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

Preventing future TSE outbreaks: exploring prion protein coding gene diversity for resistance in Portuguese Goats

PeXPTPrionGoat

2023.14526.PEX

EURL-NRLs TSE meeting

13 May 2025

https://doi.org/10.54499/2023.14526.PEX

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PeXPTPrionGoat

- 02/01/25-01/07/2026
- Budget 49 965,00 €

- INIAV Work team
  - Oeiras (Natalia Campbell, Vera Silva, Leonor Orge)
  - Santarém (Fatima S. Silva, Neuza Bacalhau, Inês Carolino, Nuno Carolino)
  - Vairão (Paula Tavares)

dgav Direção Geral de Alimentação e Veterinária

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

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Background

Active Surveillance

2002

2003

1st Portuguese atypical scrapie case

2004

1st imported classical scrapie

2007

1st Portuguese atypical scrapie case

2008

1st Portuguese classical scrapie

2023

1st Portuguese classical scrapie

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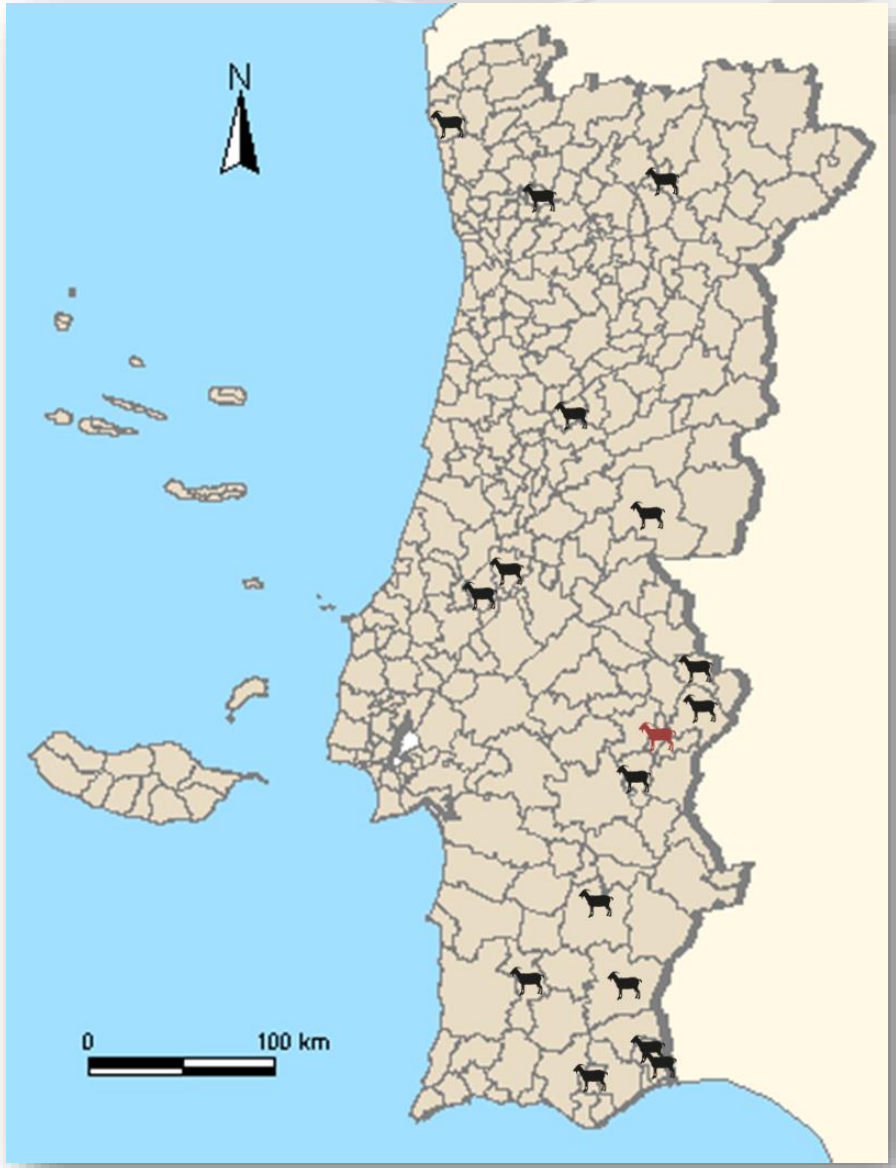
Background



