Reviewer's report

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

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Reviewer: Gabriele Vaccari

Reviewer's report:

General
The manuscript of van Poucke and collaborators “A dual fluorescent multiprobe assay for prion protein genotyping in sheep” describes an alternative method for prion protein (PrP) gene genotyping. So it can be considered as a Technical advance article. Such kind of analysis is useful for the implementation of European breeding programmes for scrapie resistance throughout all European Countries. Different methods have been already published as the authors reports. However the availability of different method for PrP genotyping using different equipments could be useful for the application of such genetic analysis in a widespread range of laboratory that possess different instrument. The proposed method uses a dual fluorescent multiprobe assay in a real time PCR. In one assay polymorphisms at codon 136 (A/V) and 154 (R/H) of the PrP gene are detected in the other at codon 171 (R/H/Q).

There are however some fundamental questions that must be answered before the manuscript can be accepted for publication:

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


Author should demonstrate that such polymorphisms will not influence the correct genotyping result of the assay. In alternative different probes should be designed or a definition of the limit of the assay should be stated.

2 The method is validated with samples of known genotypes which are determined by RFLP analysis. This should not to be considered a gold standard and sequencing should be used as reference for validating alternative genotyping methods.

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

3 In the design of the assays Authors do not mention the other PrP variants K at codon 171 which has been observed (Gombojav A. et al., 2003, J. Vet Med Sci.) and recently reported in some south European Country (Billinis et al., 2004 J. Gen. Virol., Acin et al., 2004, J. Gen. Virol., Acutis P.L. et al., 2004, J. Gen. Virol.) and in USA (DeSilva U. et al Cytogenet Genome Res. 2003). This variant is not so widespread and not yet defined with regards to scrapie susceptibility but it must be at least mentioned and it should be explained why it is not a target of the assay.
4 The acronym PRNP should be used to identify only the human PrP gene, in animals the abbreviation Prnp is preferable. This should be revised in all the article.

5 The 8 controls included in each run do not represent all the combination of the 5 most frequent allelic variant of the PrP gene. They represent all combination of variant of each polymorphic locus.

6 In the discussion is stated that the methods is intended to be used with “crude DNA as template for PCR … although the amplification plots are nicer with purified DNA”. It is not clear the definition of “crude DNA” and “purified DNA”. If DNA is prepared in different manners, a description of both should be included in the materials and methods section.

Discretionary Revisions (which the author can choose to ignore)

7 The possibility to use such a methods with other Real Time PCR apparatus is a very important information that could be mentioned if available.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests