

Identification of Prion protein genotype in sheep in Italy: eleven years of proficiency tests

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Introduction

Scrapie of sheep belongs to a group of fatal neurodegenerative diseases known as prion diseases. Scrapie is a naturally transmissible disease and sheep susceptibility is under the control of the host genotype. On this basis, the European Union has established management control strategies, to be achieved by both an *ad hoc* breeding programmes for genetic resistance of the ovine population and selective culling based upon the Prion protein genotype. To assess the accuracy of the genetic analysis performed by the Italian official laboratories appointed for PrP genotyping, the Istituto Superiore di Sanità organises proficiency tests (PTs) for sheep PrP genotype identification in accordance with the Ministerial Decree⁽¹⁾.

In sheep 4 main polymorphisms at codon 136 (A/V), 141 (L/F), 154 (R/H) and 171 (Q/R/H/K) of the PrP are those that influence sheep scrapie susceptibility (Figure 1) giving rise to seven main alleles: ALRQ, AFRQ, ALRR, ALRH, ALHQ, ALRK, VLRQ.

From 2005 to 2016, nine PTs were carried out. All the participants (from 11 to 13 laboratories depending on the year) received a set of sheep blood samples with different genotypes (Tab.1), which varied between 18 and 20 samples over the course of years.

	2005	2009	2010	2011	2012	2013	2014	2015	2016	tot
ALHQ/ALHQ	1							2	1	4
ALRQ/ALHQ	2	2	2	1	1	2	1	3	2	16
ALRQ/ALRH	1	1	1	1	1	1	1		1	8
ALRQ/ALRQ	5	3	3	5	3	4	5	4	3	35
ALRQ/VLRQ	1	1	1		1	1			1	6
ALRR/ALHQ	1				1	1	1	1	1	6
ALRR/ALRH	1	1	1	1	1		1			6
ALRR/ALRQ	5	1	1	2	5	4	5	4	4	31
ALRR/ALRR	2	1	3	5	4	3	4	4	4	30
ALRR/VLRQ	1	1	1		1	1				5
ALHQ/ALRH		2	2	1		1				6
ALRQ/AFRQ		1	1		1	1	1	1	1	7
ALHQ/VLRQ		1	1			1				3
VLRQ/VLRQ		1	1	1			1			4
ALRH/ALRH		1	1	1						3
ALRH/VLRQ		1	1	1						3
ALRR/AFRQ				1				1		2
AFRQ/AFRQ					1					1
ALRK/ALRK									1	1
ALRR/ALRK									1	1
Total	20	18	20	20	20	20	20	20	20	178

Table 1. Number and genotype of samples included in the PTs

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

REFERENCES: ⁽¹⁾ Ministerial Decree 17/12/2004 G.U. 3/3/2005 replaced with the Ministerial Decree of 5/11/2015 G.U. 27/01/2016

Polymorphisms of the PrP gene

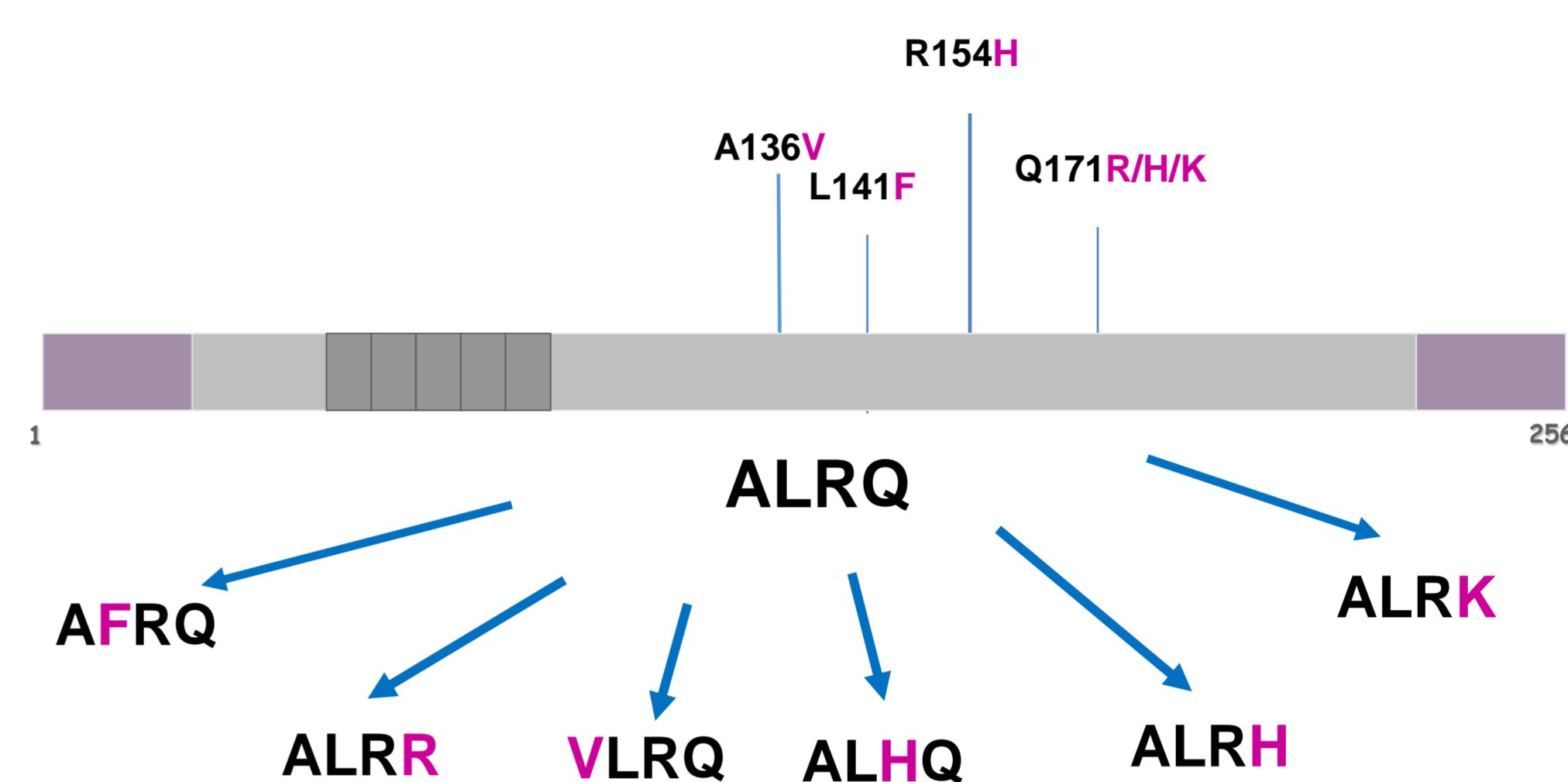


Figure 1. Principal polymorphisms of the PrP in sheep

Results and conclusion

The results obtained by each laboratory were used to evaluate the accuracy, specificity, sensibility and inter-laboratory Cohen's kappa agreement.

Over the years, a total of 22 errors in genotype identification were reported. They include either analytical (true) errors (n=16) or formal errors (n=6) not associated with analytical failure. Analytical errors were related to the use of reverse hybridization (n=4) or real-time PCR (n=12) and were associated with the erroneous definition of the genotype at codons 154 and 171. Formal errors, related to the use of sequencing (n=1) or real time PCR (n=5), were due to inaccuracy in the interpretation of the results or to the incorrect reconstruction of the allelic phase. The performance of the laboratories involved improved during the years and are reported in Figure 2.

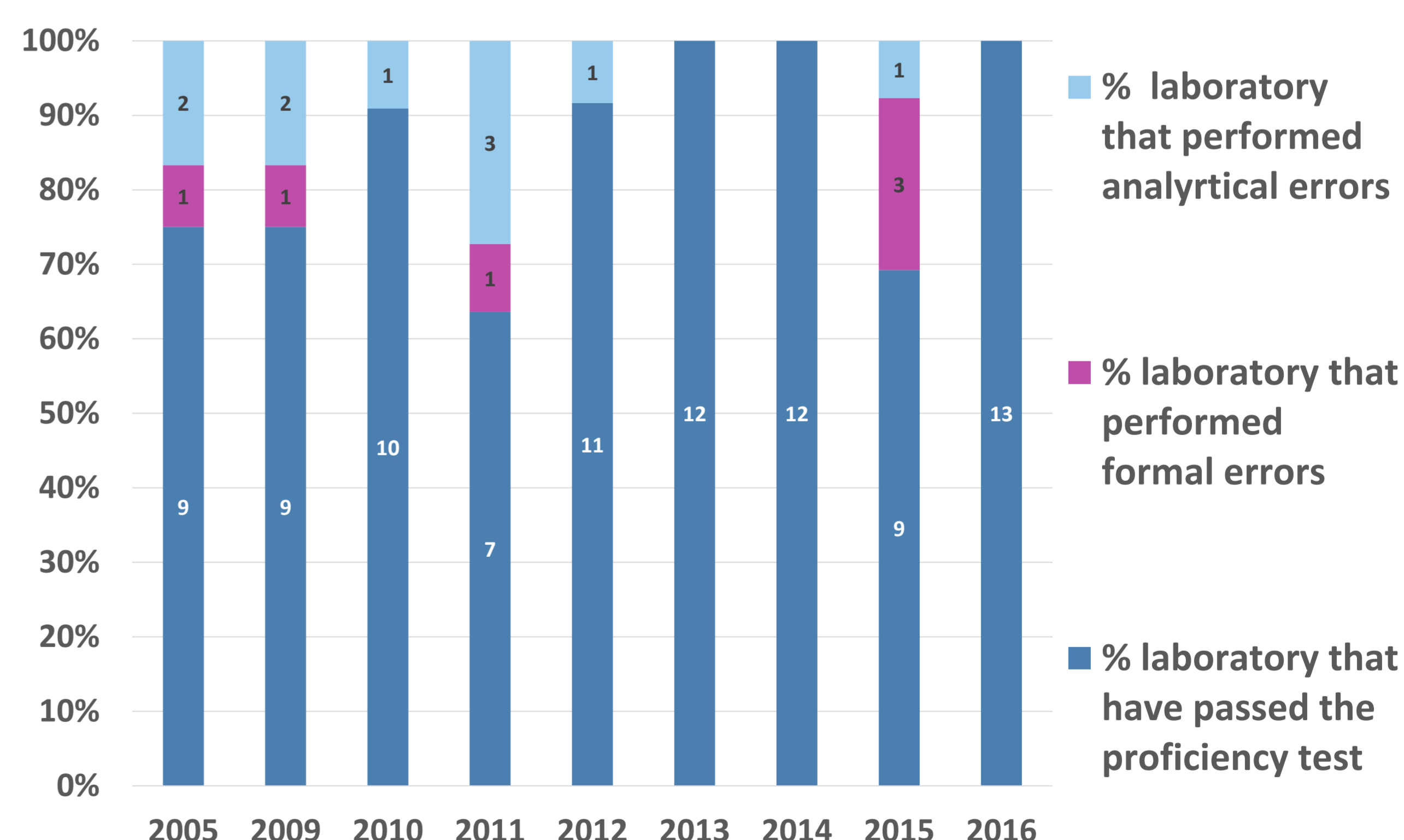


Figure 2. Percentages of laboratories passing or failing the PT

The low number of participants failing the PTs is indicative of an overall good technical level of the laboratories supporting the management of scrapie control plan. Furthermore, the proportion of laboratories reporting analytical errors significantly decreased by years (p=0.009), providing evidence of the efficacy of organising PTs to improve the overall Italian genotyping system for the identification of animals resistant or susceptible to scrapie.