

ScResGoats *Classical scrapie in genetically resistant goats: questioning current concepts and policies*

New insights on 222K from the ScResGoats ICRAD project

Consortium

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Greece



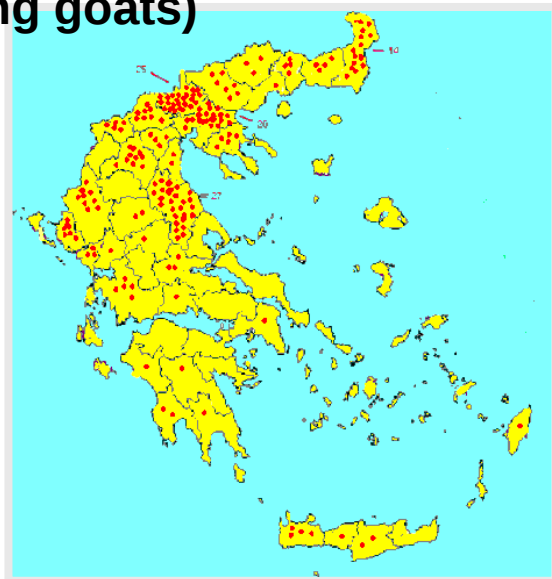
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Greece has the largest national goat flock in Europe with more than 30% of the EU milking goat herd (2.6 10⁶ milking goats)



Fragkiadaki et al. *Veterinary Research* 2011, 42:104
<http://www.veterinaryresearch.org/content/42/1/104>



SHORT REPORT

Open Access

PRNP genetic variability and molecular typing of natural goat scrapie isolates in a high number of infected flocks

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Article

Polymorphisms of Codons 110, 146, 211 and 222 at the Goat *PRNP* Locus and Their Association with Scrapie in Greece

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In Greece, an unusually high proportion of 222QK animals are associated with Classical Scrapie compared to other countries. **This increase is associated with certain herds located in specific geographical regions** suggesting that at least **under certain conditions the protective power of 222K may be compromised.**

For the first time, the protective 222K variant was detected in several scrapie-positive goats from heavily infected flocks.

The **aim** of the project is to investigate whether the occurrence of **classical scrapie cases in Greek 222QK goats can be explained by:**

- PrP-associated genetic factors,
- strain-specific properties,
- infectious load of the flocks.

- 1. Case identification and selection** (Elgo-Dimitra, Greece).
- 2. Biochemical characterisation** (ISS, Italy).
- 3. *In vitro* prion strain characterization in different PrP backgrounds, including humans** (INIA-CISA, Spain).
- 4. Strain characterization of isolates in transgenic mice and bank vole model** (APHA, UK; ISS, Italy; INIA-CISA, Spain).
- 5. Epidemiological and risk analysis** - to identify risk factors regarding the prevalence of strains that have been adapted to (potential) resistant phenotypes and the consequences or threats of such strains on breeding for resistance to scrapie schemes in goats (Elgo-Dimitra, Greece; IZS, Italy).

1. Case identification and selection

- Initially, 87 scrapie-positive samples, which were collected during the **2016-2021** period, were identified and genotyped for allelic variation at codon 222.
 - No 222QK animals were identified.
- Extended the search to include samples from **2001-2012**
 - **8 cases from 222QK animals** were identified during the 2008-2012 surveillance years out of a total of **135 samples**.
 - These cases were derived from 3 farms in geographically distinct areas.

1. Case identification and sample selection

ID ISS	Year of slaughter	SNP detection (222/211/146/110)	Age (months)	Region
241/773	2008	QK / RR/ NN/ TT	24	LARISSA
241/774	2008	QK / RR/ NN/ TT	36	LARISSA
241/775	2008	QK / RR/ NN/ TT	36	LARISSA
241/776	2010	QK / RR/ NN/ TT	24	LARISSA
241/777	2009	QK / RR/ NN/ TT	48	KALLITHEA/LARISSA
241/778	2009	QK / RR/ NN/ TT	84	KALLITHEA/LARISSA
241/779	2012	QK / RR/ NN/ TT	24	IOANNINA
241/780	2012	QK / RR/ NN/ TT	36	IOANNINA
241/781	2009	QQ / RR/ NN/ TT	24	LARISSA
241/782	2009	QQ / RR/ NN/ TT	24	LARISSA
241/783	2009	QQ / RR/ NN/ TT	60	LARISSA
241/784	2009	QQ / RQ/ NN/ TT	60	LARISSA
241/785	2009	QQ / RR/ NN/ TT	84	KALLITHEA/LARISSA
241/786	2009	QQ / RR/ NN/ TT	36	KALLITHEA/LARISSA
241/787	2009	QQ / RR/ NN/ TT	60	KALLITHEA/LARISSA
241/788	2012	QQ / RR/ NN/ TT	24	IOANNINA
241/789	2012	QQ / RR/ NN/ TT	36	IOANNINA
241/790	2012	QQ / RR/ NN/ TT	24	IOANNINA
241/791	2011	QQ / RR/ NN/ TT	60	IOANNINA

222QK n = 8

Total= 19
samples

222QQ* n = 11

*Controls: CS cases from the same herds with susceptible genotypes

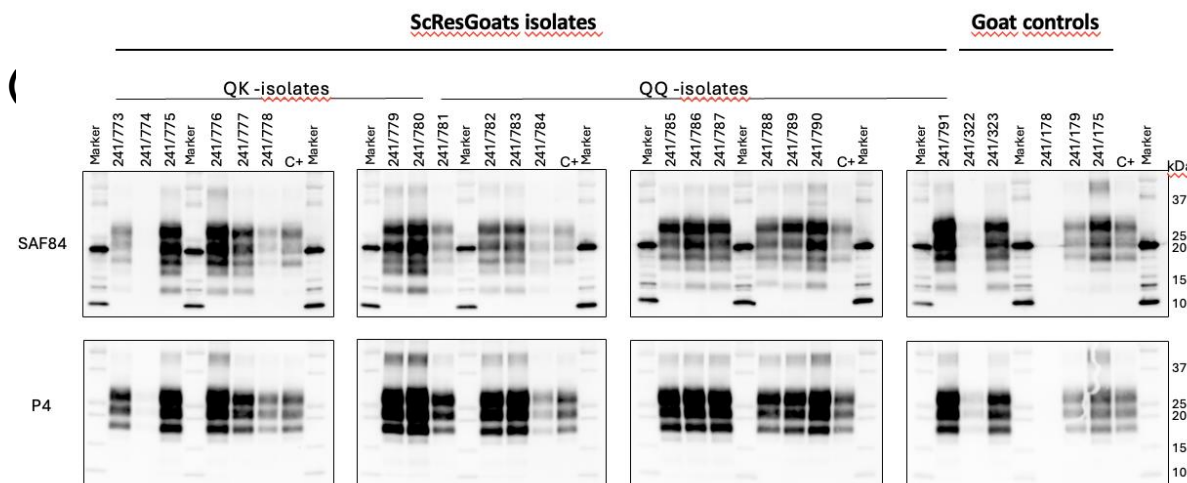
1. **Case identification and selection** (Elgo-Dimitra, Greece).
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3. *In vitro* prion strain characterization in different PrP backgrounds, including humans (INIA-CISA, Spain).
4. **Strain characterization of isolates in transgenic mice and bank vole model** (APHA, UK; ISS, Italy; INIA-CISA, Spain).
5. **Epidemiological and risk analysis** (Elgo-Dimitra, Greece; IZS, Italy).

2. Biochemical characterisation - specialised Western Blot approach

Extensive biochemical analysis of selected samples highlighted that:

1. All isolates showed a **molecular weight compatible with CS.**
2. No significant differences were observed between genetically resistant (222QK) and genetically susceptible (222QQ) goats.
3. Most isolates shared biochemical similarities with CH1641, but were **distinct from it** -> showing a C-terminal fragment of 13-14kDa, compatible with CH1641, while lacking the low molecular weight characteristic of CH1641.

4. All isolates were PK-resistant (



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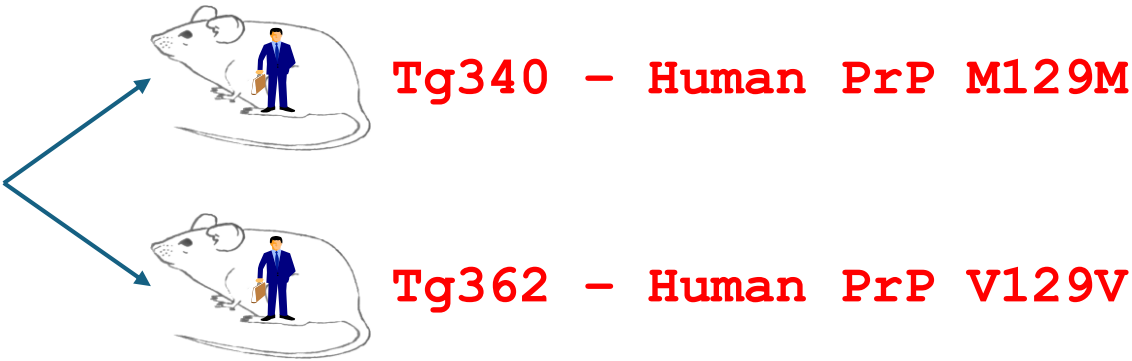
3. *In vitro* prion strain characterization in different PrP backgrounds, including humans.

PMCA is an efficient discriminatory approach to deepen the susceptibility of different species (small ruminants / cattle / humans) depending on the substrate.

In vitro propagation efficiency in different PrP backgrounds (ovine-caprine, bovine)

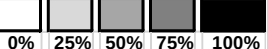


In vitro establishment of the zoonotic potential of the isolates measured by the propagation efficiency in 129MM and 129VV human PrP



During PMCA different strains may propagate differentially based on the substrate used.

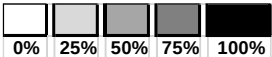
3. *In vitro* prion strain characterization in different PrP backgrounds, including humans.



Code	Scrapie	SNP detection (222/211/146/110)	 PMCA in Tg501 (Ovine PrP Q222Q)										 PMCA in Tg516 (Ovine PrP K222K)										 PMCA Tg110 (<u>bovine</u> PrP)									
			R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	/										/									/										
241/774	Positive	QK/ RR/ NN/ TT	/										/									/										
241/775	Positive	QK/ RR/ NN/ TT	/				75%						/									/										
241/776	Positive	QK/ RR/ NN/ TT	/	50%	50%								/									/										25%
241/777	Positive	QK/ RR/ NN/ TT	/	50%	75%								/									/								25%	25%	25%
241/778	Positive	QK/ RR/ NN/ TT	/										/									/							25%	25%	25%	
241/779	Positive	QK/ RR/ NN/ TT	/										/			25%	25%	25%	25%	25%	50%	/						25%	25%	25%		
241/780	Positive	QK/ RR/ NN/ TT	/										/				50%	50%	50%	75%	/											
241/781	Positive	QQ/ RR/ NN/ TT	/	50%									/									/				25%	25%	50%	50%	75%		
241/782	Positive	QQ/ RR/ NN/ TT	/	50%	50%								/									/							25%	50%	75%	
241/783	Positive	QQ/ RR/ NN/ TT	/	75%									/									/				25%	25%	25%	50%	75%		
241/784	Positive	QQ/ RQ/ NN/ TT	/										/									/					25%	50%	50%	75%		
241/785	Positive	QQ/ RR/ NN/ TT	/	50%									/									/						25%	50%	75%		
241/786	Positive	QQ/ RR/ NN/ TT	/										/								50%	50%	/									
241/787	Positive	QQ/ RR/ NN/ TT	/										/									/										
241/788	Positive	QQ/ RR/ NN/ TT	/	25%	50%	75%							/									/										
241/789	Positive	QQ/ RR/ NN/ TT	/	50%	75%								/									/									25%	
241/790	Positive	QQ/ RR/ NN/ TT	/										/									/						50%	50%	75%		
241/791	Positive	QQ / RR/ NN/ TT	/										/									/					50%	50%	75%			
241/173	Positive	PK-sen	/										/			25%	25%	50%	50%		/											
241/183	Positive	PK-sen	/										/			50%	50%	50%	50%		/											
None Zero (no inocula, negative controls PMCA)			/										/									/										
Positive control (Hu-sCJD VW type2)			/										/									/										

3. *In vitro* prion strain characterization in different PrP backgrounds, including humans.

Code	Scrapie	SNP detection (222/211/146/110)	 PMCA in Tg340 (Human PrP M129M)										 PMCA in Tg362 (Human PrP V129V)									
			R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	/										/									
241/774	Positive	QK/ RR/ NN/ TT	/										/									
241/775	Positive	QK/ RR/ NN/ TT	/										/									
241/776	Positive	QK/ RR/ NN/ TT	/										/									
241/777	Positive	QK/ RR/ NN/ TT	/										/									
241/778	Positive	QK/ RR/ NN/ TT	/										/									
241/779	Positive	QK/ RR/ NN/ TT	/										/									
241/780	Positive	QK/ RR/ NN/ TT	/										/									
241/781	Positive	QQ/ RR/ NN/ TT	/										/									
241/782	Positive	QQ/ RR/ NN/ TT	/										/						25%	25%	25%	
241/783	Positive	QQ/ RR/ NN/ TT	/										/				25%	25%	25%	25%	25%	
241/784	Positive	QQ/ RQ/ NN/ TT	/										/									
241/785	Positive	QQ/ RR/ NN/ TT	/										/									
241/786	Positive	QQ/ RR/ NN/ TT	/										/						50%	50%	50%	
241/787	Positive	QQ/ RR/ NN/ TT	/										/									
241/788	Positive	QQ/ RR/ NN/ TT	/										/									
241/789	Positive	QQ/ RR/ NN/ TT	/										/									
241/790	Positive	QQ/ RR/ NN/ TT	/										/						50%	75%	75%	75%
241/791	Positive	QQ / RR/ NN/ TT	/										/				25%	50%	75%	75%	75%	
241/173	Positive (italian)		/										/									
241/183	Positive (italian)		/										/									
None Zero (no inocula, negative controls PMCA)			/										/									
Positive control (Hu-sCJD VV type2)			/										/									



3. *In vitro* prion strain characterization in different PrP backgrounds, including humans.

Although PMCA reveals differences in amplification efficiency across substrates
-> no consistent or genotype-specific pattern distinguishing **QQ** and **QK** inocula was observed in terms of the round of first positivity or the proportion of positive replicates.



Taken together, these results indicate that scrapie isolates propagating in **QQ** and **QK** genotypes **do not appear to correspond to distinct prion strains**, as **no consistent genotype-associated differences are detected by PMCA**.

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4. Bioassays (biological strain characterization)

