12th Annual Meeting of the TSE EURL
Torino, Italy
12nd-13th May 2025

EU-wide collaboration on PrP genetics in sheep with AS: state of play and next steps

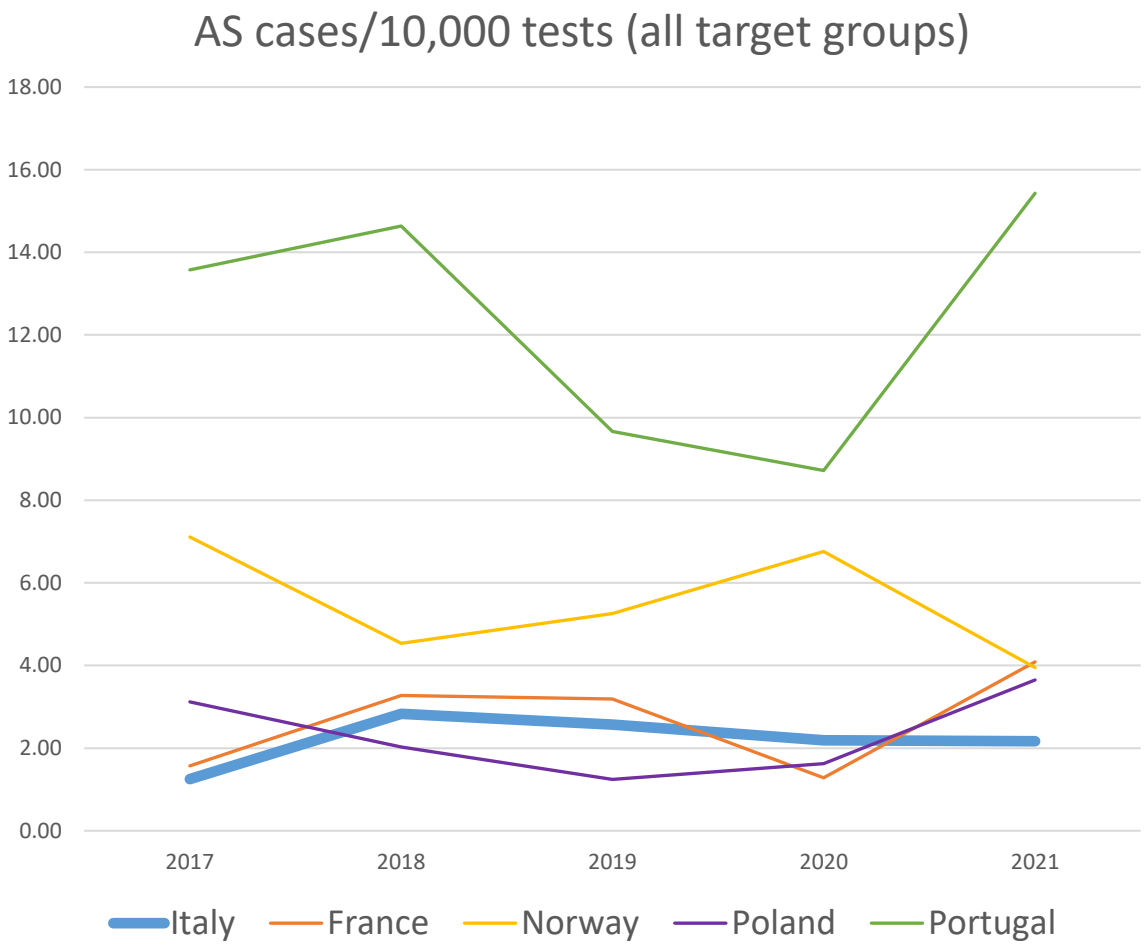
Aims of this presentation

- Recap of the project's rationale
- Update on NRLs participation and data collection
- Plan next steps

Why does AS occur?

- Can be experimentally transmitted by the oral route (Simmons et al., EID 2011) and low levels of infectivity can be detected in peripheral tissues (Andreoletti et al, Plos Path 2011)
- No temporal trends, outbreaks or clustering (Fediaevsky et al., 2008)
- The only risk factors identified are large flock size (Marier et al., Vet Rec 2017) and PrP genetics
- EFSA 2021: more likely that AS is a non-contagious, rather than a contagious disease…….but major knowledge gaps still remain
- So, why does atypical scrapie occur?

AS in Europe



Slightly different prevalences between countries persist over the years. How to explain it?

