

APPENDIX 1: LITERATURE REVIEW OF ATYPICAL SCRAPIE

LITERATURE REVIEW OF ATYPICAL SCRAPIE

This review of the technical scientific literature relating to the epidemiology and pathobiology of atypical scrapie is updated to September 2009 from an original review prepared by Lachlan McIntyre in February 2007, at the time based at the Epicentre, Massey University.

PREAMBLE

This document assumes the reader is sufficiently informed about classical scrapie and the context of New Zealand interest in atypical scrapie.

HISTORY OF ATYPICAL SCRAPIE

Classical Scrapie is a disease of sheep which has been recognized for over 250 years. Until recently classical scrapie was considered a well characterised entity, with a recognised set of clinical signs and histopathological characteristics which allowed confidence in the diagnosis. As part of the European Union wide TSE surveillance scheme, member states (EU MS) have been required to test 10 000 healthy slaughter sheep and 10 000 fallen stock (sheep) annually. This was intended to detect if it occurred, any transmission of the BSE agent in to the sheep and goat flocks of EU MS and concurrently discriminate between Scrapie and ovine BSE. What soon became apparent from testing such large numbers of adult sheep, was not all cases would classify neatly in to BSE and classical Scrapie. A small proportion of sheep would test positive to the rapid screening test, but fail to be detected by confirmatory tests designed for ovine BSE and classical Scrapie. The first reports of these were from Norway (Benestad, Sarradin et al. 2003) where it was named Nor98 scrapie. This report was rapidly followed by reports from the UK (Wilesmith, Matthews et al. 2003; Wilesmith, Ryan et al. 2004) , Germany and France (Buschmann, Biacabe et al. 2004), Portugal (Orge, Galo et al. 2004), Belgium (De Bosschere, Roels et al. 2004), Sweden (Gavier-Widen, Noremark, 2004) and then other member states, Switzerland (Seuberlich et al. 2007) and even the Falkland Islands (Epstein, Pointing et al. 2005). More recently six cases have been identified in the USA (Loiacono et al. 2009) and three cases in Canada.¹ Historical records do allude to an atypical form of scrapie (Guilhon and Lucam 1953), but the document is written in French and is not available for review. Atypical scrapie then is a newly recognized disease of sheep with the earliest confirmed detections being 1998 from Norway.

EPIDEMIOLOGY OF ATYPICAL SCRAPIE

As noted above, atypical scrapie is a relatively recent detection. The origins of atypical scrapie are uncertain but mounting evidence suggests it may be a spontaneous degenerative condition of older sheep. Because initial cases came from countries with endemic scrapie, it was

¹ <http://www.scrapiecanada.ca/UpdateJune2009.html>

assumed these were some variant of classical scrapie. However the cases from Portugal, Denmark² and Falkland Islands (Epstein, Pointing 2005) question this assumption. Portugal has never detected classical scrapie despite clinical and histopathological surveillance plans (Orge, Galo et al. 2004) and the Falkland Island case (of atypical scrapie) is also the first official report of any form of scrapie (Epstein, Pointing 2005) on the islands. Portugal has had cases of BSE but the PrP electrophoretic profile is distinct from that observed in sheep experimentally infected with BSE (Orge, Galo et al. 2004) and consequently this exposure seems unlikely. The BSE link appears even less likely in the Falkland case as the sheep concerned has likely but not unquestionably had a pasture diet. Concentrate feed is imported by the Islanders from both Chile and the UK although this is mostly for chicken, dairy cattle and pig feed. Limited amounts of concentrate are fed to a small number of “pet” sheep which graze the runway (K Lawrence personal communication).

Most cases of atypical scrapie are singleton cases with no others in the flocks of origin and are usually widely distributed across flocks in the country (Orge, Galo et al. 2004; Hopp, Omer et al. 2006; Fediaevsky, Tongue 2008).

Hopp et al conducted a case control study of Norwegian case farms and found no significant risk factors which indicated that these cases were the result of transmission between sheep flocks by animal movement or animal to animal contact. This was in contrast to previous work by the same author on risk factors for classical scrapie positive flocks, where factors such as purchase of female sheep from scrapie flocks, sharing pastures with scrapie flocks and sharing breeding rams increased the odds for scrapie in a flock. Factors which were significant on these case farms were, presence of dogs on the farm (OR=3.2), removal of all afterbirths (OR=0.14 ie protective), absence of poultry or pig feed on the farm (OR=0.14 ie presence is protective) and vitamin and mineral feed supplement used on the farm (OR=6.5).