[2003-2023]
Portugal detected a total of 23 scrapie cases in goats

16 Portuguese atypical scrapie cases

6 Imported atypical scrapie cases

1 Portuguese classical scrapie

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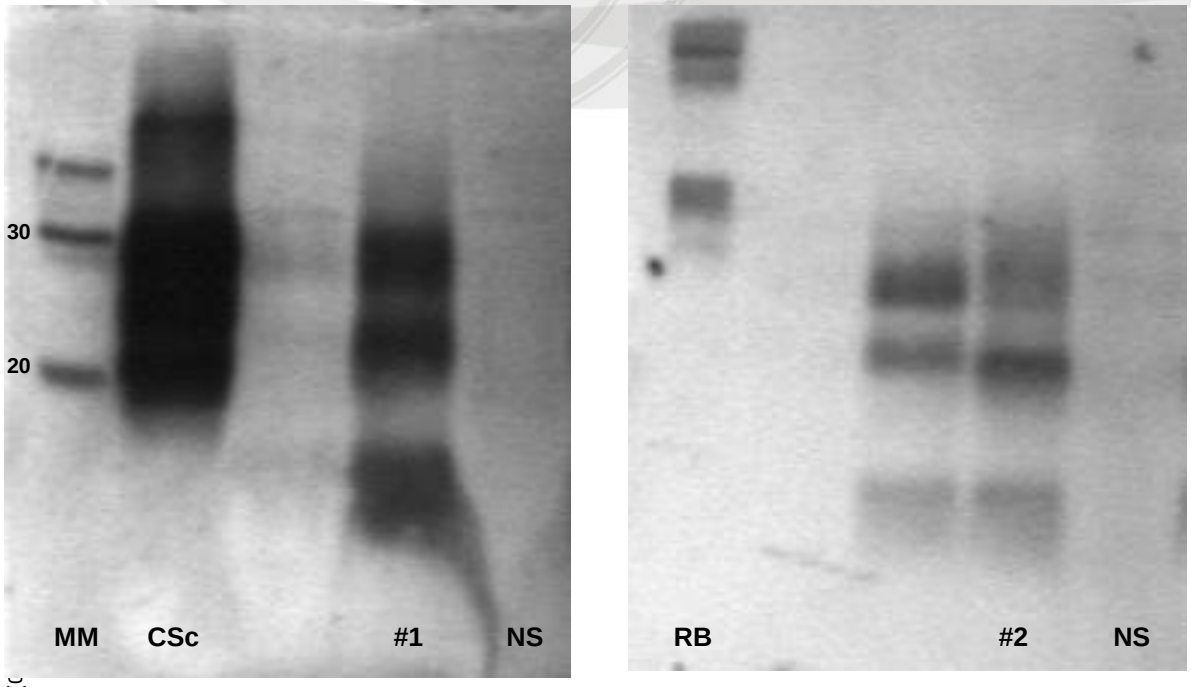
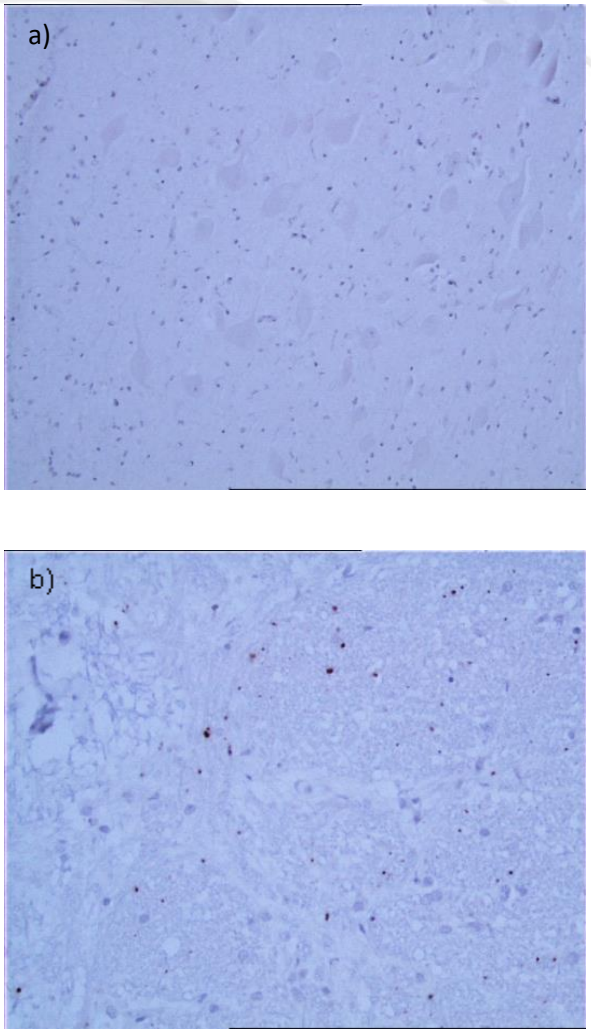
Background