PrP genotype is a major risk factor for AS

- Polymorphisms at codons 141 (L/F) and 154 (R/H) are highly associated with AS cases
- Sheep with the ALRR allele do not appear to be protected against developing atypical scrapie

Moum et al. J Gen Virol. 2005, 86, 231–235
Saunders et al. J Gen Virol. 2006;87(pt 11):3141–3149
Moreno et al. Arch Virol (2007) 152: 1229–1232
Luhken et al. Vet Res. 38 (2007) 65–80

Full sequencing of PrP in the Italian sheep population

(EC 999/2001 «random genotyping»; 2009-2022; n=9035)

- 42 alleles that combines into >100 genotypes!
- Polymorphisms *vs* mutations (alleles >1% freq are polymorhisms)
- Wt + 8 polymorphisms account for 98% of all alleles
- 33 mutations account for the remaining 2%

4-cod	others	n°	%
ALRQ-wt		**6952**	**38,5%**
ALRR		7961	44,1%
ALHQ		760	4,2%
AFRQ		480	2,7%
ALRQ	176-K	416	2,3%
ALRQ	112-T	325	1,8%
ALRQ	137-T	288	1,6%
VLRQ		268	1,5%
ALRH		255	1,4%
TOT		**17705**	**98%**

4-codon PrP genotype of AS cases in Italy

- 85% (105/123) have F141 or H154
- ALRR does not confer resistance
- Only 2,5% (3/123) ALRQ/ALRQ

✓ Recapitulate previous knowledge from other EU countries

	n°	%
ALRQ/ALRQ	3	2,4%
ALRQ/**AFRQ**	26	21,1%
ALRQ/**ALHQ**	19	15,4%
ALRQ/ALRR	4	3,3%
AFRQ/AFRQ	16	13,0%
AFRQ/ALRR	15	12,2%
AFRQ/ALHQ	3	2,4%
ALRR/ALRR	10	8,1%
ALRR/**ALHQ**	20	16,3%
ALRR/ALRH	1	0,8%
ALHQ/ALHQ	3	2,4%
ALHQ/ALRH	2	1,6%
ALHQ/ALRK	1	0,8%
TOT	123	100,0%

Full sequencing of PrP from AS cases in Italy

- **No AS cases in wt/wt sheep**

- Rare PrP mutations in AS (N146S, R159H, Y172D, E203K)

How can we substantiate quantitatively these findings?

Sequence	n°	%
ALRQ/ALRQ (wt/wt)	0	0,0%
ALRQ/ALR**H**^{159}Q	1	0,8%
ALRQ/ALRQ**D**^{172}	1	0,8%
ALRQ/ALRQ**K**^{203}	1	0,8%
ALRQ/AFRQ	23	18,7%
AL**H**^{143}RQ/AFRQ	2	1,6%
ALRQ**K**^{176}/AFRQ	1	0,8%
ALRQ/ALHQ	18	14,6%
AL**S**^{146}RQ/ALHQ	1	0,8%
ALRQ/ALRR	4	3,3%
AFRQ/AFRQ	16	13,0%
AFRQ/ALRR	15	12,2%
AFRQ/ALHQ	3	2,4%
ALRR/ALRR	10	8,1%
ALRR/ALHQ	20	16,3%
ALRR/ALRH	1	0,8%
ALHQ/ALHQ	3	2,4%
ALHQ/ALRH	2	1,6%
ALHQ/ALRK	1	0,8%
	123	100,0%

Do wt/wt sheep develop AS?

If not:

- It could be hypothesized that the necessary condition to develop AS is PrP variation (mutations/polymorphisms)

- This would possibly support a genetic, non-contagious, etiology for AS
  - genetic TSEs never described in animals
  - genetic CJD in humans, autosomal dominant, caused by spontaneous misfolding of mutated PrP; >40 PRNP mutations that confer lifetime risks ranging from <0.1 to ~100%

Collaboration

Aims:

- to determine if there are AS cases in wt/wt sheep (and how many)
- to gain insight into the causal role of rare PrP mutations

Strategy:

- to achieve full sequence data of ALRQ/ALRQ AS cases detected in the different MS

Collaboration

So far:

✓The NRLs were contacted at September 2024, asking:

- 4 codon data of AS cases (requirement by EC 999/2001)
- if available, full sequence data of ALRQ/ALRQ cases
- if available, tissue or DNA of ALRQ/ALRQ cases

✓The EURL collected 4-codon and sequence data provided by the NRLs that agreed to participate in the study

AS cases 2002-2023 (TSE annual reports)

