Veterinary Microbiology 147 (2011) 237–243

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Research article

An assessment of the efficiency of PrPsc detection in rectal mucosa and third-eyelid biopsies from animals infected with scrapie

Eva Monleón [a,*], Mª Carmen Garza [a], Rocío Sarasa [a], Javier Álvarez-Rodriguez [b], Rosa Bolea [a], Marta Monzón [a], M. Antonia Vargas [a], Juan José Badiola [a], Cristina Acín [a]

[a] Centro de Investigación en Encefalopatías y Enfermedades Transmisibles Emergentes, Universidad de Zaragoza, C/ Miguel Servet 177, 50013 Zaragoza, Spain
[b] Centro de Investigación y Tecnología Agroalimentaria, Gobierno de Aragón, Avda, Montañana 930, 50059 Zaragoza, Spain

ARTICLE INFO

Article history:
Received 27 December 2009
Received in revised form 21 June 2010
Accepted 29 June 2010

Keywords:
Scrapie
Diagnosis
Lymphoid tissue

ABSTRACT

In classical scrapie, detection of PrPsc on lymphoreticular system is used for the in vivo and post mortem diagnosis of the disease. However, the sensitivity of this methodology is not well characterised because the magnitude and duration of lymphoid tissue involvement can vary considerably. The aim of the present study was to evaluate the efficiency of detecting PrPsc in rectal mucosa and third-eyelid biopsies. A total of 474 genetically susceptible sheep and 24 goats from three scrapie infected flocks were included in this study. A sample from rectal mucosa and a sample from third-eyelid lymphoid tissue were collected from each animal. Biopsy samples were fixed in formaldehyde and processed for immunohistochemical examination. Animals with negative biopsy results were studied more closely through a post mortem examination of central nervous and lymphoreticular systems and if there was a positive result, additional biopsy sections were further tested. The sensitivity of rectal mucosa and third-eyelid assays were 36% and 40% respectively on initial examination but increased to 48% and 44% respectively after retesting. The results of this field study show a high percentage of infected animals that do not have detectable levels of PrPsc in the biopsied lymphoid tissue, due mainly to the relatively high number of animals with minimal or no involvement of lymphoid tissue in the pathogenesis of the disease.

1. Introduction

Bovine spongiform encephalopathy (BSE), scrapie and chronic wasting disease (CWD) are transmissible spongiform encephalopathies (TSEs) that affect domestic and wild ruminants. These diseases have some common characteristics, including long incubation periods, brain vacuolation and accumulation of abnormal forms (PrPsc) of a host protein (PrPc) in the central nervous system (CNS). However, there are important differences between these diseases, primarily with respect to their pathogenesis. In the case of BSE, PrPsc accumulation is restricted to the nervous system and, to a lesser extent, to the gut-associated lymphoid tissue (Buschmann and Groschup, 2005). In contrast to BSE, scrapie and CWD are associated with wide PrPsc dissemination in many non-neural tissues including the lymphoreticular system, the kidney and the placenta (Jeffrey and González, 2007; Sigurdson, 2008). Moreover, it is well known that the sheep PRNP genotype has a strong effect on the development of clinical scrapie. Susceptibility of sheep to scrapie is influenced by polymorphisms at codons 136, 154 and 171 of the prion protein gene; PRNP haplotypes VRQ and ARQ are associated with the highest susceptibility and ARR with the lowest susceptibility (Goldmann, 2008). The PRNP genotype also affects the pathogenesis of scrapie. For instance, in VRQ/ARR sheep there is little or no involvement of the lymphoid tissue in agent replication (van Keulen et al., 2008).

* Corresponding author. Present address: Dpto. Producción Animal, Universitat de LLeida, Av. de l'Alcalde Rovira Roure, 191, 25198 LLeida, Spain. Tel.: +34 976 762835; fax: +34 976 762508.
E-mail address: emonleon@unizar.es (E. Monleón).

0378-1135/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2010.06.028

PrPsc is used as a biochemical marker to indicate the presence of TSE causative agents (Mabbott and MacPherson, 2006). At present, TSE diagnosis is conducted post mortem through brain histological analysis and immunodetection of PrPsc in extracts or tissue sections of the CNS. In contrast to BSE, detection of PrPsc in lymphoid tissues allows the diagnosis of scrapie and CWD. Since PrPsc can accumulate in lymphoid tissues before spreading to the nervous system and this accumulation can be very extensive, some authors have proposed using this tissue for the in vivo and post mortem diagnosis of scrapie (Schreuder et al., 1998; O'Rourke et al., 2000; Monleón et al., 2005; Espenes et al., 2006; Langeveld et al., 2006). However, the sensitivity of this methodology is not well characterised because the magnitude and duration of lymphoid tissue involvement can vary considerably. In addition to the host PRNP genotype, the scrapie agent, the route of inoculation and the dose are thought to influence the distribution of the agent within host tissues (Jeffrey and González, 2007).