PrPres detection by Western Immunoblotting (Mab Sha31) in goat atypical scrapie cases

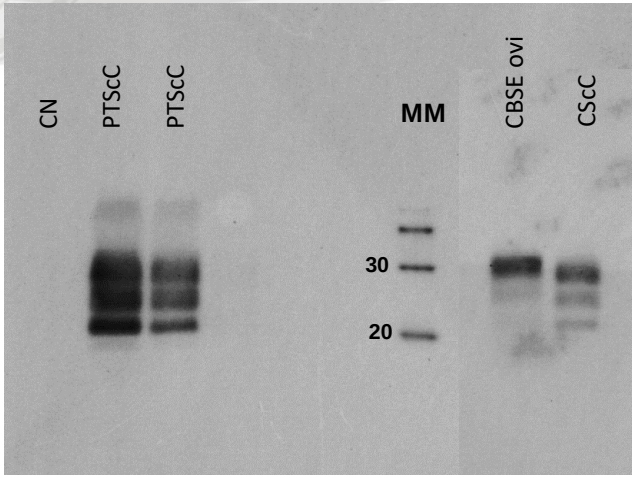
MM: Molecular weight marker (kDa); **CSc**: Classical Scrapie control; **NS**: Negative Sample: **RB**: Rainbow;

#1 e #2 : goat atypical scrapie cases



PrPres detection by immunohistochemistry (Mab 2G11; Vectstain Elite peroxidase/DAB,hematoxilina de Mayer; (a) x200; (b) x400)

Background



Discriminatory Western Immunoblotting discriminatório (Mab P4) in Portuguese classical scrapie (PTScC)

MM: Molecular weight marker (kDa); **CN**: Negative Control; CBSEovi-BSE in sheep control; **CSc**: Classical Scrapie control;

Background

prnp genotype

	Codons								
	42	138	142	143	146	154	211	222	240
Portuguese	P	S	I	H	N	H/R	R/Q	Q	P
	P	S	I	H	N	H/R	R/Q	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H	R	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H/R	R/Q	Q	P
	P	S	I	H	N	H	R	Q	S
	P	S	I	H	N	H	R	Q	S
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H	R	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P		I	H	N	HR	R	Q	P
		R	I	H	N	R	R	Q	P
	P				N	HR	R	Q	P
	P	S	I	H	N	R	R	Q	S
	P	S	I	H	N	HR	RQ	Q	P
Imported	P	S	I	H	N	H/R	R/Q	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H/R	R	Q	P
	P	S	I	H	N	H	R	Q	S

Silent

codons associated with classical scrapie

codons associated with atypical scrapie

codons associated with classical and atypical scrapie

(according to Colussi et al., 2008)

Background

Native Portuguese goat breeds

ALGARVIA

'Algarve Goat'

BRAVIA

'Wild Goat'

CHARNEQUEIRA

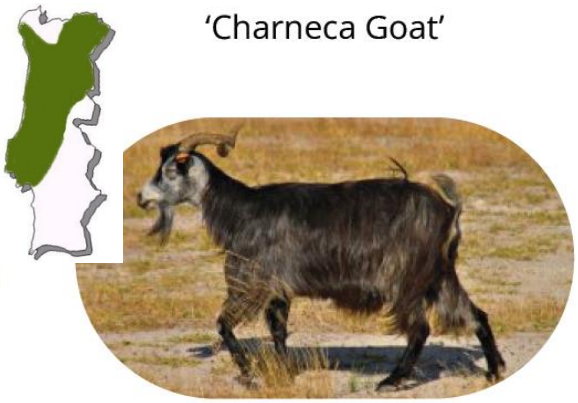
'Charneca Goat'

PRETA DE MONTESINHO

'Montesinho Black'

SERPENTINA

'Serpa Goat'



SERRANA

'Mountain Goat'

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https://www.sprega.com.pt/conteudo.php?idesp=caprinos
https://portugalpackgoats.com/blog/native-portuguese-goat-breeds/

Background

Native Portuguese goat breeds

Breed	Females (nº)	Males (nº)	Breeders	Purpose
Algarvia	3127	112	70	Dairy & Meat
Bravia	11728	432	127	Meat
Charnequeira	2057	71	42	Dairy & Meat
Preta Montesinho	1878	73	45	Dairy & Meat
Serpentina	7219	328	65	Dairy & Meat
Serrana	12846	321	132	Dairy & Meat