EU	AT	17
	BE	11
	BG	6
	CY	
	CZ	8
	DE	157
	DK	15
	EE	2
	EL	33
	ES	278
	FI	25
	FR	595
	HR	6
	HU	234
	IE	53
	IT	129
	LT	
	LU	
	LV	
	MT	
	NL	18
	PL	87
	PT	790
	RO	1
	SE	56
	SI	14
	SK	60
	Total EU27	2.595
	XI[(a)]	3
	Total EU27+XI	2.598
Other non-EU	CH	
	IS	9
	ME	
	MK	
	NO	212
	RS	
	TR	
	United Kingdom	382
	Total other non-EU	603
TOTAL	Total	3.201

Partecipating NRLs

Country	Data	N° cases 4-codon genotype	N° ALRQ/ALRQ	Sequence ALRQ/ALRQ	Tissue/DNA	Control population
Austria	Y	17	1	N	Y	Y
Belgium	Y	4	0	/	N	Y
Germany	Y	107	9	N	?	?
Spain	Y	189	6	Y (pending)	Y	N
Finland	Y	17	14	Y (6 pending)	Y	Y
Croatia	Y	5	0	/	N	N
Italy	Y	123	3	Y*	Y	Y
Netherlands	pending					
Poland	Y	108	3	Y*	Y	Y
Portugal	Y	668	21	Y (90-222)	?	Y
Slovenia	Y	15	0	/	Y	Y
Slovakia	Y	59	0	/*	N	N
Swiss	pending		0			
Iceland	Y	11	0	/	N	Y
Norway	Y	224	7	N	Y (in part)	N
UK	Y	407	0	/*	Y	Y
TOT		**1954**	**64**			

* full sequences available for all cases

PrP genotypes in AS (4 codons)

ALRR/ALHQ	340	17,4%
AFRQ/ALRR	238	12,2%
ALRQ/ALHQ	229	11,7%
AFRQ/AFRQ	216	11,1%
ALRR/ALRR	213	10,9%
ALRQ/AFRQ	213	10,9%
ALHQ/ALHQ	148	7,6%
ALRQ/ALRR	112	5,7%
AFRQ/ALHQ	102	5,2%
ALRQ/ALRQ	64	3,3%
ALHQ/ALRH	18	0,9%
AFRQ/ALRH	17	0,9%
AFRQ/VLRQ	8	0,4%
ALRQ/VLRQ	7	0,4%
ALRR/ALRH	7	0,4%
ALRQ/ALRH	6	0,3%
ALHQ/VLRQ	4	0,2%
ALRR/VLRQ	2	0,1%
ALRR/ALRK	2	0,1%
AFRQ/ALRK	1	0,1%
ALHQ/ALRK	1	0,1%
ALRH/ALRH	1	0,1%
ALRQ/ALRK	1	0,1%
ALRQ/ALLQ	1	0,1%
AFRQ/VFRQ	1	0,1%
AFHQ/AFRR	1	0,1%
ALRR/AFRR	1	0,1%
TOTAL	1954	

ALRR/ALHQ	340	17,4%
AFRQ/ALRR	238	12,2%
ALRQ/ALHQ	229	11,7%
AFRQ/AFRQ	216	11,1%
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ALRQ/AFRQ	213	10,9%
ALHQ/ALHQ	148	7,6%
ALRQ/ALRR	112	5,7%
AFRQ/ALHQ	102	5,2%
ALRQ/ALRQ	64	3,3%

Are these ALRQ/ALRQ really wt/wt?

PrP amino acid variations in ALRQ/ALRQ cases

64 ALRQ/ALRQ detected:

- ✓**12 with full sequence: ALL have additional mutations or polymorphisms: so far no wt/wt cases in samples with full PrP sequence**
- ✓21 with partial sequence (92-222; do not include 224): 13 (60%) have additional mutations/polymorphisms
- ✓At least 25/33 (75%) ALRQ/ALRQ are not wt/wt
- ✓More sequences will be available from Spain and Finland (n=12)
- ✓≈40 samples to collect for complete sequencing (not all available)