The aim of the present study was to evaluate the efficiency of detecting PrPsc in biopsies of rectal mucosa and third-eyelid lymphoid tissues. In addition, we compared the performance of third-eyelid and rectal mucosa lymphoid tissue biopsies in diagnosing scrapie in the field.

2. Materials and methods

2.1. Animals and flocks

Three scrapie-infected flocks (A, B and C) detected in the framework of the Spanish TSE surveillance programme were included in the present study. In flocks A and C, the outbreak was detected in one falling stock sheep and in three suspect animals in flock B. In accordance with the European Union regulations, all genetically TSE-susceptible animals from the infected flocks were culled. Before culling, a random sample of the genetically TSE-susceptible animals over the age of 12 months was selected for the study. With two exceptions, all animals were asymptomatic. Details of the flocks are shown in Table 1.

Table 1
Details of the flocks used in the study, including the size, breed and number of animals examined.

Flock	Flock size	No. of examined animals
A	3000 sheep[a]	231
B	1000 sheep[a]	150
	11 goats[b]	11
C	164 sheep[b]	93
	13 goats[b]	13

[a] Rasa aragonesa breed.
[b] Crossbreed.

2.2. Sampling

A total of 474 sheep and 24 goats were included in the present study. Each animal was sampled for rectal mucosa and third-eyelid biopsies using methods previously described (O'Rourke et al., 2000; González et al., 2008). Animals were restrained manually and trained veterinarians collected all biopsies on the farm of the origin. Biopsy samples were fixed in 10% neutral buffered formalin and processed for histological examination and PrPsc detection by IHC.

Animals with positive biopsy samples (No. 1–10 Table 2; one of them clinically affected, No. 3) and one clinically affected sheep with negative biopsy sample (No. 15 Table 3) were removed from the flock for monitoring. When clinical signs progressed to severe (between 15 days and 21 months post biopsy sampling), sheep were euthanised by intravenous injection of sodium pentobarbital and exsanguination. The following tissues were taken and placed into a fixative (formalin 10%): brain, tonsils, spleen, Peyer's patch of the ileum, as well as the rectum and mesenteric, retropharyngeal and mediastinic lymph nodes. Samples were processed for histological examination and PrPsc detection by IHC.

Animals with negative biopsy samples were culled approximately 7–10 days after biopsy sampling and were

Table 2
Animals with positive biopsy samples. From each animal the flock, genotype, age, immunohistochemical results from third-eyelid and rectal mucosa biopsies and from post mortem samples (central nervous system, tonsils and retropharyngeal lymph node) are included. The score of immunostaining extension is indicated. When more than one biopsy tissue section was examined, the total number of lymphoid follicles assessed is indicated.

Animal No. flock	Genotype	Age	IHC results from biopsy		IHC results in tissues collected post mortem		
			Third-eye	Rectal	CNS	Tonsil	RLN
1-A	ARQ/ARQ	2–3	+	++	+++[a]	+++[a]	+++[a]
2-A	ARQ/ARQ	2–3	++	+++	+++[a]	+++[a]	+++[a]
3-B	ARQ/ARQ	3–4	+++	+++	+++[a]	+++[a]	+++[a]
4-B	ARQ/ARQ	3–4	+++	+++	+++[a]	+++[a]	+++[a]
5-B	ARQ/ARQ	3–4	+++	+++	++[a]	+++[a]	+++[a]
6-B	ARQ/ARQ	3–4	++	+++	+++[a]	+++[a]	++[a]
7-C	ARQ/VRQ	2–3	+++	++	+++[a]	+++[a]	++[a]
8-C	VRQ/VRQ	3–4	++	+++	+++[a]	+++[a]	+++[a]
9-C	ARQ/VRQ	>6	+++	+++	+++[a]	+++[a]	++[a]
10-C	Goat	>6	+++	Unsuitable	+++[a]	+++[a]	++[a]
11-B	ARQ/ARQ	1–2	−[b](60)	+[b](>100)	−	+	+
12-C	ARQ/ARQ	1–2	−[b](40)	+[b](>100)	++	+	+
13-C	ARQ/VRQ	1–2	++[b](50)	+[b](10)	+	+++	+++

[a] Samples were collected and score when animals presented severe clinical signs (15 days–21 months post biopsy sampling).
[b] Immunohistochemical results when 4 biopsy sections were tested.