An overview of the ongoing ScResGoats ICRAD project

John Spiropoulos
On behalf of the consortium members

Animal & Plant Health Agency

Genetic susceptibility/resistance to scrapie in small ruminants

- Polymorphisms at specific codons of PRNP gene are associated with susceptibility or resistance to scrapie (classical or atypical for sheep, classical for goats)
- Polymorphisms that confer resistance to the disease can be used to breed for resistance to the disease

In sheep

- National Scrapie Plan has been successful for almost eliminating classical scrapie (CS) in sheep wherever it was possible to be applied

Genotype (codons 136, 154, 171)	NSP Risk Group
ARR/ARR	1
ARR/AHQ ARR/ARH ARR/ARQ	2
AHQ/AHQ AHQ/ARH ARH/ARH AHQ/ARQ ARH/ARQ ARQ/ARQ	3
ARR/VRQ	4
AHQ/VRQ ARH/VRQ ARQ/VRQ VRQ/VRQ	5

Susceptibility to classical scrapie

Susceptibility to atypical scrapie

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Genetic susceptibility/resistance to CS in small ruminants

In goats

- 2 codons have been associated with resistance to the disease
  Codon 146 and 222
- N (asparagine) in 146 confers susceptibility
  D (aspartic acid) or S (serine) are associated with resistance
  D or S are have been encountered in Cyprus (so far).
- Q (glutamine) in 222 confers susceptibility
  K (lysine) is associated with resistance
- A lot of hope has been placed at the K222 polymorphism
  which can confer resistance even a heterozygosity (QK222)

ICRAD announced the 2nd call for expressions of interest

Call announced in 2021

Approached by Dr Loukia Ekateriniadou, via Jan Langevelt, who proposed to investigate a high incidence of classical scrapie in QK222 goats in Greece [1, 2]

ISS also got involved since the conception of the project as they had identified CS cases in goats with resistant polymorphisms at 222

From then on, the consortium expanded to include INIA-CISA-CSIC and IZSTO

Expertise of the consortium

- Partner 1 - UK team (APHA)
  Project coordination, strain typing (bioassays)
- Partner 2 - Greek team (NRL, ELGO-DIMITRA)
  Sourcing materials, genotyping, epidemiology
- Partner 3 - Italian team (IZSTO, ISS)
  Epidemiology, Risk analysis
  Biochemical characterization and strain typing (bioassays)
- Partner 4 Spanish team (CISA-INIA-CSIC)
  In vitro propagation of prions and investigation of zoonotic potential in vitro and by – bioassay in humanized transgenic mice

1. Fragiadaki *et al. Veterinary Research* 2011, 42:104
2. Gelasakis *et al. Animals* 2021, 11:2340

The project

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

ScResGoats

WP1 - PRNP genetics

- **Sourcing samples, identifying QK222 cases, open reading frame sequencing (P2)**

WP2 - Prion strain characterisation

- **Characterisation of biochemical properties by Western blot (P3)**
- ***In vitro* propagation of prions in animal and human substrates (P4)**
- **Bioassays in ovinised (P1, P3); bovinised (P3) and humanised transgenic mice (P4), and in bank voles (P3)**

WP3 - Epidemiological and risk analysis

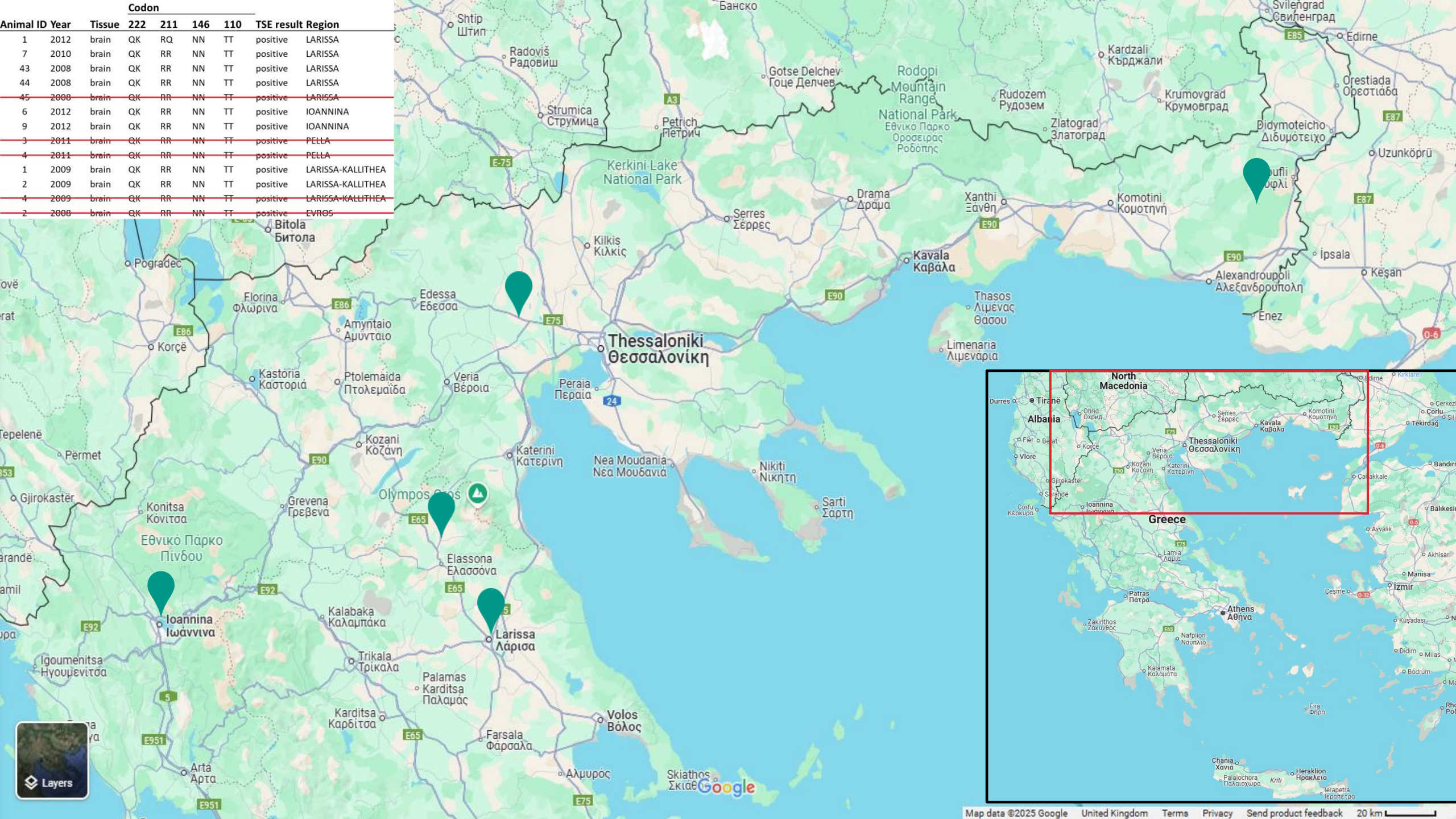
- **Gathering data from affected and neighbouring farms (P2, P3)**
- **Data analysis (P3)**