New PrP polymorphisms/mutations potentially associated with AS

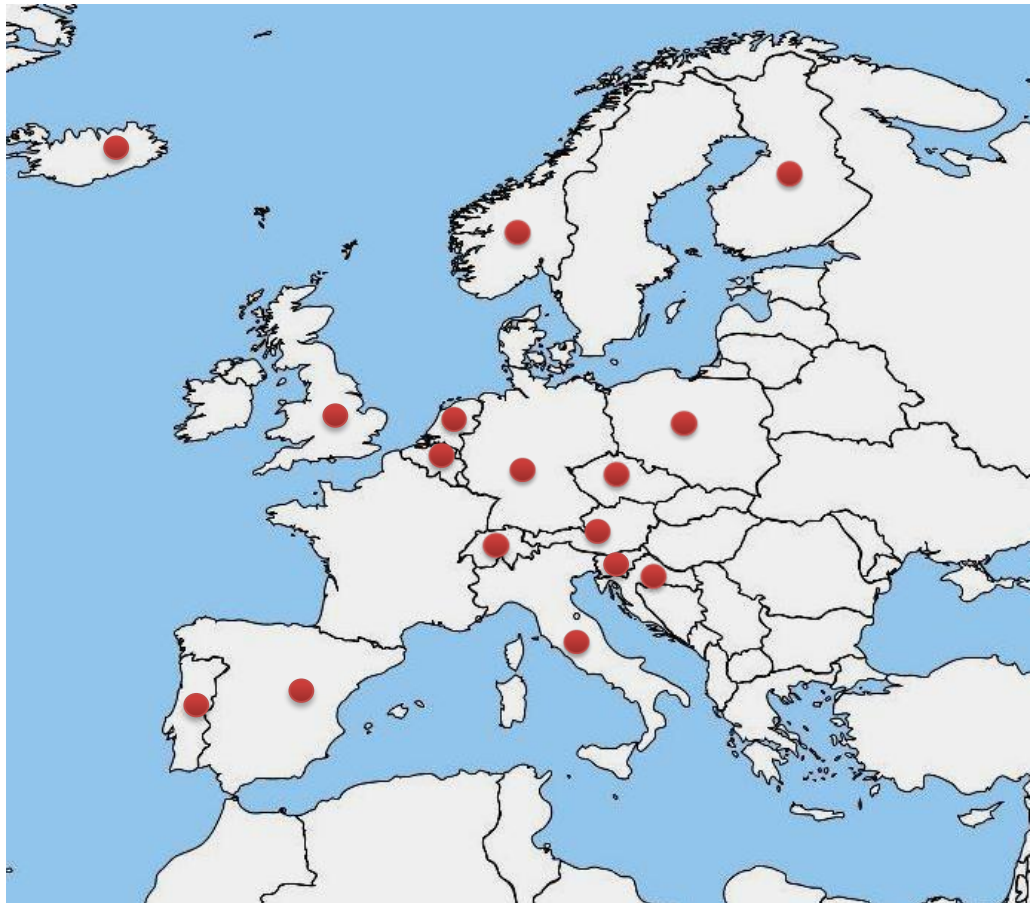
- Italy: R159H, Y172D, E203K
  - Very rare mutations in the general population in Italy (collectively, 0.02% of all alleles)
- Portugal (partial sequernce: codon 224 not included in the sequence): H143R; Y172D; N176K
- Finland: E224K and R151C (both heterozygous and homozygous!)
  - Both are frequent polymorphisms in Finnish Landrace sheep (Hautaniemi et al. BMC Veterinary Research 2012)
- Poland: E224K, P241S
  - E224K is a polymorphism associated to AS in Romanov sheep in Poland
  - P241S is probably rare in Poland (Piestrzynska-Kajtoch et al., Mol Biol Rep 2012); it can be however a frequent polymorphism is some breed (Goldman et al., Journal of Applied Microbiology 2005)

Next steps

- Last call for NRLs (FR, HU, IE, SE..)
- NRLs having ALRQ/ALRQ will be contacted individually to share tissues/DNA for sequencing
- NRLs that have full sequence of all AS cases (Poland, Italy, UK): to check for polymorhisms/mutations other than at 4-codons
- It would be useful that any new ALRQ/ALRQ is considered for sequencing
- 4-codons datasets: descriptive, but we will discuss with Giuseppe on the possible use of control populations

Aknowledgments

- ✓ Jelka Zabavnik Piano - Slovenia
- ✓ Zuzana Dirbáková, Miroslav Mojzis - Slovakia
- ✓ Agata Piestrzynska-Kajtoch - Poland
- ✓ Alba Marin-Moreno - Spain
- ✓ Katrina McCrory, John Spiropoulos - UK
- ✓ Auri Kauppinen, Lauri Kareinen - Finland
- ✓ Christine Fast, Gesine Luhken - Germany
- ✓ Alexandra Bastian - Austria
- ✓ Alex Bossers - Netherlands
- ✓ Leonor Orge, Renata Carvalho - Portugal
- ✓ Stefanía Þorgeirsdóttir - Iceland
- ✓ Sylvie Benestad, Torfinn Moldal - Norway
- ✓ Severine Matthijs - Belgium
- ✓ Karmen Branović Čakanić - Croatia
- ✓ Thorsten Seuberlick - Swiss
- ✓ Gabriele Vaccari, Barbara Chiappini - Italy
- ✓ Giuseppe Ru, EURL



AS prevalence in sheep with different genotypes in Italy