# Six years of proficiency tests for the identification of Prion protein genotype in sheep

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## Introduction

Scrapie of sheep belongs to a group of fatal neurodegenerative diseases known as prion diseases. Other members of the group are Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy in cattle. Scrapie is an infectious disease and sheep susceptibility is under the control of the host genotype. On this basis, the European Union has established management strategies for scrapie, to be achieved both by means of ad hoc breeding programmes and selective culling, in the case of an outbreak based upon the Prion protein (PrP) genotype. Since 2005, the Istituto Superiore di Sanità organises proficiency tests (PT) focused on genotype identification from sheep blood for the detection of genetically susceptible or resistant animals, aimed to assess the analytical accuracy of the tests conducted by the participants.

In sheep 4 main polymorphisms at codon 136, 141, 154 and 171 of the PrP are those that influence sheep scrapie susceptibility (Figure 1) giving rise to six main alleles: ALRQ, ALRR, ALHQ, VLRQ, ALRH, AFRQ.
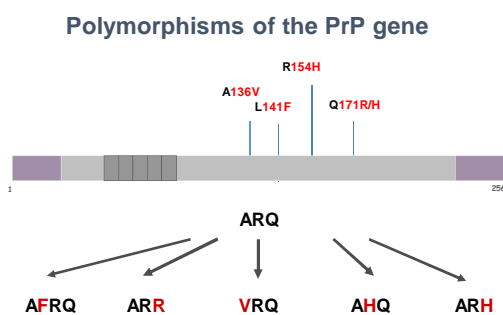
**Polymorphisms of the PrP gene**



Figure 1. Principal polymorphisms of the PrP

## Results

A total of six PT were organized between 2005 and 2013. On each occasion, except one in which 18 samples were used, 20 blood samples with different genotypes (Table 1) were submitted for analysis to the 11 or 12 laboratories (depending on the year) performing such tests on a routine basis.

Participants were allowed to use their own method, (Real-Time PCR, Sanger sequencing, Primer extension, Pyrosequencing or a combination of Real-Time PCR and Restriction fragment length polymorphism).

Table 1.
Number of sample for each genotype included in the PT in each year

| | 2005 | 2009 | 2010 | 2011 | 2012 | 2013 |
|---|---|---|---|---|---|---|
| ALHQ/ALHQ | 1 | | | | | |
| ALRQ/ALHQ | 2 | 2 | 2 | 1 | 1 | 2 |
| ALRQ/ALRH | 1 | 1 | 1 | 1 | 1 | 1 |
| ALRQ/ALRQ | 5 | 3 | 3 | 5 | 3 | 4 |
| ALRQ/VLRQ | 1 | 1 | 1 | | 1 | 1 |
| ALRR/ALHQ | 1 | | | | 1 | 1 |
| ALRR/ALRH | 1 | 1 | 1 | 1 | 1 | |
| ALRR/ALRQ | 5 | 1 | 1 | 2 | 5 | 4 |
| ALRR/ALRR | 2 | 1 | 3 | 5 | 4 | 3 |
| ALRR/VLRQ | 1 | 1 | 1 | | 1 | 1 |
| ALHQ/ALRH | | 2 | 2 | 1 | | 1 |
| ALRQ/AFRQ | | 1 | 1 | | 1 | 1 |
| ALHQ/VLRQ | | 1 | 1 | | | 1 |
| VLRQ/VLRQ | | 1 | 1 | 1 | | |
| ALRH/ALRH | | 1 | 1 | 1 | | |
| ALRH/VLRQ | | 1 | 1 | 1 | | |
| ALRR/AFRQ | | | | 1 | | |
| AFRQ/AFRQ | | | | | 1 | |
| Total | 20 | 18 | 20 | 20 | 20 | 20 |

The results obtained by each laboratory were used to evaluate the accuracy, specificity, sensibility and inter-annotator agreement using the Cohen's kappa coefficient of the method used. The performance of the laboratories involved improved during the years are reported in Figure 2.
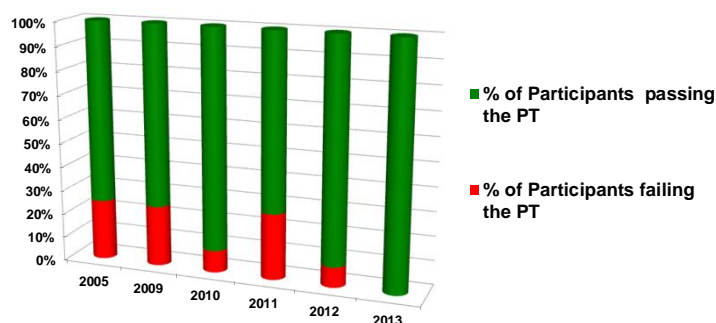


Figure 2. Percentages of laboratories passing or failing the PT

## Conclusion

The low number of laboratories failing the PT is indicative of an overall good technical level of the laboratories involved in the management of scrapie. Moreover the positive trend of the percentages of laboratories passing the PT suggests an improved performance on the correct identification of animals resistant or susceptible to scrapie.