WP1 - PRNP genetics
Sourcing samples, Identifying QK222 cases

- 87 CS caprine cases from 2016-2021 surveillance years were screened to identify allelic variation at codons 146 and 222
  They were all QQ222 – all susceptible to CS
- Extended the search to 2008-2014 surveillance years
- Out of 282 CS cases 13 were QK
  Located in 5 farms
- Material was available from
  8 cases
  Located in 3 farms

			Codon					
Animal ID	Year	Tissue	222	211	146	110	TSE result	Region
1	2012	brain	QK	RQ	NN	TT	positive	LARISSA
7	2010	brain	QK	RR	NN	TT	positive	LARISSA
43	2008	brain	QK	RR	NN	TT	positive	LARISSA
44	2008	brain	QK	RR	NN	TT	positive	LARISSA
~~45~~	~~2008~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~LARISSA~~
6	2012	brain	QK	RR	NN	TT	positive	IOANNINA
9	2012	brain	QK	RR	NN	TT	positive	IOANNINA
~~3~~	~~2011~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~PELLA~~
~~4~~	~~2011~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~PELLA~~
1	2009	brain	QK	RR	NN	TT	positive	LARISSA-KALLITHEA
2	2009	brain	QK	RR	NN	TT	positive	LARISSA-KALLITHEA
~~4~~	~~2009~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~LARISSA-KALLITHEA~~
~~2~~	~~2008~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~EVROS~~

			Codon					
Animal ID	Year	Tissue	222	211	146	110	TSE result	Region
1	2012	brain	QK	RQ	NN	TT	positive	LARISSA
7	2010	brain	QK	RR	NN	TT	positive	LARISSA
43	2008	brain	QK	RR	NN	TT	positive	LARISSA
44	2008	brain	QK	RR	NN	TT	positive	LARISSA
~~45~~	~~2008~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~LARISSA~~
6	2012	brain	QK	RR	NN	TT	positive	IOANNINA
9	2012	brain	QK	RR	NN	TT	positive	IOANNINA
~~3~~	~~2011~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~PELLA~~
~~4~~	~~2011~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~PELLA~~
1	2009	brain	QK	RR	NN	TT	positive	LARISSA-KALLITHEA
2	2009	brain	QK	RR	NN	TT	positive	LARISSA-KALLITHEA
~~4~~	~~2009~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~LARISSA-KALLITHEA~~
~~2~~	~~2008~~	~~brain~~	~~QK~~	~~RR~~	~~NN~~	~~TT~~	~~positive~~	~~EVROS~~



WP1 - PRNP genetics
Sequencing of open reading frame

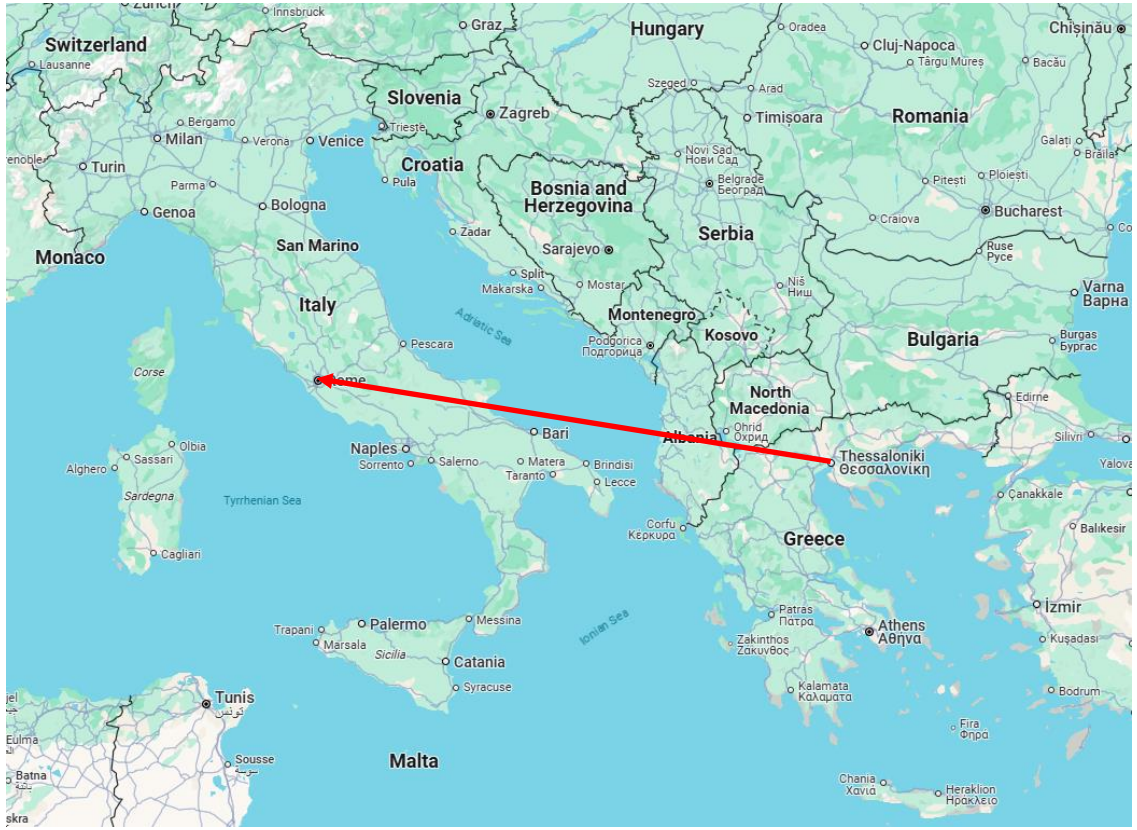
- **Open reading frame determination where samples are still available**
- **Shipment of these samples to ISS for biochemical characterisation**

