



Instituto Nacional de
Investigação Agrária e
Veterinária, I.P.

Preliminary results from the PeXPrionGoat project: genetic diversity of *PRNP* gene in Portuguese Goat

2023.14526.PEX

EURL-NRLs TSE meeting

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INIAV Work team

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Consultants

- DGAV (Renata Carvalho)
- EURL (Gabriele Vaccari, Giuseppe Ru)

Aims



- Define the frequency of *PRNP* allelic variant in Portuguese and exotic goat breeds
- Estimate these allelic variants in the general goat population
- Identify Portuguese breeds with a potential genetic resistance to scrapie as well as those from exotic breeds
- Determine the complete *PRNP* genotypes of goat scrapie cases

Organization of the proposed Tasks – PeXPTPrionGoat



Task 1 – Sampling organization and collection and DNA extraction (INIAV/DGAV/EURL-TSEs)

280 Portuguese goat breeds (40 Algarvia; 40 Charnequeira; 40 Serpentina ; 40 Bravia ; 40 Preta de Montesinho and 80 Serrana).

120 Exotic goat breeds (40 Saanen, 40 Alpine and 40 Murciana Granadina).
(Blood samples)

TSE negative goats

- **150** randomly collected **under Active Surveillance** (brainstem samples)
- **75-100** from **affected flocks** (blood/brainstem/ear tissue samples)

23 goat scrapie cases (16 Portuguese atypical scrapie; 6 imported atypical scrapie; 1 Portuguese classical scrapie) (ear tissue/brainstem samples)

Task 2 – Determination of *PRNP* genotypes (INIAV)

Task 3 – Implementation of a rapid methodology for *PRNP* genotyping (INIAV)

Task 4 Statistical analysis of data and defining putative goat risk groups for both classical and atypical scrapie (INIAV/DGAV/EURL-TSEs)

Task 5 – Dissemination of results and establishment of a collaborative work platform and a database for TSEs in goats (INIAV/DGAV/EURL-TSEs)



Task 1 – Sampling organization and collection and DNA extraction (INIAV/DGAV/EURL-TSEs)



23 goat scrapie cases
(16 Portuguese atypical scrapie; 6 imported atypical scrapie; 1 Portuguese classical scrapie)
(ear tissue/brainstem samples)



[2003-2023]

A total of 23 scrapie cases detected in goats in Portugal



16 Portuguese atypical scrapie cases



6 Imported atypical scrapie cases



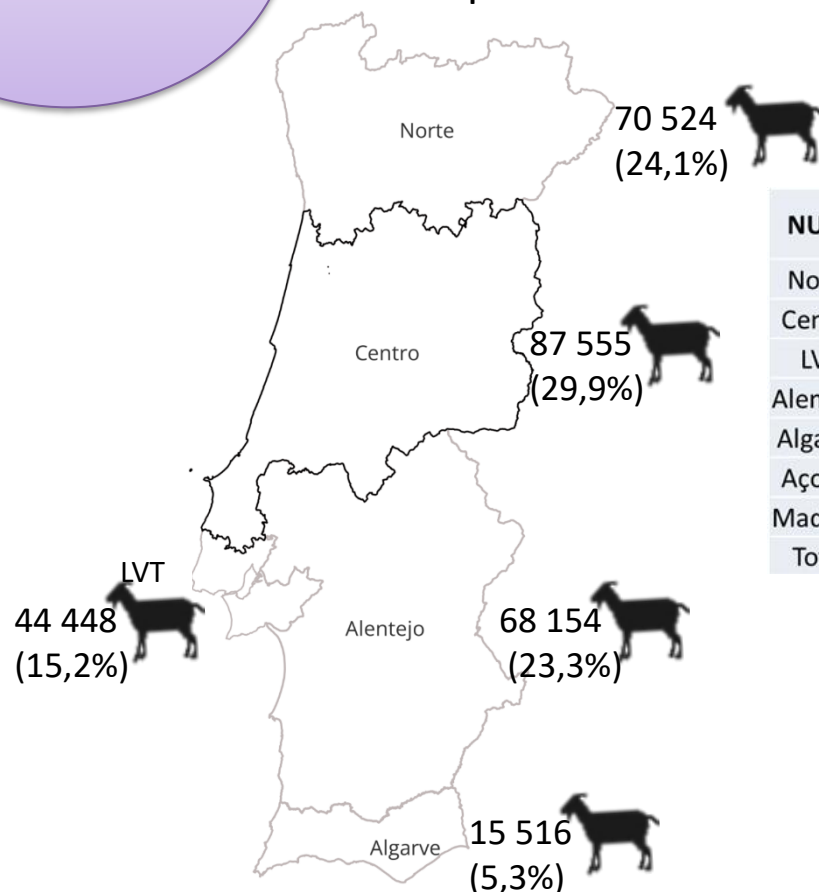
1 Portuguese classical scrapie

Task 1 – Sampling organization and collection and DNA extraction (INIAV/DGAV/EURL-TSEs)

TSE negative goats

- **150** randomly collected **under Active Surveillance** (ear tissue samples)
- **75-100** from **affected flocks** (blood)

Goat Population



Sampling (nº)

NUTS	Goat Population	%				DNA ext	PCR	Seq
Norte	70524	24,1	Norte	36	37	43	42	42
Centro	87555	29,9	Centro	45	46	46	44	44
LVT	44448	15,2	LVT	23	23	34	31	30
Alentejo	68164	23,3	Alentejo	35	35	40	35	35
Algarve	15516	5,3	Algarve	8	9	4	4	4
Açores	6088	2,1	Total	147	150	167	156	155
Madeira	399	0,1						
Total	292694	100						

**10 from 2 CSc affected flocks
(6 Centro and 4 Alentejo) (blood samples)**

NUTS (Nomenclature of Territorial Units)

Task 1 – Sampling organization and collection and DNA extraction (INIAV/DGAV/EURL-TSEs)



- Genomic DNA extraction (ear tissue, brainstem, blood)

Instructions for use



MB135_IFU_EN_V2401

NZY Tissue gDNA Isolation kit

Catalogue number
MB13502

Presentation
50 columns

- [DNA extraction procedure form](#)

Task 2 – Determination of *PRNP* genotypes (INIAV)

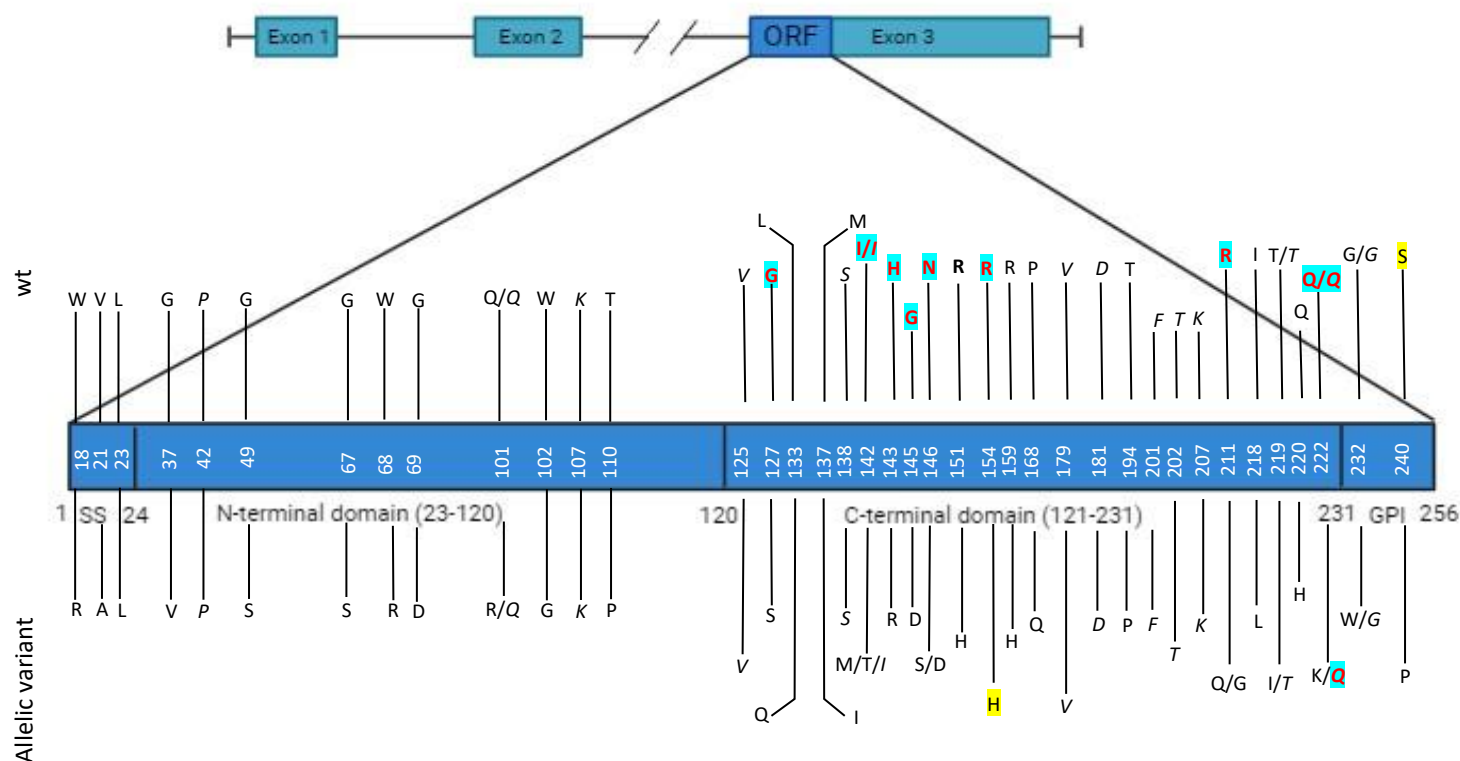
PCR and sequencing:

- [Methodology for whole *PRNP* \(INIAV OEIRAS/EURL-NRL; Chapinini *et al.*, 2019\)](#) and INIAV Santarém methodology (Solis Biodyne HOT FIREPol® MultiPlex Mix with 10 mM MgCl₂ ref: 04-34-00120, BioPortugal)
- [PCR registration form](#)
- External sequencing service (STAB VIDA) <https://www.stabvida.com/pt>
- Validation of methodology using goat DNA controls of known *PRNP* genotypes (EURL TSE)

Task 2 – Determination of *PRNP* genotypes (INIIV)



Sequences were aligned to the *Capra hircus* gene for *PRNP* (Accession number GenBank EU870890.1_cds_ACJ66711.1_1) with ClustalW tool of BioEdit 7.0.9 software. In addition, electropherograms were checked at each investigated mutation point to identify heterozygous peaks.



Van Pocke *et al.*, 2009; Vaccari *et al.*, 2009; EFSA, 2017; Teferedegn *et al.*, 2020

**23 goat scrapie cases
(16 Portuguese
atypical scrapie; 6
imported atypical
scrapie; 1 Portuguese
classical scrapie)**

Task 2 – Determination of *PRNP* genotypes (INI_{AV})

[illegible]

	codons associated with classical scrapie						
	codons associated with atypical scrapie						
	codons associated with classical and atypical scrapie (according to Colussi et al., 2008)						
silent							

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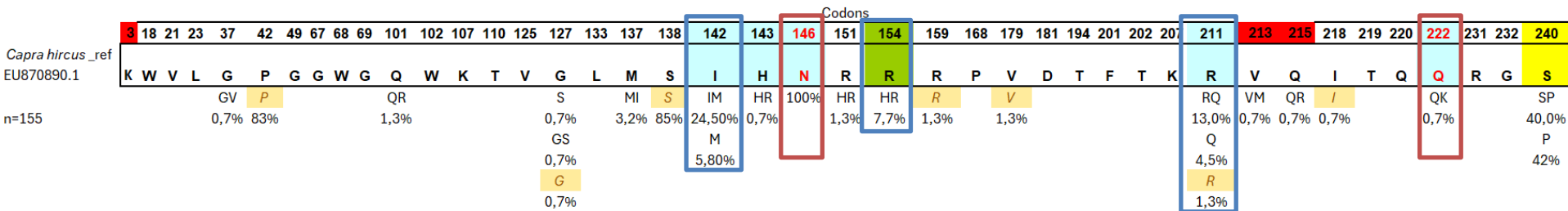
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silent							

Task 2 – Determination of *PRNP* genotypes (INIAV)



TSE negative goats

- 150 randomly collected under Active Surveillance (ear tissue samples)



Ranking of resistant alleles for Classical Scrapie:

K222>D146=S146>Q211=H154=M142>S127=R143>wild-type (EFSA recommendations¹)

	codons associated with classical scrapie	
	codons associated with atypical scrapie	
	codons associated with classical and atypical scrapie	
	(according to Colussi et al., 2008)	
silent		