For optimal determination of scrapie



Tg501 - Ovine PrP ARQ/ARQ



Tg338 - Ovine PrP VRQ/VRQ



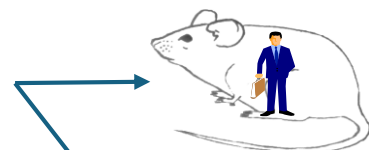
Bank vole

For optimal determination of BSE



Tg110 - Bovine PrP

For determination of zoonotic potential



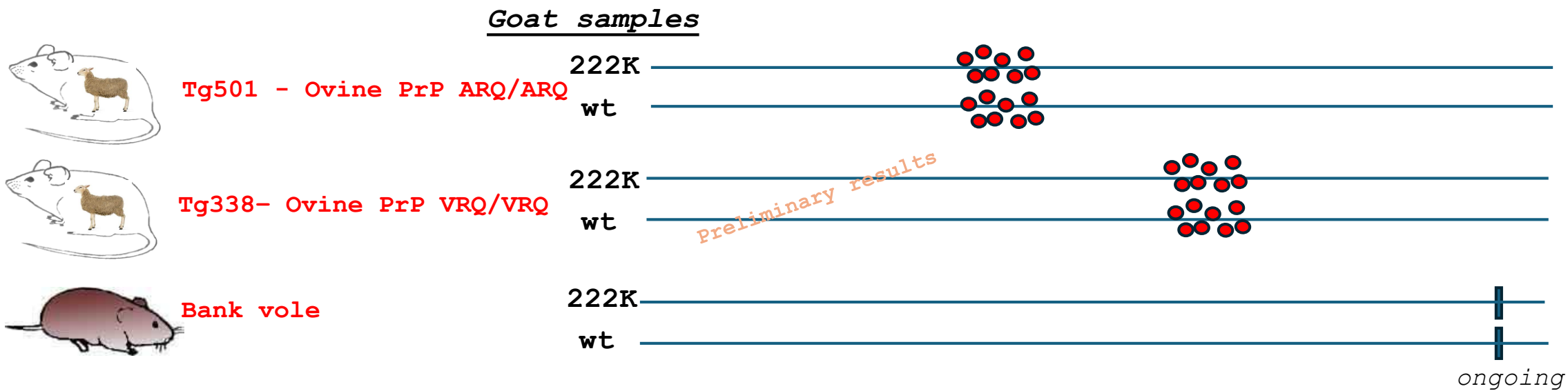
Tg340 - Human PrP M129M



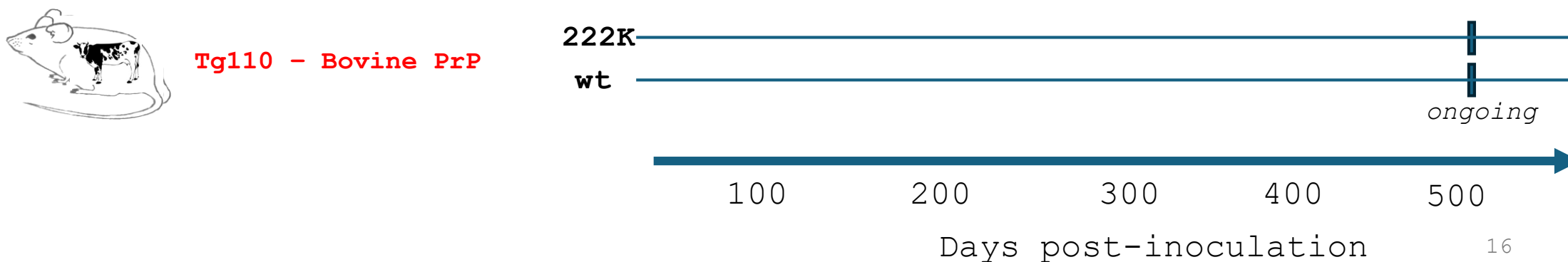
Tg362 - Human PrP V129V

4. Bioassays (biological strain characterization)