The authors discussed the possible role of the placenta in transmission of Nor98 as is the case with classical scrapie but noted they were unaware of any literature showing the presence of Nor98 material outside the central nervous system, in particular the lymphoid system, and argued this may mean Nor98 is not found in the placenta. They did suggest examining placental tissues to give insight in to this. Unfortunately until a reliable model can be developed which will allow collection of placenta in advance of confirmed diagnosis of atypical scrapie, there will be no opportunity for this approach as case material is very rare, with the only report associated with pregnancy being the New Zealand sourced ewe at Arthur Rickwood Secure Unit. The authors could offer no explanation for the role of dogs and left this question open for further work although they noted the small sample size of their study (28 cases and 102 randomly selected controls).

The authors concluded the study supported the hypothesis of the epidemiology of scrapie Nor98 (atypical) differing from classical scrapie. In particular they identified the low transmissibility of the agent and suggested it may not be transmissible between animals under natural conditions. They also considered the association between mineral intake and Nor98 is biologically plausible and would be in accordance with a spontaneous aetiology.

More recently Fediaevsky et al (2008) have described the epidemiological situation with respect to atypical scrapie in 20 European countries. They noted many similarities to the findings of Hopp et al. and came to similar conclusions ie atypical scrapie is remarkably

² <http://www.uk.foedevarestyrelsen.dk/NR/rdonlyres/5B47E89B-DA58-4534-BBB5-AEA1385A0A1B/0/StatusreportonBSEandscrapieinDenmarkMarch2009.pdf> accessed 21 Sept 2009

homogeneous across countries and areas within a country and concluded the aetiology is not the same as classical scrapie.

More recently (Luhken et al 2007, Green et al 2007, Simmons et al 2009 and Fediaevsky et al 2009) provide evidence to show that scrapie and atypical scrapie are separate and distinct diseases. The absence of atypical scrapie in countries like New Zealand with a long history of freedom from classical scrapie or the presence of atypical scrapie in countries like Portugal and Denmark which have also had no classical scrapie is corroborating evidence for the position that they are separate diseases. The detection of a single case in New Zealand would be suggestive of, but not proof of, a spontaneous aetiology (McIntyre 2007).

This position has been accepted by the OIE and the Animal Health Code Article 14.9.1 states

“The chapter does not cover so-called ‘atypical’ scrapie which is clinically, pathologically, biochemically and epidemiologically unrelated to ‘classical’ scrapie, may not be contagious and may, in fact, be a spontaneous degenerative condition of older sheep.”

This position has been supported by the scientific panel on biological hazards of the European Food Safety Authority³.

PREVALENCE

Atypical scrapie has been detected at low prevalence from continental European countries and from the more geographically isolated British Isles and Falkland Islands. More recently it has also been detected in Canada, USA and Denmark. Prevalence estimates derived from published material (Anonymous; Orge, Galo et al. 2004) are shown in table 1.

TABLE 1: ESTIMATED PREVALENCE OF ATYPICAL SCRAPIE IN SHEEP FOR PORTUGAL AND UK

	Positives	Tests	Apparent prevalence
Portugal (2002- 2003)	7	30269	0.0002
UK-2002	18	31028	0.0006
UK-2003	52	75309	0.0007
UK-2004	16	14960	0.0011
UK-2005	22	19384	0.0011
UK-2006	50	>54281	~ 0.0009
UK-2007	33	na	--
UK-2008	7	na	--
UK-2009	5	na	--
UK Total	210	na	--

The number of positive cases in the UK has declined in recent years in line with the number tested.

³ (Opinion of the Scientific Panel on Biological Hazards on a request from the European Commission on certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals, The EFSA Journal (2007), 466, 1-10)

DETECTION

Clinical signs have been reported by several more recent authors (Benestad, Sarradin et al. 2003; Onnasch, Gunn et al. 2004; Epstein, Pointing et al. 2005; Konold, Davis et al. 2006, 2007; Dagleish, Rodger et al 2008), even though most cases have been detected by active surveillance methods which did not readily allow for the collection of clinical signs data. Benestad et al reported progressive symmetrical ataxia ending in death after about two months in five sheep with a mean age of 5 years. Onnasch et al 2004 on the other hand reported incoordination, a hopping gait and weight loss culminating in circling and a nervous appearance in a Texel/Suffolk ewe of six to seven years age. Epstein et al reported the case of a seven year Corriedale or Corriedale Polwarth cross ewe showing loss of body condition relative to flock mates and farmer reporting of weakness, lack of flight response and attempted biting at a hind leg. The authors were not able to observe clinical neurological signs during two days of observation.

