Reviewer's report

Title: A dual fluorescent multiprobe assay for prion protein genotyping in sheep

Version: 2   Date: 25 November 2004

Reviewer: Michael Andreas A Tranulis

Reviewer's report:

General
This contribution deals with the establishment and validation of a method for analysis of polymorphisms at codons 136, 154 and 171 in the ovine PrP-gene (PRNP). The method consists of dual-labelled fluorescent allele specific probes that are used for detection of allelic variation with real-time PCR. A great number of different methods have been used for PrP-genotyping, most of which are relatively time-consuming. The method described in this communication is fast and robust and to the best of my knowledge not previously published.

The method is well described and proper controls have been carried out. Data are clearly presented and discussed in a relevant manner. However, this referee thinks that the paper can be significantly improved prior to publication. Some suggestions are given below.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Sheep scrapie is a complex disease. Oversimplifications concerning the genetic influence on scrapie occurrence should be avoided in original papers like this. Authors fail to address the up-coming reports from several countries in Europe of atypical scrapie, affecting sheep with "neutral" and even "highly resistant" PrP-genotypes. These data can prove to have major impact on the ongoing breeding programmes for reduced scrapie susceptibility throughout Europe. Thus, the authors must address this problem properly in the introduction and discussion of this communication. Here are some papers of relevance.

1: Benestad SL, Sarradin P, Thu B, Schonheit J, Tranulis MA, Bratberg B.

Identification of putative atypical scrapie in sheep in Portugal.


4: Luhken G, Buschmann A, Groschup MH, Erhardt G.
Prion protein allele A136 H154Q171 is associated with high susceptibility to


Authors must thus discriminate between classical scrapie and atypical scrapie.

Table 1 appears unnecessary and can be removed. If not, it must be clearly stated that the validity of the information is limited to classical scrapie.

The transition between the “Methods” and “Results” part of the paper is unclear. The first sub-title in the “Results” section is “Dual fluorescent multiprobe assay setup”, while an identical sub-title, except for the word “setup” is also present in the “Methods” section. This must be sorted out and presented more accurate. Furthermore, the authors fail to properly present the amount of DNA used in their reactions, when 3 µl of a solution with approximately 50 ng/µl of DNA is used, one should give ~ 150 ng of DNA as the amount used, each time this is of relevance. The authors should also briefly comment on why PCR reactions are run for 40 cycles, which is, at least 5 cycles more than necessary – is there a reason for this – if so, it could be of interest to the readers.

Finally, the terms BHQ1, BHQ2 (Black hole quenchers), FAM, HEX, LNA-probes and Cy5 appears to be missing in the list of abbreviations. There are still a considerable number of typos in the text.

I recommend the publication of this paper.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests