Review of 20 years of *prnp* genotyping in Portuguese Atypical scrapie sheep cases

Orge, L.[1,2], Cristovão, P.[1], Tavares, P.[3], Paiva, V.[3], Pacheco, M.[3] e Carvalho, R.[4]

[1] Pathology Laboratory, UEISPSA, National Institute for Agricultural and Veterinary Research (INIAV), Oeiras; [2] Animal and Veterinary Research Centre (CECAV) and Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Universidade de Trás-os-Montes e Alto Douro; [3] Pathology Laboratory, UEISPSA, INIAV, Vairão [4]Direção Geral de Alimentação e Veterinária (DGAV), Division of Epidemiology and Animal Health, Directorate of Animal Protection Services

Background

Within TSE EURL activities, it was proposed to gather data on the study of the genetic profile of cases of atypical scrapie for the identification of mutations in animals homozygous ALRQ. The TSE NLRs were invited to collaborate in this study by doing the full genotype of the open reading frame or sent the data TSE EURL for analysis. The hypothesis is that atypical scrapie could be an genetic spontaneous disease like genetic CJD due to mutations in a single individual (EFSA, 2023).

This work gathers the kick-off data for this genetic study.

Figure 1. Timeline of identification of scrapie in Portugal

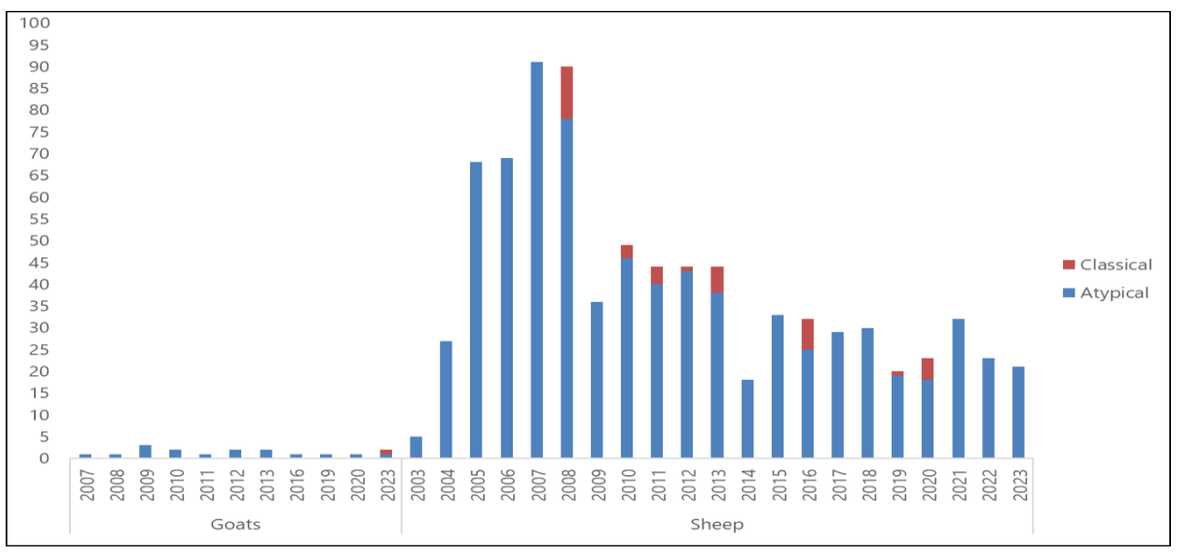
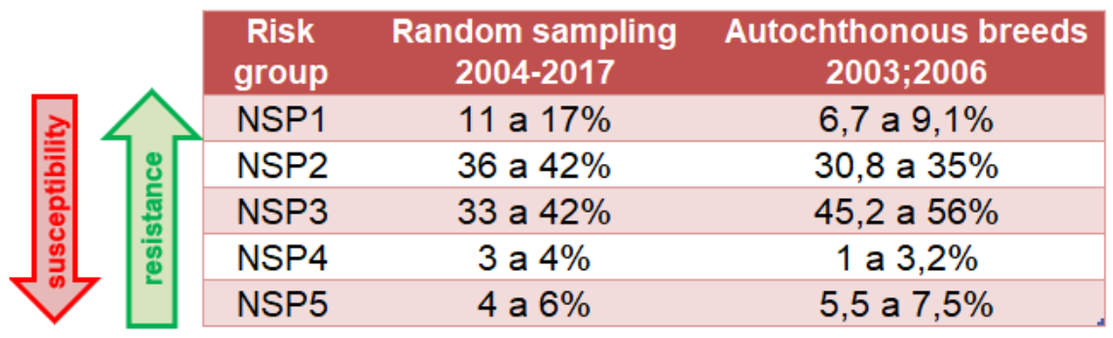


Figure 2. Scrapie evolution (2003-2023)



Risk group	Random sampling 2004-2017	Autochthonous breeds 2003;2006
NSP1	11 a 17%	6,7 a 9,1%
NSP2	36 a 42%	30,8 a 35%
NSP3	33 a 42%	45,2 a 56%
NSP4	3 a 4%	1 a 3,2%
NSP5	4 a 6%	5,5 a 7,5%

Figure 3. Portuguese *prnp* sheep profile Gama *et al.*, 2006; Orge *et al.*, 2003)

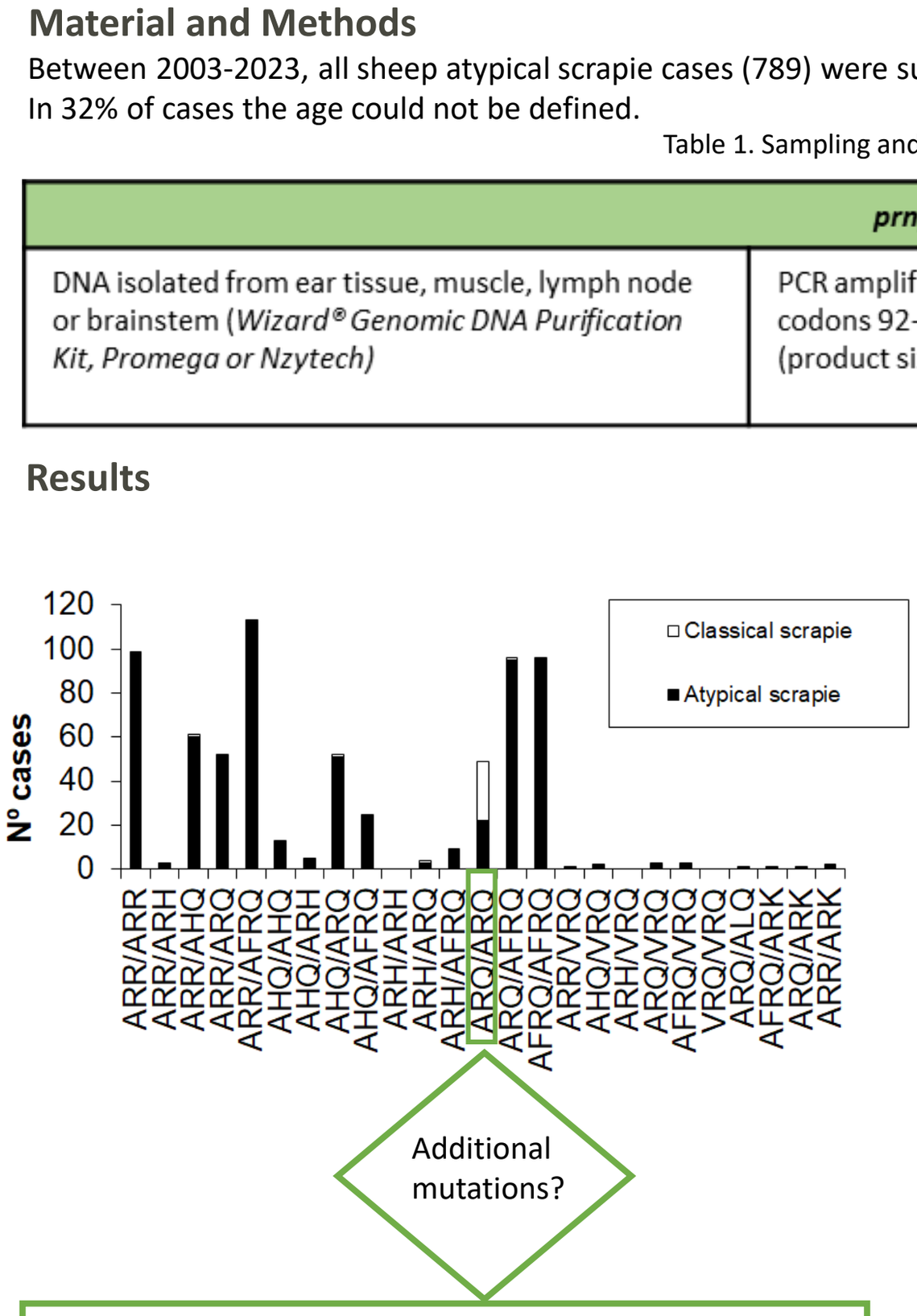
Material and Methods

Between 2003-2023, all sheep atypical scrapie cases (789) were submitted to *prnp* genotyping but 87 were unsuitable (11%).
In 32% of cases the age could not be defined.

Table 1. Sampling and prnp genotyping method

***prnp* analysis**		
DNA isolated from ear tissue, muscle, lymph node or brainstem (*Wizard® Genomic DNA Purification Kit, Promega or Nzytech)*	PCR amplification (*prnp* coding region codons 92-222) (Moum et al., 2004) (product size 393 bp)	Sequencing DNA ABI3730xl (Stabvida, Eurofins and in-house)

Results



- 8 no additional polymorphisms;
- 13 with additional polymorphims (H143R; Y172D; N176K)

Figure 4. *prnp* distribution of scrapie cases (2003-2023)

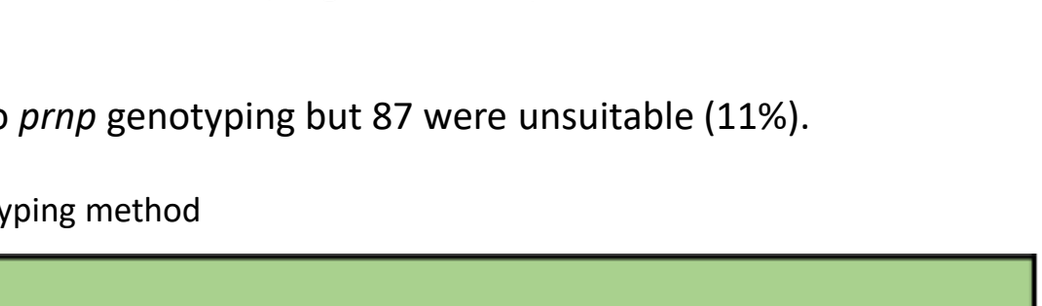


Figure 5. Evolution of *prnp* genotypic profile of PT ASc

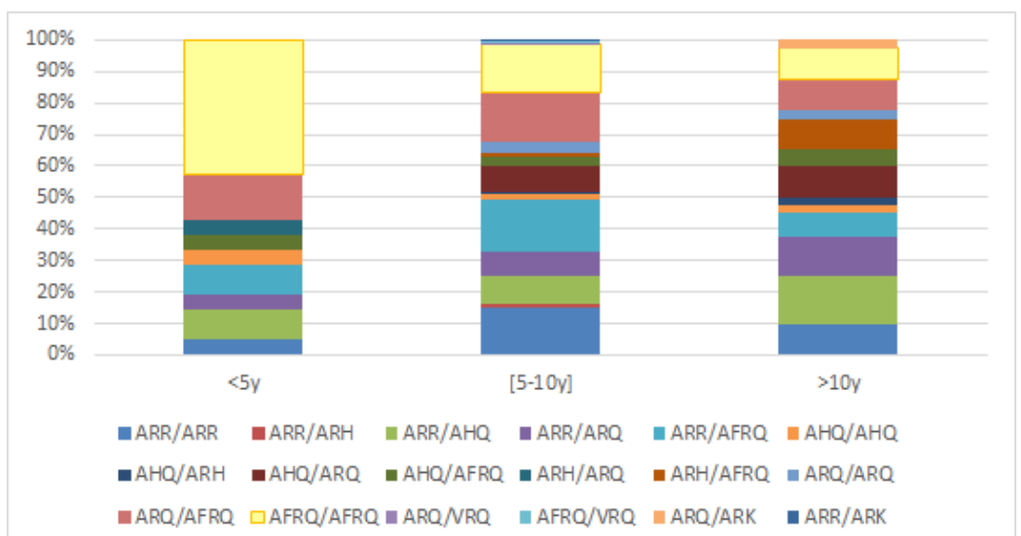


Figure 6. Age distibution of *prnp* genotypic profile of PT ASc (2003-2023)

Conclusions

- Most frequent genotypes: ARR/AFRQ, ARR/ARR, AFRQ/AFRQ and ARQ/AFRQ;
- Only 21 Wt- ARQ/ARQ (2,8%) for further genetic analysis;
- No changes in the diversity of *prnp* profile over the years, but AFRQ/AFRQ was predominant in cases below 5 years of age;
- No VRQ/VRQ nor ARH/ARH cases (only VRQ alelle present with AFRQ, ARQ, AHQ and ARR alelles);
- Single cases in rare genotypes ARQ/ALQ; AFRQ/ARK; ARQ/ARK and ARR/ARK.

References: EFSA, 2023 doi: 10.2903/sp.efsa.2023.EN-8386; Gama *et al.* Livestock Science 99 (2006) 175-184; Orge *et al*. The Veterinary Record 2003 Oct 18;153(16):508