For optimal determination of scrapie

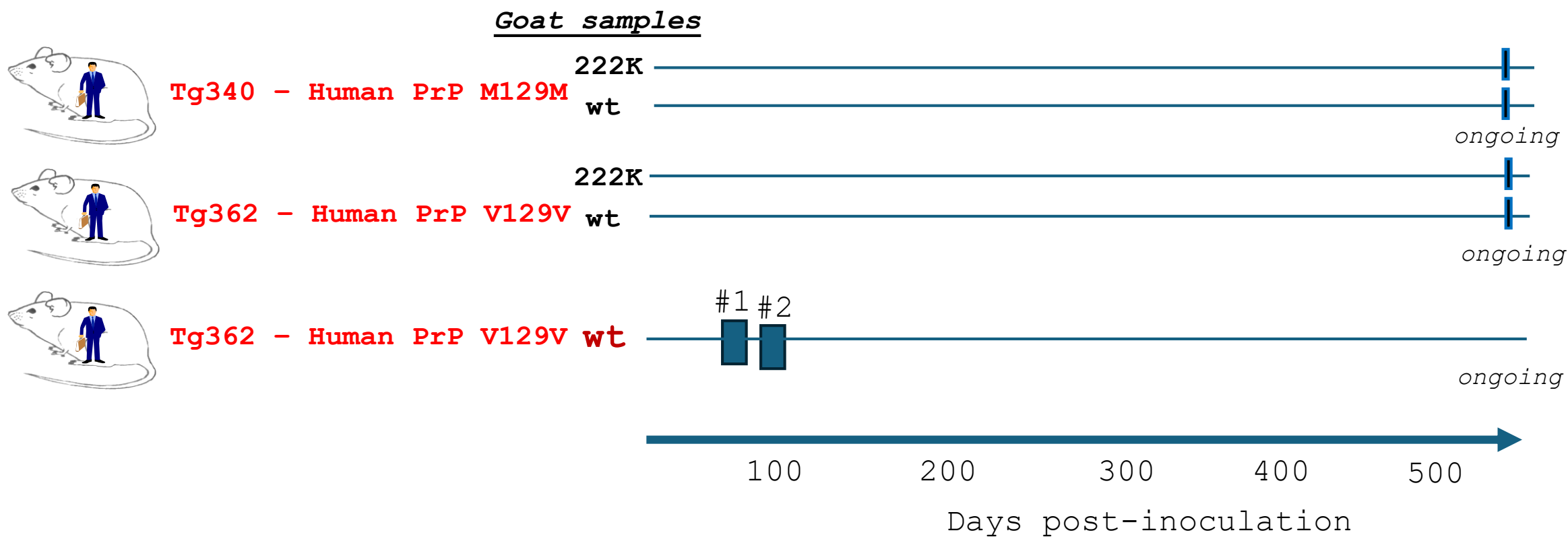


For optimal determination of BSE



4. Bioassays (biological strain characterization)

For determination of **zoonotic potential**



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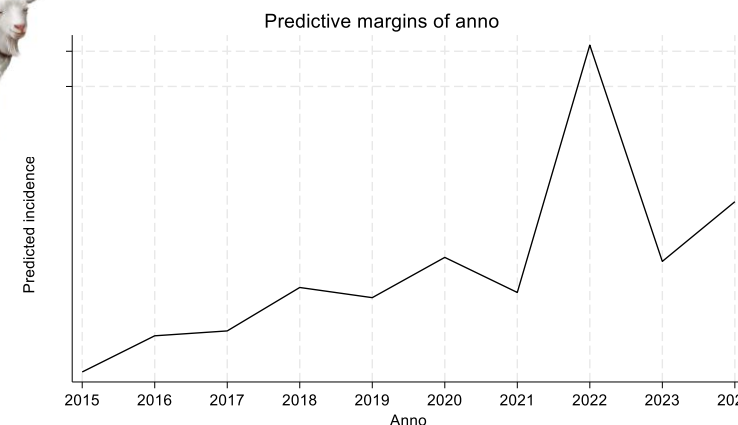
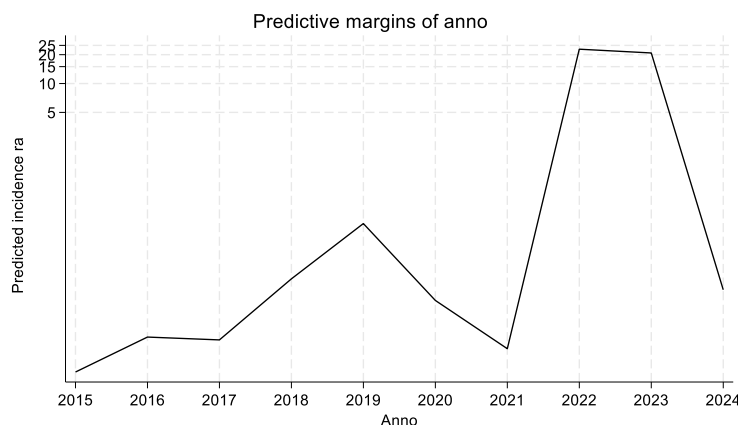
5. Epidemiological and risk analysis

