A biopsy procedure suitable for large scale screening for scrapie in live sheep and goats

Introduction

• Definitive diagnosis of TSE disease e.g. scrapie in sheep and goats and chronic wasting disease (CWD) in deer relies on analysis of brain material from dead animals.

• Lymphoid tissues, including tonsil and third eyelid, have been investigated to develop a screening test suitable for use in live animals at farm level. Tissues are technically difficult to sample, require anaesthesia and expensive specialist tools and also result in significant discomfort to the animal and may retard growth rate.

• We have developed a novel, simple biopsy technique to obtain lymphoid tissue from the recto-anal mucosa-associated lymphoid tissue or RAMALT, which does not require general anaesthesia, sedation or expensive tools and can be used to undertake large scale screening for scrapie in live sheep and chronic wasting disease in live deer.

Materials and Methods

Location of the RAMALT

The RAMALT is located immediately beneath the last 1cm of rectal mucosa at the end of the alimentary tract. The lymphoid follicles are found in aggregates underneath the crypts of the rectal mucosa folds (Fig 1) and become more visible when treated with glacial acetic acid (Fig. 2) and trans-illuminated (Fig. 3).

Biopsy Procedure in Live Animals

We have developed a biopsy procedure to obtain RAMALT tissue from live sheep including the design, manufacture and testing of a disposable speculum to gain access to the target 1cm² area of rectal mucosa (Fig. 5).

• The speculum is inserted in the anus after liberal application of analgesic cream which also doubles as a lubricant. The folds of the rectal mucosal are now easily identified (Figs. 6 & 7).

• A sample of RAMALT tissue is taken by picking up the base of a crypt with forceps and cutting off the resultant 'tent' of tissue with scissors (Fig. 8).

• This precise location of sampling ensures adequate RAMALT lymphoid follicles are present that is essential for the detection of scrapie².

• Biopsy sample fixed and processed, thin sections cut and mounted on glass slides. Tissue sections examined for the presence of the disease associated marker PrP²(Fig. 9)

• Brown staining within the lymphoid follicles is a positive result for scrapie (Fig. 9). The same would apply for deer with CWD.

Results

• Test 100% specific and 98.7% sensitive for detecting scrapie in clinically affected sheep confirmed positive by standard diagnostic test.

• No detectable adverse effect on health and welfare of animals.

• Test can detect asymtomatic infected animals up to one year prior to onset of clinical signs.

Conclusions and Future Work

• Biopsy procedure is quick, easy to perform, does not require expensive tools, general anaesthesia or sedation and has no adverse effects on the animal.

• Current limiting factor is the tissue processing and examination required for immunohistochemistry. This is being addressed by development of an ELISA test which would enable a roboticised procedure with a high throughput of samples.

• A parallel ELISA is also being developed to detect the presence of RAMALT to avoid false negative results from incorrect tissue sampling.

• Once this development has been completed we would be in a position to undertake large scale screening of the national sheep flock.

References


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