classical scrapie. The Norwegian Scientific Committee for Food Safety, Panel on Biological Hazards and panel on Animal Health and Animal Welfare, consider PrP-genetic information from flocks with atypical scrapie to be of value and therefore supports the Afssa recommendation for PrP-genotyping. In accordance with the view of the minority of the Afssa committee, the Norwegian Scientific Committee for Food Safety, Panel on Biological Hazards and panel on Animal Health and Animal Welfare, does not recommend the destruction of animals based upon PrP-genetic data.

Restrictions on the sale and movement of breeding stock
It is currently unknown whether atypical scrapie is contagious under natural conditions. In concordance with the suggested 5-year duration of surveillance of flocks with atypical scrapie, the Norwegian Scientific Committee for Food Safety, Panel on Biological Hazards and panel on Animal Health and Animal Welfare, support restrictions on the sale and movement of breeding stock from atypical scrapie flocks to flocks with a different status.

Testing with relevant tests of culled/slaughtered animals (> 18 months),
The Norwegian Scientific Committee for Food Safety agrees with the Afssa recommendation for the testing of culled/slaughtered animals (> 18 months) for atypical scrapie with an appropriate test (currently the Bio-Rad and IDEXX tests).

Discriminatory tests
Atypical scrapie gives a characteristic Western Blot (WB) profile, which is easily distinguishable from that obtained with BSE. The characteristic atypical WB profile obtained using confirmatory WB (i.e. Bio-Rad WB) is sufficient to exclude BSE from atypical scrapie. The Norwegian Scientific Committee for Food Safety, Panel on Biological Hazards and panel on Animal Health and Animal Welfare, support the suggestion that non-atypical scrapie TSE-positive animals should be subsequently tested with a discriminatory test to exclude BSE from classical scrapie, if and whenever such tests are available.

Scientifically novel finding in the Afssa report
The Afssa report does not discuss any new scientific data. However, the report is important in that it underlines the uncertainties in the limited knowledge, and recommends a more precautionary approach for the control of TSEs in sheep.

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