Final selection of samples for biochemical characterisation

ISS Code	SNP detection (222/211/146/110)	Unit	Year of slaughter	Age (months)	Region
241/773	QK/ **RR/ NN/ TT**	42302306	2008	24	LARISSA
241/774	QK/ **RR/ NN/ TT**	42302306	2008	36	LARISSA
241/775	QK/ **RR/ NN/ TT**	42302306	2008	36	LARISSA
241/776	QK/ **RR/ NN/ TT**	42302306	2010	24	LARISSA
241/777	QK/ **RR/ NN/ TT**	42302856	2009	48	KALLITHEA/LARISSA
241/778	QK/ **RR/ NN/ TT**	42302856	2009	84	KALLITHEA/LARISSA
241/779	QK/ **RR/ NN/ TT**	33304462	2012	24	IOANNINA
241/780	QK/ **RR/ NN/ TT**	33304462	2012	36	IOANNINA
Wild type controls from same farms					
241/781	QQ/ RR/ NN/ TT	42302306	2009	24	LARISSA
241/782	QQ/ RR/ NN/ TT	42302306	2009	24	LARISSA
241/783	QQ/ RR/ NN/ TT	42302306	2009	60	LARISSA
241/784	QQ/ RQ/ NN/ TT	42302306	2009	60	LARISSA
241/785	QQ/ RR/ NN/ TT	42302856	2009	84	KALLITHEA/LARISSA
241/786	QQ/ RR/ NN/ TT	42302856	2009	36	KALLITHEA/LARISSA
241/787	QQ/ RR/ NN/ TT	42302856	2009	60	KALLITHEA/LARISSA
241/788	QQ/ RR/ NN/ TT	33304462	2012	24	IOANNINA
241/789	QQ/ RR/ NN/ TT	33304462	2012	36	IOANNINA
241/790	QQ/ RR/ NN/ TT	33304462	2012	24	IOANNINA
241/791	QQ / RR/ NN/ TT	33304462	2011	60	IOANNINA

Total of QQ cases from Larissa 11; from Kallithea/Larissa 4; from Ioannina 12

WP1 – WP2 transition



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WP2 – Prion strain characterisation
Biochemical characterisation

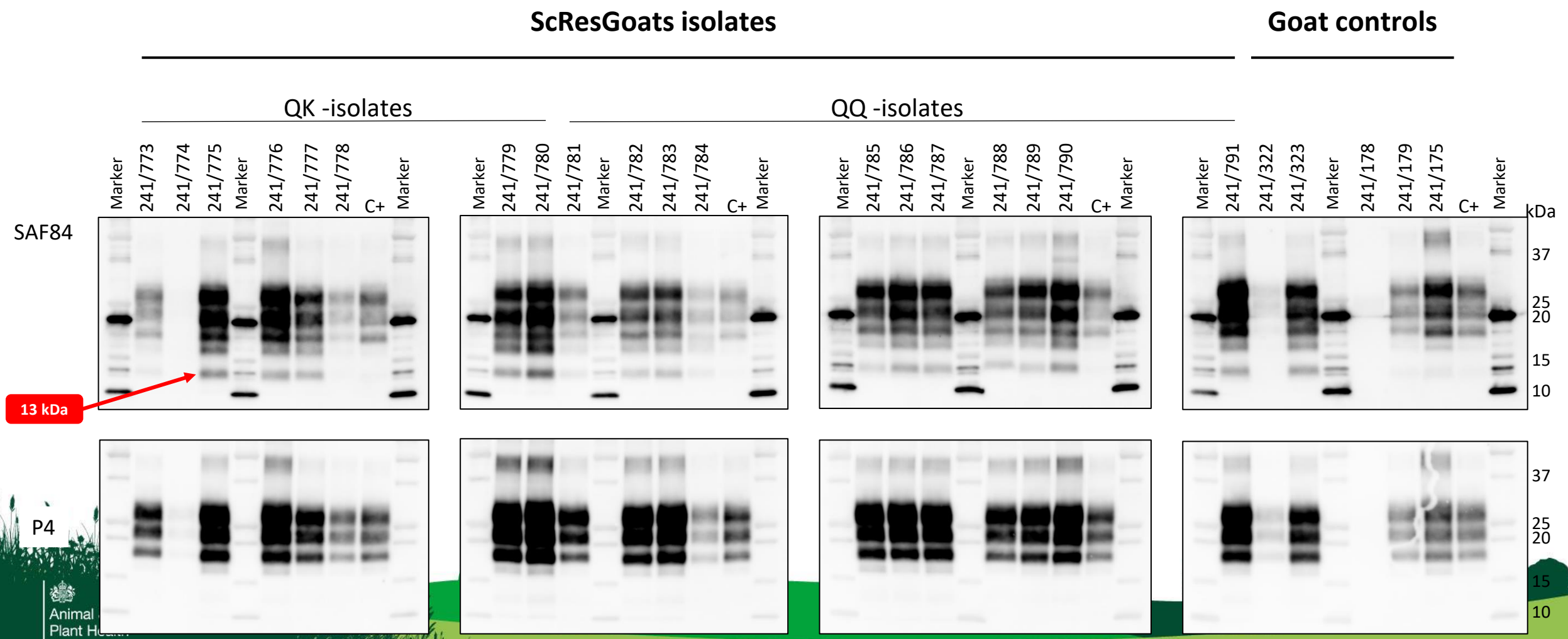
ISS WB method
Approved as discriminatory WB (scrapie-BSE) for TSE Surveillance activity EU Reg. 999/2001