Konold et al reported various clinical signs including ataxia in all four cases, three cases with temperament changes, including one with extreme agitation in a pen with tight circling in both directions. Only one was reportedly pruritic although none showed wool loss. All were five years of age. At this time no one appears to have made a link between the typically later age of onset (than classical scrapie) and the genotype of these cases, although others have noted the role of genotype in delaying onset of clinical signs and relative resistance to classical scrapie cases (Hunter and Cairns 1998; Bossers, Harders et al. 1999). Moum et al noted the earliest onset of scrapie Nor98 as about 30 months of age (Moum, Olsaker et al. 2005) with a mean age of 6 years and ranging from 36 to 100 months.

The BioRad test was used as the primary test in the majority of published cases (Benestad, Sarradin et al. 2003; Wilesmith, Matthews et al. 2003; Buschmann, Biacabe et al. 2004; De Bosschere, Roels et al. 2004; Onnasch, Gunn et al. 2004; Orge, Galo et al. 2004; Wilesmith, Ryan et al. 2004; Epstein, Pointing et al. 2005). Only for the Falklands case was it not possible to conclude the use of this test from the written literature. Because this sample was tested at VLA Weybridge, it is probable that it was also subjected to this test. This is not surprising as the European Food Safety Authority (EFSA) reported the relative diagnostic sensitivities of the available tests and concluded that BioRad tests had the greatest sensitivity because of an ability to detect lower concentrations of PrP^{Sc} (Anonymous 2005).

Molecular profiling of the various atypical strains and comparing these with classical scrapie and BSE case material has identified that there are significant differences between each of these forms of TSE (Benestad, Sarradin et al. 2003; De Bosschere, Roels et al. 2004; Orge, Galo et al. 2004). In particular electrophoretic profiling by Western Blot detection after proteinase K treatment revealed a small (12 kDa) band which is not normally seen in classical scrapie (Benestad, Sarradin et al. 2003; Onnasch, Gunn et al. 2004; Orge, Galo et al. 2004; De Bosschere, Roels et al. 2006). Benestad also noted the detection of this small band was independent of the region of the brain the sample came from.

The use of tests on peripheral tissues such as rectal biopsy (Espenes, Press et al. 2006), third eyelid biopsy (O'Rourke, Duncan et al. 2002) or other lymphoid tissues (Ryder, Dexter et al. 2004) are not suitable at this time because of the lack of evidence of PrP^{Sc} outside the central nervous system.

GENOTYPIC RESISTANCE AND SUSCEPTIBILITY

One aspect of the presentation which has concerned the managers of the UK National Scrapie Plan (NSP) (Baylis and McIntyre 2004) is the presence of atypical scrapie in sheep genotypes previously thought to be relatively resistant to classical scrapie. The national scrapie plan was predicated on the use of these resistant genotypes. Gradually through controlled breeding and culling of susceptible genotypes, it was intended to move the national flock to a status thought to be more likely to resist classical scrapie. Researchers have found most of the atypical cases studied so far have predominantly been of the genotypes thought to give relative resistance to classical scrapie.

Of relevance to the New Zealand situation, Bossers et al (Bossers, Harders et al. 1999) conducted a study of randomly selected samples from fourteen different Romney Marsh flocks scattered all over the country. How the sampling frame was determined was not stated. They reported the frequency of various genotypes resistant and susceptible to classical scrapie. The observed and expected frequencies of the three most resistant genotypes (NSP types 1, 2 and 3) was 90%. This is essentially identical to the work of Lee et al (Lee, Manley et al. 2007) and Hickford et al 2008 (Hickford, Zhou et al 2008). The work of Lee et al and Hickford et al both suffered from a lack of randomisation, but covered a wider range of breeds including Romney, Texel, Coopworth, Merino and crossbreeds and used a sample size approximately twenty times larger than the work of Bossers et al. This alone is not sufficient to explain the presence or absence of atypical scrapie and very recent research has identified another codon, 141 which appears to have an as yet only partially understood role in the susceptibility to atypical scrapie. Moum et al (Moum, Olsaker et al. 2005) have reported the relative frequencies of various genotypes and shown that for the Nor98 cases detected in Norway, over 50% were either homozygous or heterozygous for phenylalanine at codon 141 (F₁₄₁). In contrast this allele was only present in 10.5% of flock mates and 4.5% of a random sample of slaughter sheep. They also noted the H₁₅₄ allele was present in 63.2% of Nor98 cases as opposed to 27% of flock mates and 17% of the slaughtered sheep. Likewise Goldmann notes the role of codons 141 and 154 (Goldmann 2008). Bossers et al have identified that New Zealand sheep have relatively high frequencies of the allelic variant at codon 141 (F₁₄₁). They found 5% of the animals they tested had this variant in two genotypes (ARQ/AF₁₄₁RQ & ARR/ AF₁₄₁RQ). This may have implications for the potential susceptibility of New Zealand sheep to atypical scrapie whether it is infectious or non infectious in origin.

