

PAPERS & ARTICLES

isolates which have distinct human profiles. Although no species-specific ruminant isolates of *Cryptosporidium* have been characterised, the observation that this outbreak was limited to goats suggests that such isolates may exist.

At present, there is no acceptable treatment for cryptosporidiosis. However, in this outbreak the antibiotic amosidine sulphate had some effect in reducing both the severity of the disease and the faecal shedding of the organism (unpublished observations).

This is the first report of caprine cryptosporidiosis in the Sultanate of Oman. In contrast with the generally established clinical picture of cryptosporidiosis as a disease of neonates, this outbreak was unusual because of the extreme virulence of the organism, its apparent species-specificity, and its shedding by animals of all age groups.

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SHORT COMMUNICATIONS

Immunohistochemical evaluation of tonsillar tissue for preclinical screening of scrapie based on surveillance in Belgium

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SCRAPIE is a fatal neurodegenerative disease that occurs naturally in sheep and goats, and is the archetype of transmissible spongiform encephalopathies (TSEs) or prion

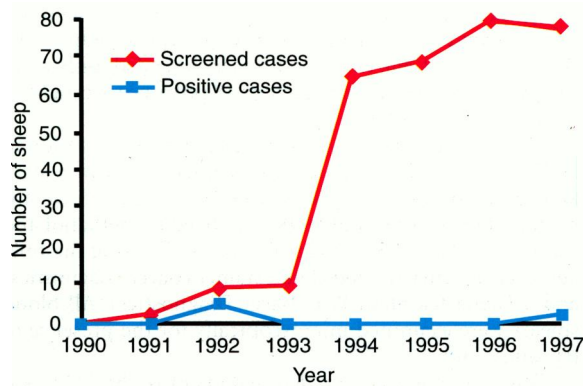
diseases (Parry 1962, Bossers and others 1996). Currently, the aetiology is considered as an infectious disease with a maternal and lateral contagious transmission, where host genetic factors play a central role (Dickinson and others 1974, Belt and others 1995, Smits and others 1997). Confirmation of the diagnosis of TSE disease is given by conventional microscopy of haematoxylin and eosin (HE) stained brainstem tissue sections. However, two additional diagnostic criteria are also widely employed; the detection of disease-specific fibrils by transmission electron microscopy (Scott and Stack 1994) and the detection of the main constituent of the fibrils, the abnormal protein PrP^{Sc} (Prusiner 1992).

Eleven (3.5 per cent) clinically positive and histologically confirmed cases were found in Belgium, between May 31, 1990 and December 31, 1997, from a total of 314 screened sheep (Fig 1). Of the 11 positive cases, five occurred in 1992 on one farm (Vanopdenbosch and others 1993) and six on two farms (used in this study) in 1997. Forty-six of the screened animals were clinically suspected, while the other 268 were clinically healthy animals submitted for investiga-

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FIG 1: Annual number of sheep screened and those found clinically positive for scrapie between May 31, 1990 and December 31, 1997 in Belgium



tion for herd certification. Details of the epidemiosurveillance network for TSEs in Belgium are described by Vanopdenbosch and others (1998).

One hundred and eleven sheep, at least one year old, were removed from the two farms diagnosed as scrapie positive in 1997, based on the examination of three sheep from each farm, which were clinically suspected of scrapie. The sheep were divided into two groups. The farms were placed under close surveillance (Vanopdenbosch and others 1998) from the day of the clinical suspicion, but no other animals showed clinical signs of scrapie.

Once the clinical suspicion was confirmed by histopathological, immunohistochemical (IHC) and electron microscopic examination, approximately 14 days after restrictions were placed on the farm, all the animals were euthanased. The first group consisted of 89 Flevolandiers and the second comprised 22 sheep of mixed Texel origin. As a result of observations on the use of tonsillar tissue (Schreuder and others 1998) for IHC diagnosis in the preclinical stage, both brainstem and tonsils were removed from all of the sheep. The immunoperoxidase technique and the primary antibody R524 (rabbit anti-PrP-peptide serum) used are described by van Keulen and others (1995).

Three animals from the first farm appeared positive in immunostaining for both brainstem and tonsils without any histopathological lesions in the brainstem or clinical symptoms. Another three sheep stained positive in both tonsils, but did not stain or show any histopathological lesion in the brainstem. They also lacked clinical symptoms (Table 1). The second farm did not exhibit PrP^{Sc} positivity apart from the three cases on which the farm was diagnosed. The PrP^{Sc} staining of all the positive tonsils was present in more than four follicles in the cytoplasm and between the cells of the lymphoid population of the tonsils (germinal centres).

IHC is a practical diagnostic method that can be performed in every histopathological laboratory and studies are currently

being undertaken to find a way of diagnosing TSEs in live animals. Schreuder and others (1998) suggested that IHC of tonsillar biopsies could be used, based on surveys under experimental circumstances.

In this study, the same technique is described but under natural circumstances, that is in two flocks from farms which were diagnosed as being scrapie positive.

The results from the present study indicate that the presence of PrP^{Sc} in the CNS precedes histopathological lesions and clinical signs, and that PrP^{Sc} can be detected in tonsils in the absence of PrP^{Sc} in the CNS. The lack of clinical symptoms and histopathological lesions in the three cases with positive immunohistochemical staining only in the tonsils, suggests that these sheep could have been preclinically infected and may have developed histopathological changes and clinical symptoms in the future. They could have been a potential source of infection to other animals within their own flock, or in other flocks if sold.

In conclusion, tonsil tissue IHC or PrP^{Sc} can be used as a supplementary test to screen flocks for the presence of scrapie. This is not only based on data originating from experimental conditions, but also based on results in the field. This technique appears to be of importance in the detection of preclinical cases of scrapie, proving it to be an asset in limiting the spread of scrapie throughout the sheep population.

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TABLE 1: Results of the screening of sheep at least one year old, from the two farms

	Number of sheep	Clinical symptoms	Histopathological lesions in the brainstem	Immunohistochemistry	
				Obex	Tonsils
Farm 1 (Flevolander breed)	3*	Yes	+	+	+
	3	No	-	+	+
	3	No	-	-	+
	80	No	-	-	-
Farm 2 (mixed Texel breed)	3*	Yes	+	+	+
	19	No	-	-	-

* Number of sheep sent in as suspected, and on which the initial diagnosis of scrapie was based + Positive, - Negative



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