ISS PK method
High PK concentration (200mg)
Quantification of SAF84/P4 ratio (BSE-like when >2)
MW determination of un-glycosylated band of PrPres (BSE-like <0,5 kDa)

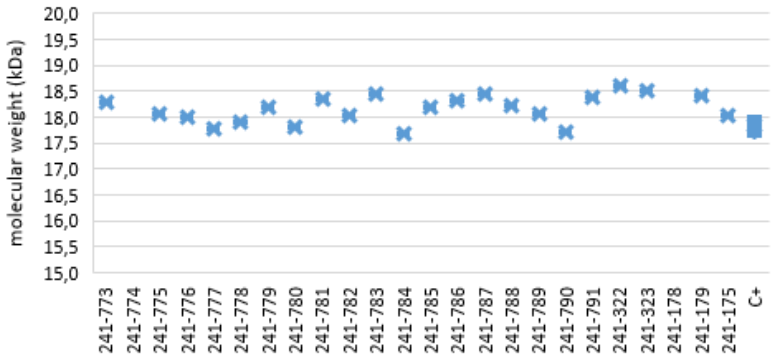
- **Classical scrapie (CS)**
  - **CS1**
  - **CS2**
- **CH1641-like**
- **Atypical scrapie/Nor98**
- **BSE**

WP2 – Prion strain characterisation
Biochemical characterisation

ISS western blot

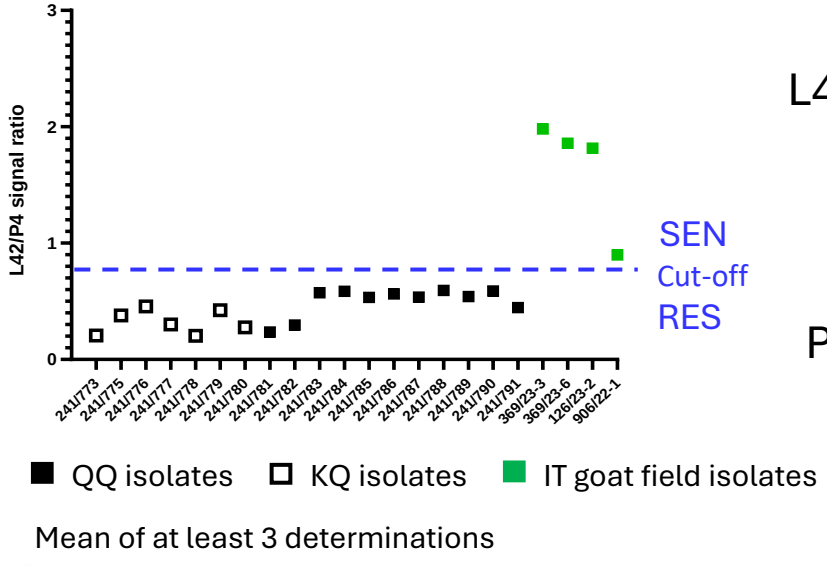


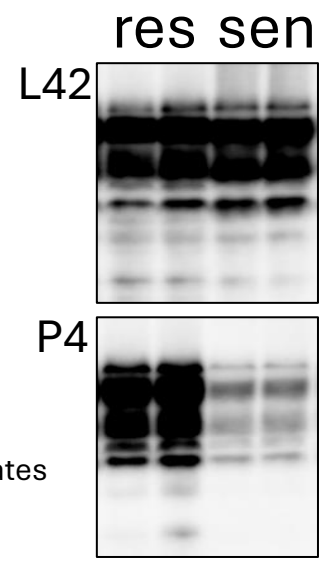
C+ = ovine classical scrapie



Determination of molecular weight of unglycosylated band of PrPres (by SAF84 mAb)

- Slight variability in the range of CS isolates





ISS PK method

- Even if a slight variability is observed, all isolates showed to be resistant to PK treatment
- In the range of «res/CS1» types and different from «italian/sen/CS2» types

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Summary of biochemical characterisation

1. **Standard discriminatory WB (ISS WB method)**
   - **All isolates are classical scrapie**
   - **Most of them showed a C-terminal fragment of 13-14kDa as CH1641-like isolates but they don't show the low MW of CH1641**

2. **PK resistance analysis (ISS-PK method)**
   - **All isolates showed to be resistant (variability but under the «experienced/empirical» cut-off)**
   - **Different from «italian/sen type»**

3. **Conclusion**
   - **No differences between QQ and QK isolates**
   - **They are CS (CS1) although they have some similarity to CH1641**
   - **Are different to CS2 (PK sensitive or Italian CS) type (known to have specific biological properties)**
   - **We cannot conclude that these new CS2 types (PK resistant) correspond to one or more strains**

Selection of samples for PrP^{Sc} propagation *in vitro* and *in vivo*

ISS Code	SNP detection (222/211/146/110)	Unit	Year of slaughter	Age (months)	Region	Selection for PMCA	Selection for bioassay		
						INIA-CISA-CSIC (P4)*	APHA (P1)*	ISS (P3)*	INIA-CISA-CSIC (P4)*
241/773	**QK/ RR/ NN/ TT**	42302306	2008	24	LARISSA	**X**	**X**	**X**	**X**
241/774	**QK/ RR/ NN/ TT**	42302306	2008	36	LARISSA	**X**	**X**		
241/775	**QK/ RR/ NN/ TT**	42302306	2008	36	LARISSA	**X**	**X**		
241/776	**QK/ RR/ NN/ TT**	42302306	2010	24	LARISSA	**X**	**X**		
241/777	**QK/ RR/ NN/ TT**	42302856	2009	48	KALLITHEA/LARISSA	**X**	**X**	**X**	**X**
241/778	**QK/ RR/ NN/ TT**	42302856	2009	84	KALLITHEA/LARISSA	**X**	**X**		
241/779	**QK/ RR/ NN/ TT**	33304462	2012	24	IOANNINA	**X**	**X**	**X**	**X**
241/780	**QK/ RR/ NN/ TT**	33304462	2012	36	IOANNINA	**X**	**X**		
Wild type controls from same farms									
241/781	**QQ/ RR/ NN/ TT**	42302306	2009	24	LARISSA	**X**	**X**		
241/782	**QQ/ RR/ NN/ TT**	42302306	2009	24	LARISSA	**X**			
241/783	**QQ/ RR/ NN/ TT**	42302306	2009	60	LARISSA	**X**	**X**		
241/784	**QQ/ RQ/ NN/ TT**	42302306	2009	60	LARISSA	**X**	**X**	**X**	**X**
241/785	**QQ/ RR/ NN/ TT**	42302856	2009	84	KALLITHEA/LARISSA	**X**	**X**		
241/786	**QQ/ RR/ NN/ TT**	42302856	2009	36	KALLITHEA/LARISSA	**X**	**X**	**X**	**X**
241/787	**QQ/ RR/ NN/ TT**	42302856	2009	60	KALLITHEA/LARISSA	**X**			
241/788	**QQ/ RR/ NN/ TT**	33304462	2012	24	IOANNINA	**X**			
241/789	**QQ/ RR/ NN/ TT**	33304462	2012	36	IOANNINA	**X**	**X**	**X**	**X**
241/790	**QQ/ RR/ NN/ TT**	33304462	2012	24	IOANNINA	**X**	**X**		
241/791	**QQ / RR/ NN/ TT**	33304462	2011	60	IOANNINA	**X**	**X**		
241/173**	**240-PP**	NA	NA	NA	LARISSA	**X**	**X**		
241/183**	**240-PP**	NA	NA	NA	KATERINI	**X**	**X**		

* P1 and P3 received 3% W/V homogenates; P4 received 10% W/V

** PrP^{Sc}-Sen from CS cases from Greece. Sourced via another ISS project .

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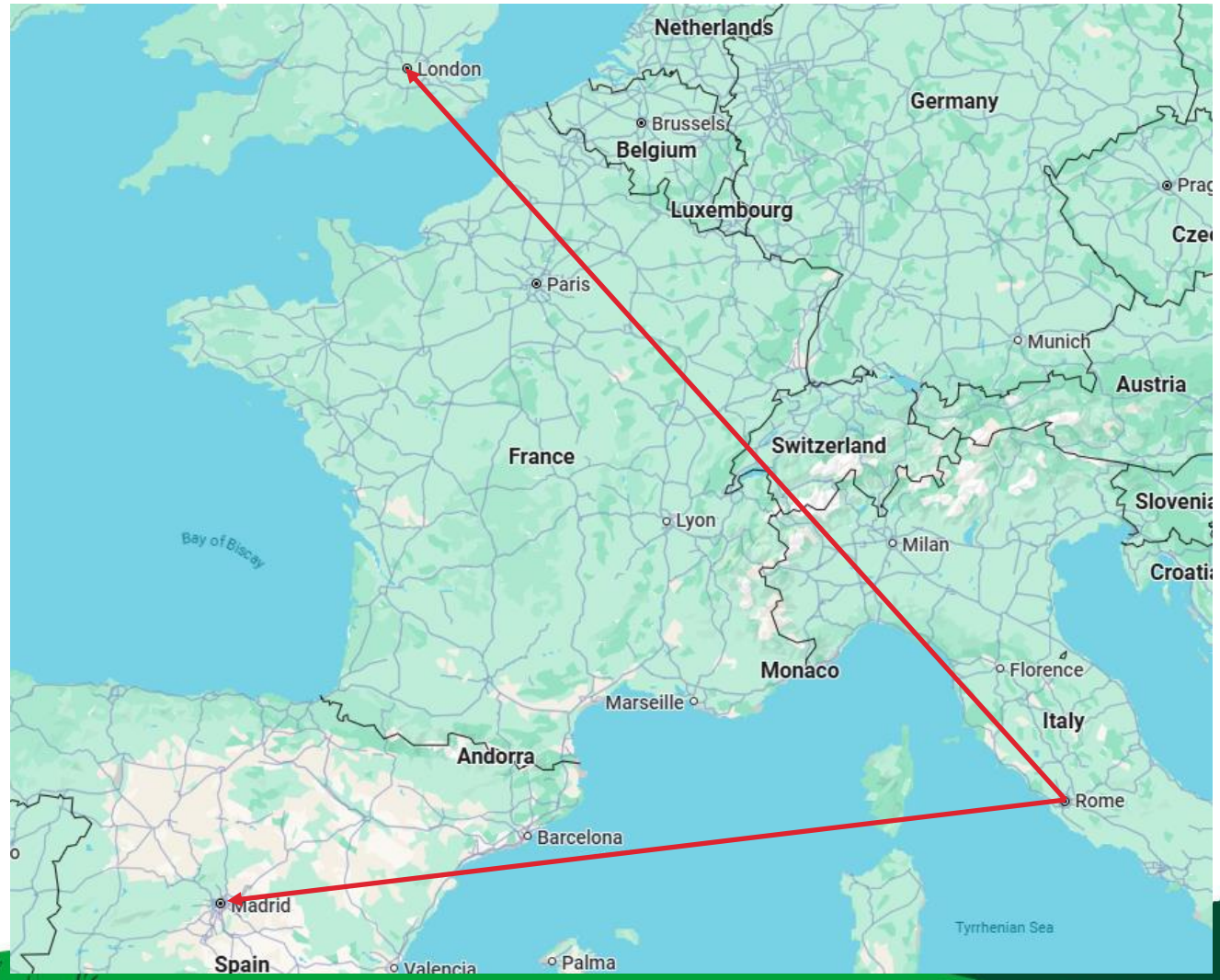
Samples were sent to P1, P3 and P4



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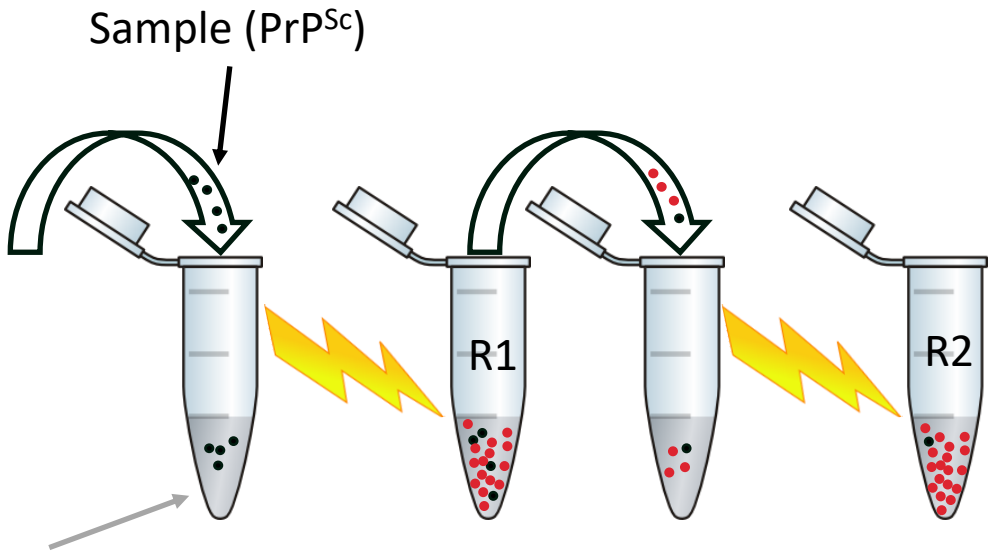
WP2 – Prion strain characterisation

In vitro prion strain characterisation in different backgrounds, including human



For each isolate

- **4 replicates**
- **10 Rounds**

Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg501 (Ovine PrP Q222Q)									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/			75%						
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/	50%	50%							
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/	50%	75%							
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/									
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/									
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/	50%								
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/	50%	50%							
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/	75%								
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/									
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/	50%								
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/									
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/									
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/	25%	50%	75%						
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/	50%	75%							
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/									
241/173	Positive (italian)		--	LARISSA	/									
241/183	Positive (italian)		--	KATR	/									
None Zero (no inocula, negative controls PMCA)					/									
Positive control (sheep-BSE)					/									

0% 25% 50% 75% 100%

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0%	25%	50%	75%	100%

Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg516 (Ovine PrP K222K)									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/									
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/				25%	25%	25%	25%	25%	50%
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/						50%	50%	50%	75%
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/									
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/									
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/									
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/									
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/								50%	50%
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/									
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/									
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/									
241/173	Positive (italian)		--	LARISSA	/					25%	25%	50%	50%	
241/183	Positive (italian)		--	KATR	/					50%	50%	50%	50%	
None Zero (no inocula, negative controls PMCA)					/									
Positive control (sheep-BSE)					/									

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0%	25%	50%	75%	100%

Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg110 (Bovine PrP)									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									25%
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/									25%
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									25%
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/									25%
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/									
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/				25%	Analysis pending				
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/					Analysis pending				75%
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/					Analysis pending				75%
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/					Analysis pending				75%
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/					Analysis pending				75%
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/									
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/									
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/					Analysis pending				25%
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/					Analysis pending				75%
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/					Analysis pending				75%
241/173	Positive (italian)		--	LARISSA	/									
241/183	Positive (italian)		--	KATR	/									
None Zero (no inocula, negative controls PMCA)					/									
Positive control (sheep-BSE)					/									

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0%	25%	50%	75%	100%

Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg407 (Bank vole PrP)									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773		QK/ RR/ NN/ TT	24	LARISSA	/									
241/774		QK/ RR/ NN/ TT	36	LARISSA	/									
241/775	Positive	RR/ NN/ TT	36	LARISSA	/									
241/776	Positive	N/ TT	24	LARISSA	/									
241/777	Positive	QK/	48	KALLITHEA/LARISSA	/									
241/778	Positive	QK/ RR/ N	84	KALLITHEA/LARISSA	/									
241/779	Positive	QK/ RR/ NN/ TT		IOANNINA	/									
241/780	Positive	QK/ RR/ NN/ TT		INA	/									
241/781	Positive	QQ/ RR/ NN/ TT	24	L	/									
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/									
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA										
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA										
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/									
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/									
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/									
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/									
241/173	Positive (italian)		--	LARISSA	/									
241/183	Positive (italian)		--	KATR	/									
None Zero (no inocula, negative controls PMCA)					/									
Positive control (Bank vole-BSE)					/									

ONGOING

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Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg340 (Human PrP M129M)									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/									
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/									
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/									
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/									
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/									
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/									
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/									
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/									
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/									
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/									
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/									
241/173	Positive (italian)		--	LARISSA	/									
241/183	Positive (italian)		--	KATR	/									
None Zero (no inocula, negative controls PMCA)					/									
Positive control (sheep-BSE)					/									

0% 25% 50% 75% 100%

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Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg362 (Human PrP V129V)									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/									
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/									
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/									
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/									
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/									
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/									
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/							25%	25%	25%
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/					25%	25%	25%	25%	25%
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/									
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/									
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/							50%	50%	50%
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/									
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/									
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/									
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/						50%	75%	75%	75%
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/					25%	50%	75%	75%	75%
241/173	Positive (italian)		--	LARISSA	/									
241/183	Positive (italian)		--	KATR	/									
None Zero (no inocula, negative controls PMCA)					/									
Positive control (Hu-sCJD VV type2)					/									

