Atypical scrapie in Australia

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Background  Since its initial detection in Norway in 1998, atypical scrapie (‘atypical/Nor98 scrapie’) has been reported in sheep in the majority of European countries (including in regions free of classical scrapie) and in the Falkland Islands, the USA, Canada, New Zealand and Australia.

Case series  The diagnosis in Australia of atypical scrapie in four Merino and one Merino-cross sheep showing clinical signs of neurological disease was based on the detection of grey matter neurolonal vacuolation (spongiform change) in the brain (particularly in the molecular layer of the cerebellum cortex) and associated abnormal prion protein (PrPSc) deposition in both grey and white matter. Changes were minimal in the caudal brainstem, the predilection site for lesions of classical scrapie.

Conclusion  The distinctive lesion profile of atypical scrapie in these five sheep highlights the diagnostic importance of routine histological evaluation of the cerebellum for evidence of neuropil vacuolation and associated PrPSc deposition in adult sheep with suspected neurological disease.

Keywords  atypical scrapie; prion disease; sheep; transmissible spongiform encephalopathy

Abbreviations  ANZSDP, Australian and New Zealand Standard Diagnostic Procedure; CNS, central nervous system; DMNV, dorsal motor nucleus of the vagus nerve; H&E, haematoxylin and eosin; IHC, immunohistochemistry; NTSESP, National TSE Surveillance Program; PrPSc, abnormal prion protein isomer; TSE, transmissible spongiform encephalopathy.

Scrapie is a transmissible spongiform encephalopathy (TSE) or prion disease of small ruminants that occurs in classical and atypical forms.

Classical scrapie is characterised histologically by vacuolation and abnormal prion protein (PrPSc) immunolabelling of neuronal cytoplasm and grey matter neuropil within the central nervous system (CNS), with a predilection for the caudal brainstem, mainly at the level of the obex and typically involving the dorsal motor nucleus of the vagus nerve (DMNV). The Western immunoblot pattern (‘molecular signature’) of PrPSc extracted from brains of classical scrapie cases has three bands comprising unglycosylated, monoglycosylated and diglycosylated PrPSc residues with molecular masses between 18 and 30 kDa.

In contrast, atypical scrapie (‘atypical/Nor98 scrapie’) is defined by a characteristic neuroanatomical distribution of neuropil vacuolation and PrPSc immunolabelling in the brain, and a multiband PrPSc Western immunoblot pattern with a fast-migrating, lower band of molecular mass <15 kDa. Vacuolation of grey matter neuropil (spongiform change) is detected mainly in the cerebellar cortex (particularly in the molecular layer), cerebral cortex and basal ganglia. Immunohistochemistry (IHC) demonstrates PrPSc deposition at these sites and in the spinal tract nucleus of the trigeminal nerve, and also within white matter throughout the brain.

There is no intracytoplasmic vacuolation or PrPSc immunolabelling of neurons.

Since its initial detection in Norway in 1998, atypical scrapie has been reported in sheep in the majority of European countries (including in regions free of classical scrapie) and in the Falkland Islands, the USA, Canada, New Zealand and Australia. Usually occurs as single cases within sheep flocks, in an older age range than classical scrapie, and is often found in sheep with prion protein genotypes associated with resistance to classical scrapie.

Worldwide, most cases of atypical scrapie have been detected during surveillance testing of apparently healthy sheep at slaughter or fallen stock (diseased or dead animals) by rapid immunochemical methods introduced for testing of small ruminants for TSE in the European Union from 2002. Prevalence estimates of atypical scrapie from these two test populations (slaughter and fallen stock) were remarkably uniform across 14 European countries (average 6.1 and 8.2 cases, respectively, per 10,000 tests), in contrast to the more variable and clustered occurrence of classical scrapie.

The National TSE Surveillance Program (NTSESP) in Australia includes passive surveillance for classical scrapie by histological screening of brains from sheep (at least 18 months of age) with clinical signs of progressive neurological disease. In this report, we describe the clinical and pathological findings in five NTSESP cases of atypical scrapie detected in New South Wales (in 1999 and 2016), Western Australia (in 2009) and Victoria (in 2011 and 2014).