Background

Exotic goat breeds

Saanen

Murciana Granadina

Alpine

Aims

- Define the frequency of *prnp* polymorphisms in Portuguese and exotic goat breeds
- Estimate these polymorphims in the general goat population
- Identify Portuguese breeds potentially resistant to scrapie as well as resistant goats 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)

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Timeline

Task	Members of Research Team											Year 1												Year2					
												02/01/2025											31/12/2025	01/01/2026					30/06/2026
Nº	Denomination	Leonor Orge	Vera Silva	Natália Campbell	Paula Tavares	Fatima Santos Silva	Neuza Bacalhau	M. Inês Carolino	Nuno Carolino	Renata Carvalho	Gabriele Vaccari	Total Person/month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
1	Sampling design and DNA extraction	X	X	X	X	X	X	X	X	X	X	7,30																		
2	Determination of prnp in goat population in Portugal and TSE positive cases	X	X	X		X	X	X				7,10																		
3	Implementation of a rapid methodology for prnp genotyping	X	X	X		X	X	X	X	X	X	6,00																		
4	Statistical analysis of data and defining putative goat risk groups for both classical and atypical scrapie	X	X	X	X	X	X	X	X	X	X	4,70																		
5	Dissemination of results and establishment of a collaborative work platform and a database for TSEs in goats	X	X	X	X	X	X	X	X	X	X	6,30																		
	Milestones																M1				M2							M3		M4

Milestones

1- Conclusion of sample collection 01/06/2025
2- End of genotyping assays 01/10/2025
3- Rapid prnp genotyping method validated in goats 31/03/2026
4- Integration of genotyping data and dissemination results 01/07/2026

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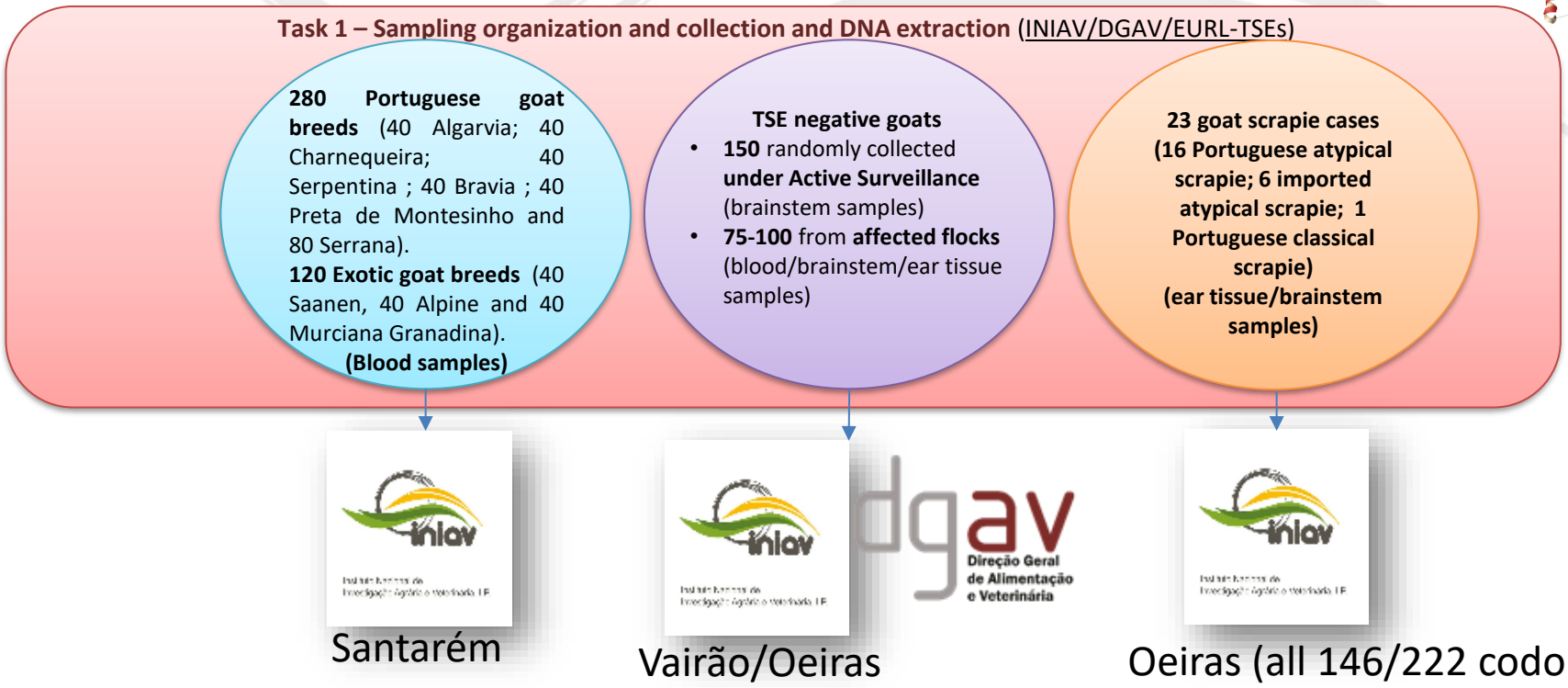