0% 25% 50% 75% 100%

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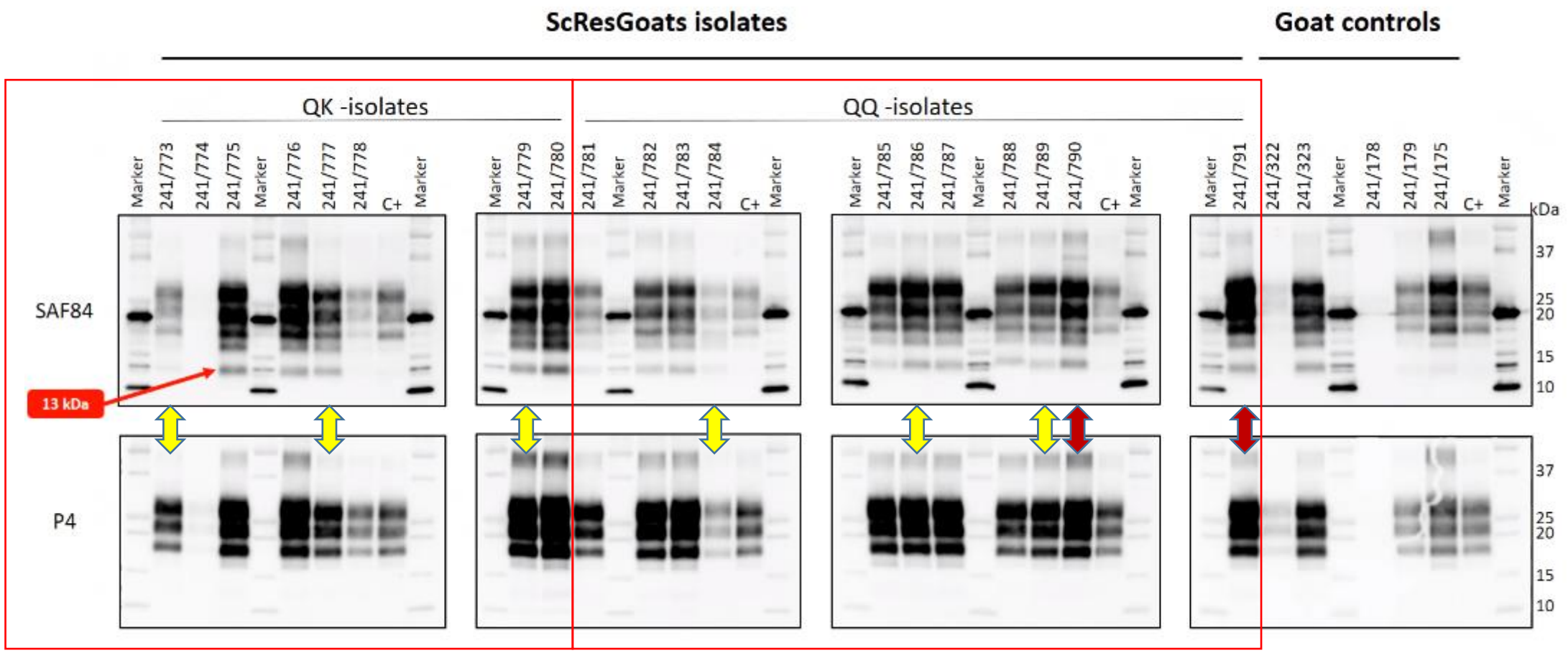
Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg340 (Human PrP M129M)										PMCA in Tg340 (Human PrP M129M) REPETITION									
					R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
241/773	Positive	QK/ RR/ NN/ TT	24	LARISSA	/																			ND
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/																			ND
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/										/									
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/										/									
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/																			ND
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/																			ND
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/																			ND
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/																			ND
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/																			ND
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/																			ND
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/																			ND
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/																			ND
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/																			ND
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/																			ND
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/																			ND
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/																			ND
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/																			ND
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/										/									
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/										/									
241/173	Positive (italian)		--	LARISSA	/																			ND
241/183	Positive (italian)		--	KATR	/																			ND
None Zero (no inocula, negative controls PMCA)					/										/									
Positive control (sheep-BSE)					/										/									

Code	Scrapie	SNP detection (222/211/146/110)	Age (months)	Region	PMCA in Tg362 (Human PrP V129V)										PMCA in Tg362 (Human PrP V129V) REPETITION									
					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	24	LARISSA	/																			ND
241/774	Positive	QK/ RR/ NN/ TT	36	LARISSA	/																			ND
241/775	Positive	QK/ RR/ NN/ TT	36	LARISSA	/										/									25%
241/776	Positive	QK/ RR/ NN/ TT	24	LARISSA	/										/									
241/777	Positive	QK/ RR/ NN/ TT	48	KALLITHEA/LARISSA	/																			ND
241/778	Positive	QK/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/																			ND
241/779	Positive	QK/ RR/ NN/ TT	24	IOANNINA	/																			ND
241/780	Positive	QK/ RR/ NN/ TT	36	IOANNINA	/																			ND
241/781	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/																			ND
241/782	Positive	QQ/ RR/ NN/ TT	24	LARISSA	/							25%	25%	25%										ND
241/783	Positive	QQ/ RR/ NN/ TT	60	LARISSA	/					25%	25%	25%	25%	25%										ND
241/784	Positive	QQ/ RQ/ NN/ TT	60	LARISSA	/																			ND
241/785	Positive	QQ/ RR/ NN/ TT	84	KALLITHEA/LARISSA	/																			ND
241/786	Positive	QQ/ RR/ NN/ TT	36	KALLITHEA/LARISSA	/							50%	50%	50%										ND
241/787	Positive	QQ/ RR/ NN/ TT	60	KALLITHEA/LARISSA	/																			ND
241/788	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/																			ND
241/789	Positive	QQ/ RR/ NN/ TT	36	IOANNINA	/																			ND
241/790	Positive	QQ/ RR/ NN/ TT	24	IOANNINA	/						50%	75%	75%	75%	/				50%	50%	75%	75%	75%	75%
241/791	Positive	QQ / RR/ NN/ TT	60	IOANNINA	/					25%	50%	75%	75%	75%	/									25%
241/173	Positive (italian)		--	LARISSA	/																			ND
241/183	Positive (italian)		--	KATR	/																			ND
None Zero (no inocula, negative controls PMCA)					/										/									
Positive control (Hu-sCJD VV type2)					/										/									