History and clinical findings

Case reports

Case 1 (euthanased 2 August 1999, New South Wales: archival NTSESP material). A 2-year-old Merino ewe in mid-gestation developed hindlimb ataxia, muscle tremors, weakness and depression, with progression to recumbency in 2 weeks.
Case 2 (euthanased 23 December 2009, Western Australia). Within a pen of 1.5- to 5-year-old sheep undergoing inspection for live export, a Merino wether was circling.

Case 3 (euthanased 19 July 2011, Victoria). A 5-year-old Merino ewe with a cross-legged, ataxic gait was wandering aimlessly (“can’t seem to stop walking; almost appears to be circling”) and had lost weight.

Case 4 (euthanased 18 June 2014, Victoria). An 8-year-old Merino-cross ewe was observed to be ataxic, with a head tilt. After 2 months it had lost weight and showed signs of ataxia, head pressing and circling.

Case 5 (euthanased 1 April 2016, New South Wales). A 5-year-old Merino ewe displayed depression, separation from the flock, aimless wandering, and circling for at least 2 months. Its gait was slow but not ataxic. Weight loss was minimal.

No evidence of pruritus (rubbing or wool loss) was detected in any of these sheep.

Histopathology

Whole brains were collected from each sheep in the field, preserved in 10% neutral buffered formalin and submitted for histological screening for classical scrapie in accordance with the NTSESP and the Australian and New Zealand Standard Diagnostic Procedure (ANZSDP) for TSEs. Transverse sections of the caudal brainstem of all animals were sampled at the three standard sites specified in the ANZSDP: medulla through the obex, medulla through the caudal cerebellar pudenules (usually extended to cerebellar cortex in small ruminants) and midbrain through the rostral colliculi. In addition, a representative selection of other brain sites (including cerebellum, cerebral cortex, basal ganglia and thalamus) was sampled.

The formalin-fixed brain samples were processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (H&E) and assessed for histological evidence of neuronal or grey matter neuropil vacuolation suggestive of scrapie.

In all five animals, neural tissue was well preserved. The most consistent and prominent changes were detected in the cerebellum. They comprised multifocal to diffuse vacuolation of grey matter neuropil in the molecular layer of the cerebellar cortex and scattered vacuoles in the granule cell layer and underlying white matter, without associated evidence of inflammation. Sites of more variable and less prominent vacuolation of grey matter neuropil included the cerebral cortex, basal ganglia (caudate nucleus, globus pallidus and putamen), thalamus and substantia nigra. In the caudal brainstem at the three standard ANZSDP sites, only occasional vacuoles were detected within grey matter neuropil. Intracytoplasmic vacuolation of neurons was not detected in any animal.

At all levels in these brains, the white matter contained variable but usually small numbers of scattered, individual vacuoles, including at the sites of grey matter vacuolation.

Immunohistochemistry

Sections from the three standard ANZSDP sites and a selection of other brain sites were examined by IHC to detect PrPSc deposition. Separate sections from each sheep were immunolabelled using mouse monoclonal antibody F99/97.6.1 (VMRD, Pullman, WA, USA), which recognises an epitope of PrPSc; further confirmatory immunolabelling used other antibodies that recognise PrPSc epitopes, including monoclonal antibodies F89.160.1.5 (VMRD, Pullman), 2G11 (Novus Biologicals, Littleton, CO, USA), L42 (R-Biopharm AG, Darmstadt, Germany) and R145 (APHA Scientific, UK).

At each brain site, identifiable neuroanatomical areas were examined and the distribution and type of any PrPSc immunolabelling were recorded.

In all five sheep, immunolabelling (mainly fine granular type) of grey matter neuropil was most abundant in the molecular and granule cell layers of the cerebellum. Less prominent and more variable immunolabelling (fine granular and aggregate types) of grey matter neuropil was detected in the cerebral cortex and rostral brainstem, including the basal ganglia, thalamus and substantia nigra. Only scant immunolabelling of grey matter was detected in the caudal brainstem of the five sheep, including in the DMNV, solitary tract nucleus, spinal tract nucleus of the trigeminal nerve, reticular formation, midline raphe and olivary nuclei. Intracytoplasmic PrPSc immunolabelling of neurons was not detected.

Distinctive immunolabelling (globular and punctate types) was detected in the white matter at all levels of the brain in all five sheep (Figure 2).

Other types of PrPSc immunolabelling were not detected in these brains.

Biochemistry

Samples of fresh cervical spinal cord from each sheep and of cerebellum from cases 4 and 5 were submitted from the field in accordance with the NTSESP and ANZSDP (TSEs) for rapid immunohistochemical testing. The spinal cords from all five sheep tested negative and the cerebells from cases 4 and 5 tested inconclusive and negative,
respectively, in the Prionics®-Check WESTERN (Prionics AG, Schlieren, Switzerland) (Table 1). The spinal cords from the sheep in cases 4 and 5 tested negative and their cerebellums tested positive in the BioRad® TeSeE™ Western Blot (BioRad Laboratories Pty Ltd, NSW, Aust). The spinal cord from case 2 tested negative and the spinal cords and cerebellums from cases 4 and 5 all tested positive in the BioRad® TeSeE™ ELISA (BioRad Laboratories), with the ELISA reading for the cerebellum approximately 3-fold higher than that for the spinal cord sample in both sheep.

Table 1. Summary of biochemical results from five clinical cases of atypical scrapie in sheep

<table>
<thead>
<tr>
<th>Sample</th>
<th>Prionics®-Check WESTERN</th>
<th>BioRad® TeSeE™ Western Blot</th>
<th>BioRad® TeSeE™ ELISA</th>
</tr>
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<tbody>
<tr>
<td>Spinal cord</td>
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<tr>
<td>Case 1</td>
<td>Neg</td>
<td>NT</td>
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<td>Case 2</td>
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<td>Case 3</td>
<td>Neg</td>
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<tr>
<td>Case 4</td>
<td>Neg</td>
<td>Neg</td>
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<tr>
<td>Case 5</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
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<tr>
<td>Cerebellum</td>
<td></td>
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</tr>
<tr>
<td>Case 1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>Case 2</td>
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<tr>
<td>Case 4</td>
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<td>Pos*</td>
</tr>
<tr>
<td>Case 5</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos*</td>
</tr>
</tbody>
</table>

* Cerebellum ELISA reading threefold higher than spinal cord reading. Inc, inconclusive; Neg, negative; NT, not tested; Pos, positive.

Figure 2. Case 3: Cerebellum. Diffusely throughout the molecular layer (ML) and less prominently in the granule cell layer (GCL) there is fine granular PrPSc immunolabelling (light-brown). Purkinje cells (P) are not immunolabelled. White matter (WM) contains globular and punctate PrPSc immunolabelling (dark-brown). (IHC PrP Sc Mab F99/97.6.1. Bar = 110 μm.)

Discussion

Atypical scrapie (‘atypical/Nor98 scrapie’) is clinically, pathologically, biochemically and epidemiologically different from classical scrapie.²

The five sheep in this report showed clinical signs of progressive neurological disease consistent with those reported for atypical scrapie, including ataxia, behavioural change (including circling) and weight loss, but without the pruritus and associated wool loss seen in classical scrapie.⁵,¹⁴,¹⁵

The lesion profile of classical scrapie (the neuroanatomical distribution of vacuolation and PrPSc immunolabelling of neuronal cytoplasm and grey matter neuropil within the CNS) reflects the pathogenesis of neuro-invasion following oral infection. The scrapie agent spreads from the gut via the vagus nerve to the DMNV in the caudal brainstem and via the splanchnic nerve to the intermediolateral column (lateral horn) in the thoracic spinal cord; from these CNS sites, infection and associated lesions spread in ascending and descending directions throughout the neuraxis.¹⁶–¹⁸ At clinical endpoint, PrPSc immunolabelling occurs throughout the neuraxis at a moderate to marked severity.³

In contrast, in atypical scrapie, which appears to be a spontaneous disease that occurs worldwide, neuropil vacuolation and PrPSc immunolabelling are more restricted in the CNS, with the cerebellum and forebrain most affected.²–⁵ The overall severity of immunolabelling is generally mild to moderate.⁷ It is speculated that the PrPSc deposition and histopathological lesions evolve from the cerebrum to the cerebellum and brainstem.¹⁸,¹⁹ It is noteworthy that within the six subtypes of sporadic Creutzfeldt-Jacob disease, the most frequently occurring human prion disease, which has a worldwide distribution, grey matter neuropil vacuolation and PrPSc deposition also have a predilection for the cerebral cortex, cerebellar cortex and basal ganglia.²⁰

Most cases of atypical scrapie have been detected during surveillance testing of apparently healthy sheep at slaughter or of fallen stock, where usually only caudal brainstem and occasionally also the cerebellum were tested.⁶,⁸,⁹ However, pathological studies of whole brains from mainly clinically affected sheep with atypical scrapie have identified a characteristic distribution of neuropil vacuolation and PrPSc deposition.⁵,¹⁸ The distinctive lesion profile was detected in the brains of the five sheep in this report. It comprised vacuolation of grey matter neuropil in the cerebellar cortex (particularly in the molecular layer), cerebral cortex, basal ganglia, thalamus and substantia nigra, with associated PrPSc deposition in both grey and white matter. Notable were the paucity of neuropil vacuolation and PrPSc deposition within grey matter in the caudal brainstem, including the DMNV, usually affected in classical scrapie, and the absence of the intracytoplasmic vacuolation and PrPSc immunolabelling of neurons that occur in classical scrapie.

Deposition of PrPSc within white matter throughout the brain, detected as globular and punctate immunolabelling by IHC, appears to be unique to atypical scrapie among the TSEs and is possibly associated with oligodendrocytes;¹²,²¹ its demonstration in all five sheep supported a specific diagnosis of atypical scrapie. However, the associated vacuolation of white matter detected in the H&E sections was variable, often minimal, and indistinguishable from that reported as...
an incidental CNS change in clinically normal sheep\textsuperscript{22} or as a more severe change in toxic or metabolic myelinopathies\textsuperscript{23} and in autolysed CNS samples. Consequently, routine histological examination of brains for atypical scrapie (or any prion disease) should focus on detecting the characteristic vacuolation of grey matter neuropil, which is best appreciated in well-preserved, non-autolysed CNS sections (Figure 1).\textsuperscript{13}

The failure to detect PrP\textsuperscript{Sc} in fresh samples of cervical spinal cord from three of the five sheep was consistent with the paucity of PrP\textsuperscript{Sc} immunolabelling detected by IHC in the caudal brainstems of all five animals. The results of testing the fresh CNS samples from cases 4 and 5 confirmed a relatively larger accumulation of PrP\textsuperscript{Sc} in the cerebellum compared with cervical spinal cord, and also suggested an increasing level of test sensitivity from Prionics\textsuperscript{TM}-Check WESTERN through BioRad\textsuperscript{®} TeSeETM Western Blot to BioRad\textsuperscript{®} TeSeETM ELISA in these cases. The rapid immunochromatic and IHC immunolabelling results for PrP\textsuperscript{Sc} in the cerebellums from cases 4 and 5 confirmed the diagnostic relevance of the neuropil vacuolation detected in H&E sections of cerebellar cortex from these animals. Also, by identifying the distinctive neuroanatomical distribution and type of PrP\textsuperscript{Sc} immunolabelling in the cerebellum, IHC provided a specific diagnosis of atypical scrapie in all five sheep.

These five animals with atypical scrapie were identified during the NTSESP testing of 9260 sheep from 1 January 1998 to 1 April 2016 inclusive\textsuperscript{24} and represent a prevalence of 5.4 clinical cases per 10,000 brains examined. This prevalence estimate is not directly comparable with the prevalence of atypical scrapie reported from European countries, because of differences in population sampling and the lower sensitivity of histological screening used in the NTSESP compared with the mass screening by rapid immunochemical methods in Europe.\textsuperscript{5} The diagnosis of five atypical but no classical scrapie cases during the NTSESP histological screening of brains from adult sheep with clinical signs of neurological disease confirms the value of the NTSESP in detecting prion disease in Australian sheep. Importantly, the prompt formalin fixation of whole brains collected by field veterinarians following euthanasia of these sheep minimised autolytic CNS changes and enabled histological detection of the subtle vacuolation of grey matter neuropil characteristic of prion disease.

Although histological screening of sheep brains in the NTSESP focusses on the caudal brainstem to detect lesions of classical scrapie, veterinary pathologists should be mindful of the distinctive lesion profile of atypical scrapie, as sporadic cases of this prion disease will continue to occur in Australian sheep. The ANZSDP (TSEs) includes information on the diagnosis of scrapie, including the current OIE recommendation to examine the cerebellum in addition to the caudal brainstem in sheep and goats within surveillance programs to detect and differentiate cases of classical and atypical scrapie.\textsuperscript{13,25}

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