- 15/01/25: Starting point for acquisition of reagents and consumables (INIAV Oeiras)
- Breeds – DNA available (Portuguese Animal GermPlasm Bank – INIAV); sampling strategy (Torricelli *et al.,* 2021)
- TSE negative goats
  - Country distribution of the 150 Active surveillance determined by DGAV;
  - 75-100 from affected flocks: atypical scrapie flocks- possibility of collecting blood samples from health activities in those flocks

    Classical scrapie flock only 6 goats (146/222 codons genotyped);

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

PeXPTPrionGoat

Timeline													Year 1												Year2					
Task		Members of Research Team											02/01/2025											31/12/2025	01/01/2026					30/06/2026
Nº	Denomination	Leonor Orge	Vera Silva	Natália Campbell	Paula Tavares	Fatima Santos Silva	Neuza Bacalhau	M. Inês Carolino	Nuno Carolino	Renata Carvalho	Gabriele Vaccari	Total Person/month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
2	Determination of prnp in goat population in Portugal and TSE positive cases	X	X	X		X	X	X				7,10																		

2- End of genotyping assays 01/10/2025

- Revision of the methodology for whole prnp (INIAV OEIRAS/EURL-NRL)
- Implementation of methodology (INIAV Santarém);
- Preparation for methodology accreditation (INIAV Oeiras and Santarém);
- External sequencing service
- Goat DNA controls known prnp genoytpes (validation) (EURL TSE)

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

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Timeline

Task		Members of Research Team											Year 1												Year2					
													02/01/2025											31/12/2025	01/01/2026					30/06/2026
Nº	Denomination	Leonor Orge	Vera Silva	Natália Campbell	Paula Tavares	Fatima Santos Silva	Neuza Bacalhau	M. Inês Carolino	Nuno Carolino	Renata Carvalho	Gabriele Vaccari	Total Person/month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	5	6			
3	Implementation of a rapid methodology for prnp genotyping	X	X	X		X	X	X	X	X	X	6,00																		

3- Rapid prnp genotyping method validated in goats 31/03/2026

- Implementation of a fast PCR-based SNP'S methodology to detect 146/222 codons and others according to results on Task 2 (INIAV Santarém);
- Preparation for methodology accreditation

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

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Timeline

Task		Members of Research Team										Year 1												Year2					
												02/01/2025											31/12/2025	01/01/2026					30/06/2026
Nº	Denomination	Leonor Orge	Vera Silva	Natália Campbell	Paula Tavares	Fatima Santos Silva	Neuza Bacalhau	M. Inês Carolino	Nuno Carolino	Renata Carvalho	Gabriele Vaccari	Total Person/month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
4	Statistical analysis of data and defining putative goat risk groups for both classical and atypical scrapie	X	X	X	X	X	X	X	X	X	X	4,70																	

4- Integration of genotyping data and dissemination results 01/07/2026

Important role

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

PeXPTPrionGoat

Timeline														Year 1												Year2					
Task		Members of Research Team												02/01/2025										31/12/2025		01/01/2026					30/06/2026
Nº	Denomination	Leonor Orge	Vera Silva	Natália Campbell	Paula Tavares	Fatima Santos Silva	Neuza Bacalhau	M. Inês Carolino	Nuno Carolino	Renata Carvalho	Gabriele Vaccari	Total Person/month		1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
5	Dissemination of results and establishment of a collaborative work platform and a database for TSEs in goats	X	X	X	X	X	X	X	X	X	X	6,30																			4- Integration of genotyping data and dissemination results 01/07/2026

1st half 2025 for project promotion:

- 1 review paper Scrapie in goats
- Participation in 13th Iberian Prion Meeting (ICBAS, Oporto, 22/23 May2025)
- Participation in Genetic/production meetings
- Set up of the project website in INIAV page and its link to EURL-NRL TSEs website, DGAV, SPOC, thematic network of Anidop- Centre of Competence of goat breeding
- A flyer

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PeXPTPrionGoat

Thank you
See you next year

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