ISS western blot



C+= ovine classical scrapie

Inoculations determined by the consortium

Inoculations determined by PMCA results in human substrates

WP2 – Prion strain characterisation
Bioassays in transgenic mice and bank voles

Criteria for sample selection and sample allocation to various partners has been covered

Summary of animals models to be used

- **P1. APHA 18 isolates in**
  - **tg338 (ovine $V_{136}R_{154}Q_{171}$ PrP^C)**
  - **tg110 (bovine PrP^C)**
- **P3. ISS 6 isolates in**
  - **tg501 (ovine $A_{136}R_{154}Q_{171}Q_{222}$ PrP^C)**
  - **bank voles**
- **P4. INIA-CISA-CSIC 8 isolates in**
  - **tg340 (human M_{129} PrP^C)**
  - **tg361 (human V_{129} PrP^C)**

All bioassays have been initiated and are currently ongoing

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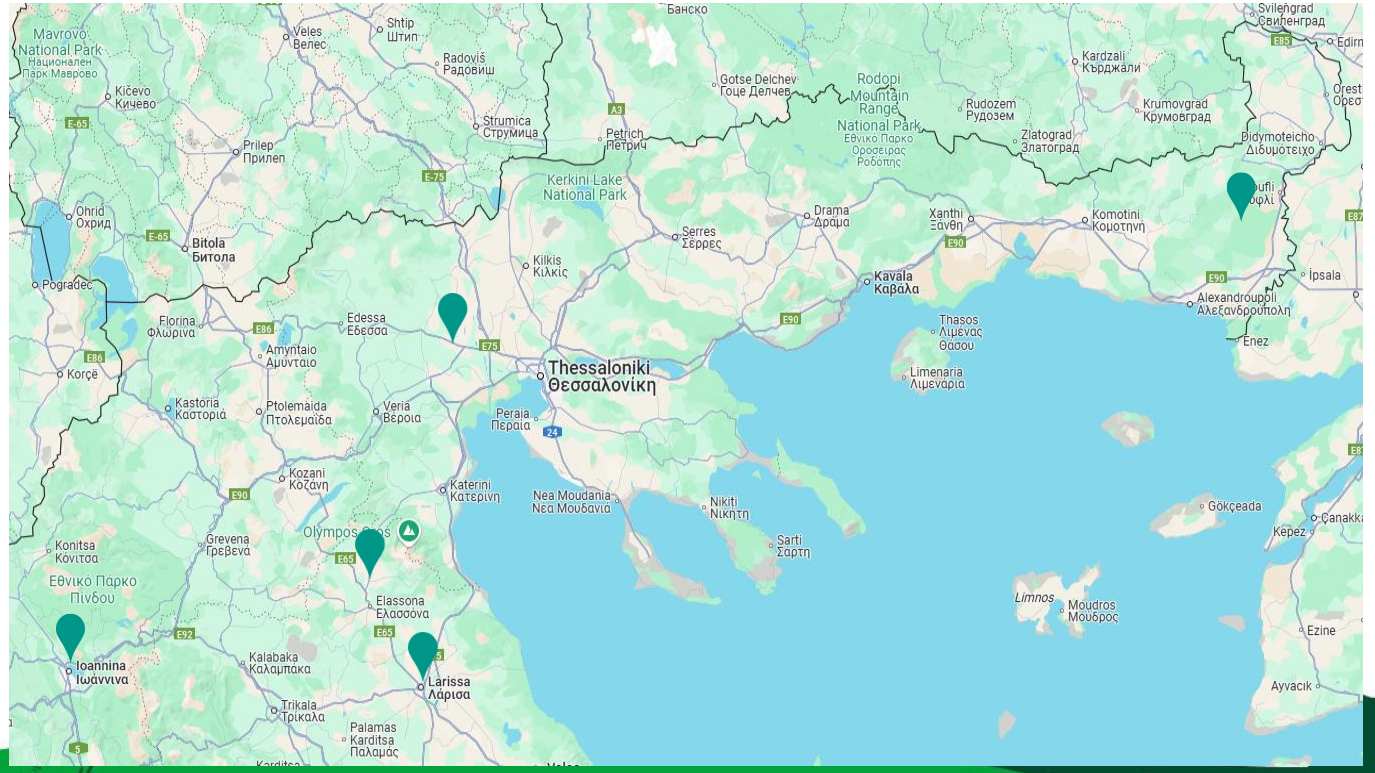
WP3 – Epidemiological and risk analysis

It involves

- **Development of questionnaires (P3)**
- **Sourcing and Interviewing relevant farmers (P2)**
- **Compiling and analysing data (P3)**



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Current progress

WP1

- **Sample identification: Completed**
- **Sequencing of ORF: ongoing. It will be completed within the timeline of the project**

WP2

- **Biochemical characterisation of sources: completed**
- **In vitro propagation of prions in various PrP^C substrates: ongoing. It will be completed within the timeline of the project**
- **Bioassays**
  Initiated data will not be available by the end of the project. ICRAD has been notified and an extension will be requested

WP3

- **Ongoing. It will be completed within the timeline of the project**

The teams

P1. APHA: John Spiropoulos, Janet Hills, Katrina McCrory

P2. Elgo-Dimitra: Evridiki Boukouvala, Ilias Bouzalas
NRL: Vaia Palaska

P3. IZSTO: Giuseppe Ru, Rossana Desiato, Matia Begovoeva, Paola Barzanti

ISS: Michele Di Bari, Laura Pirisinou, Romolo Nonno

P4. INIA-CISA-CSIC: Natalia Fernádez-Borges, Juan Maria Torres, Juan Carlos Espinosa

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

Animal & Plant Health Agency

ELGO - DIMITRA
HELLENIC AGRICULTURAL ORGANIZATION - DIMITRA

icrad
International coordination of research on infectious animal diseases

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ISTITUTO ZOOPROFILATTICO SPERIMENTALE del Piemonte Liguria e Valle d'Aosta

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
Υπουργείο Αγροτικής Ανάπτυξης και Τροφίμων

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