¹EFSA Panel on Biological Hazards (BIOHAZ); Ricci, A.; Allende, A.; Bolton, D.; Chemaly, M.; Davies, R.; Escámez, P.S.F.; Gironés, R.; Herman, L.; Koutsoumanis, K.; et al. Genetic resistance to transmissible spongiform encephalopathies (TSE) in goats. EFSA J. **2017**, 15, e4962

Task 2 – Determination of *PRNP* genotypes (INIAV)

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ON GOING

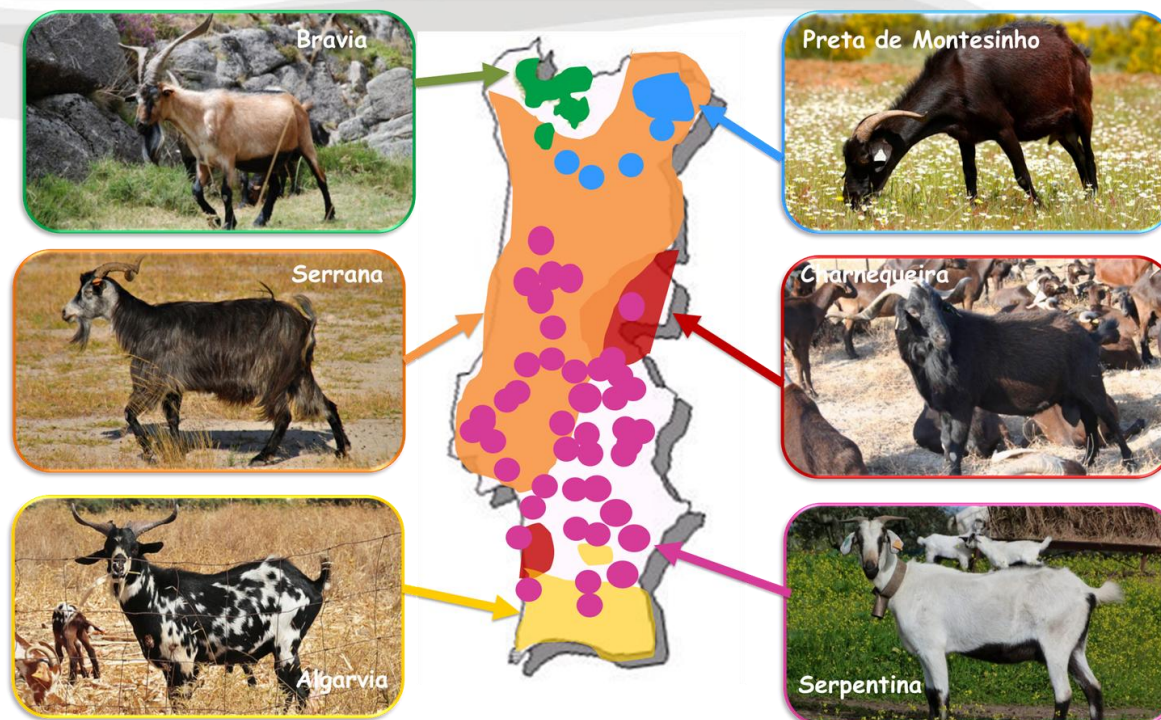


Figure 2 - Native Portuguese goat breeds and their geographic distribution



Figure 3 -Exotic goat breeds most frequently raised in Portugal

Task 2 – Determination of *PRNP* genotypes (INIIV)



Provisional conclusions:

- Scrapie cases were almost exclusively wild-type at all key codons; in particular, no K222 or D/S146 allele was detected in any scrapie case;
- Several Portuguese atypical scrapie cases showed H154 hetero/homozygosity, a codon associated with atypical scrapie susceptibility;
- Cohabitant goats from classical scrapie affected flocks showed a predominantly wild-type profile at resistance codons, confirming that scrapie-exposed flocks are largely composed of genetically susceptible animals;
- The 155 TSE-negative surveillance animals displayed a measurable frequency of resistance alleles for classical scrapie (142,154, 211);
- Genotyping of 280 Portuguese breed animals and 120 exotic breed animals is ongoing; results will enable breed-specific resistance allele frequencies and population-level risk group definition for both classical and atypical scrapie.



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Thank you
See you next year



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