Aims:

- **to describe the national patterns of scrapie:** analysis of active surveillance data for scrapie in sheep and goats considering also genotyping activity. As sheep can be in close contact with goats, the prevalence of scrapie of both species compared to identify potential spillage of strains.
- **to investigate risk factors:** case-control study at herd level through ad hoc questionnaires on both potential risk factors and confounders.

5. Epidemiological and risk analysis - Greek patterns of scrapie

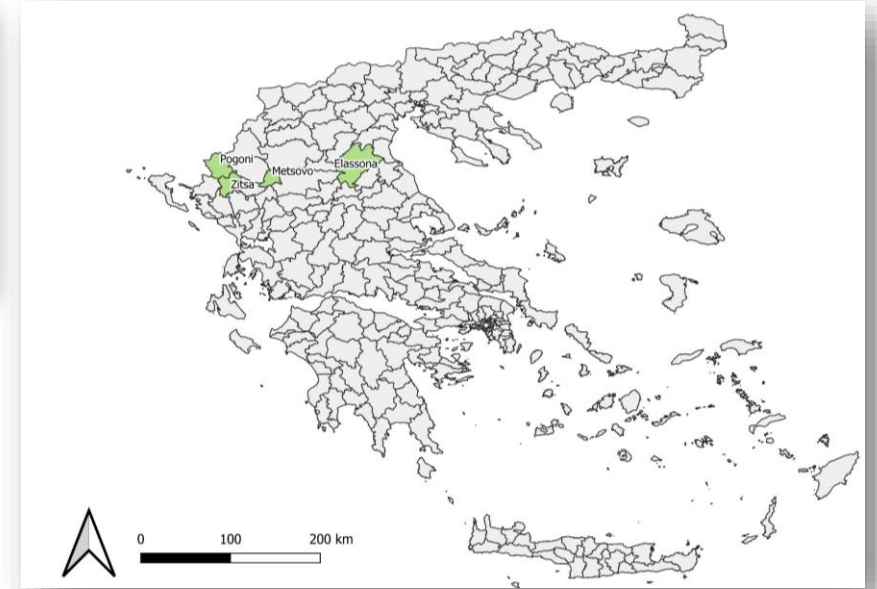
- Greece is consistently one of the Member States reporting the highest number of classical scrapie cases in sheep within the EU
- **Increasing 10-year trends of scrapie cases stream-adjusted prevalence (cases/tests)**
- In goats, although **absolute numbers are lower**, Greece has recently become **a major contributor to EU cases**, highlighting increasing epidemiological relevance.
- Genetic testing at codons 146 and 222 (2021-2024) has **not identified any resistant alleles in Greek goat cases.**



5. Epidemiological and risk analysis - Case-control interviews

To date, **12 interviews** have been carried out:

- 9 scrapie flocks
- 3 unaffected flocks
- Ionnina province
 - Metsovo
 - Pogoni
 - Zitsa
- Larissa province
 - Elassona



➡ Identified risk factors:

1. Animal introductions (common but not directly linked to index cases);
2. Pasture exposure and possible indirect contacts;
3. Management practices at lambing (e.g., pooled colostrum, placenta handling, indoor group pens)

What conclusions can we draw from these findings?

Our data support the conclusion that the circulating strain detected in 222K goats is consistent with classical scrapie; however, transmission studies are still ongoing to further characterize the strain. We also aim to increase the sample size to strengthen the epidemiological and risk analysis.

No-Cost

Extension:

September 2027

**Thanks to all colleagues
involved in the project**



**Thessaloniki - 27-28 April,
2026**