TRANSMISSION STUDIES

The epidemiological pattern of disease observed so far suggests that infectivity between sheep under natural conditions is very low or non existent (Hopp, Omer et al. 2006, Green et al 2007, Luhken, Buschmann et al 2007, Fediaevsky, Morignat et al 2009). Most cases reported are the only case found in the flock even when the entire flock is subsequently depopulated and tested (Hopp, Omer et al. 2006). The Norwegian experience is such that they no longer insist on complete depopulation instead relying on other controls (Moum, Olsaker et al. 2005). Benestad et al report finding no signs of PrP outside the central nervous system using current testing methodologies (Benestad, Sarradin et al. 2003).

To examine the potential transmissibility of brain tissues from cases of Nor98 scrapie from Norway and atypical cases from sheep and a goat of French origin, Le Dur et al (Le Dur, Beringue et al. 2005) used intra-cerebral inoculation in 6-8 week old female mice genetically

modified to over express PrP and transgenic for the ovine VRQ allele. They also inoculated various lines of inbred mice. All inbred mice line failed to develop disease. All genetically modified mice succumbed in a fairly homogenous time period of approximately 200 – 300 days. Secondary passage of material showed no change from the already observed characteristics of the agent. No infectivity was detected in second passage of spleen homogenates. They concluded the Nor98 and French atypical strains were essentially identical and represent one or more closely related strain(s). They found there was a distinct tropism for neural tissue and found this unprecedented for natural scrapie isolates previously studied. They concluded the strain or strains must be considered potentially transmissible irrespective of the potential origin whether acquired or spontaneous.

Recent studies have shown experimental transmission of atypical scrapie in sheep and laboratory animals (Simmons et al 2007) but there is as yet no evidence to confirm that transmission can occur naturally in the field. Another recent paper by Espinosa et al (Espinosa, Herva, 2009) has shown that atypical scrapie can be transmitted with low efficiency to genetically engineered mice over expressing the porcine prion protein. They concluded there was a marked species transmission barrier. Further the agent appeared to undergo a strain phenotype shift upon transmission to the transgenic mice. This is the first report of this occurring.

FOOD SAFETY

There is no evidence that atypical scrapie abnormal prion protein is found in any tissues other than central nervous system (Benestad et al 2003, Le Dur 2005, EFSA 2008).

INFORMATION TO INFORM SURVEILLANCE DESIGN

CHOICE OF RAPID SCREENING TEST

Onnasch et al (Onnasch, Gunn et al. 2004) note the role of the BioRad Western Blot at detecting cases which would have escaped detection with Prionics-Check Western Blot and the Enfer test, had only the medulla and spinal cord respectively, been tested. The Prionics-Check Western Blot used on cerebellum samples did detect PrP from these atypical cases.

LOCATION OF LESIONS

All authors have noted the location of PrP reactivity in the cerebellum and how inappropriate choice of sample site could reduce the chance of detecting lesions. Also noted was the lack of histological evidence in the historical location examined to detect classical scrapie. Nentwig, Oevermann et al (2007), have reported on the neuroanatomical distribution of abnormal prion protein in six sheep and two goats of Swiss origin. They found the biodiversity of abnormal prion distribution was much more variable than expected and highlighted that this had implications for sampling and testing strategies.

INFORMATION TO INFORM POSSIBLE CONTROL OR ERADICATION

A number of authors have indicated the similarity between atypical scrapie and sporadic CJD. Soto (2006) has stated that 85% of all CJD cases are sporadic and have no known associations with infectious sources or prior or subsequent generations of the patients family. There is a very real possibility that atypical scrapie is indeed spontaneous in origin. If that is the case,

then it is equally possible to find it in New Zealand as we appear to have the susceptible genotypes. If indeed it is present the primary reasons for not detecting it before now would appear to be, the sample size examined is possibly too small, the location sample tissues are collected from is definitely not ideal and the rapid screening test used is the Prionics test which has not featured in published reports of detection of these cases.

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