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Prion protein genetics of atypical scrapie cases in Italy

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Atypical scrapie: contagious or not?

- Do the scientific data on the 2-year intensified monitoring collected by the EC provide any evidence on the contagiousness of atypical scrapie?

- "…. it is considered **more likely (subjective probability range 50-66%) that AS is a non-contagious, rather than a contagious disease**."

- "The analysis of the data of the EU……confirmed some of the known epidemiological features of AS but identified that **major knowledge gaps still remain**."

EFSA Scientific report on the analysis of the 2-year compulsory intensified monitoring of atypical scrapie. EFSA J. 2021;19(7):e06686

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

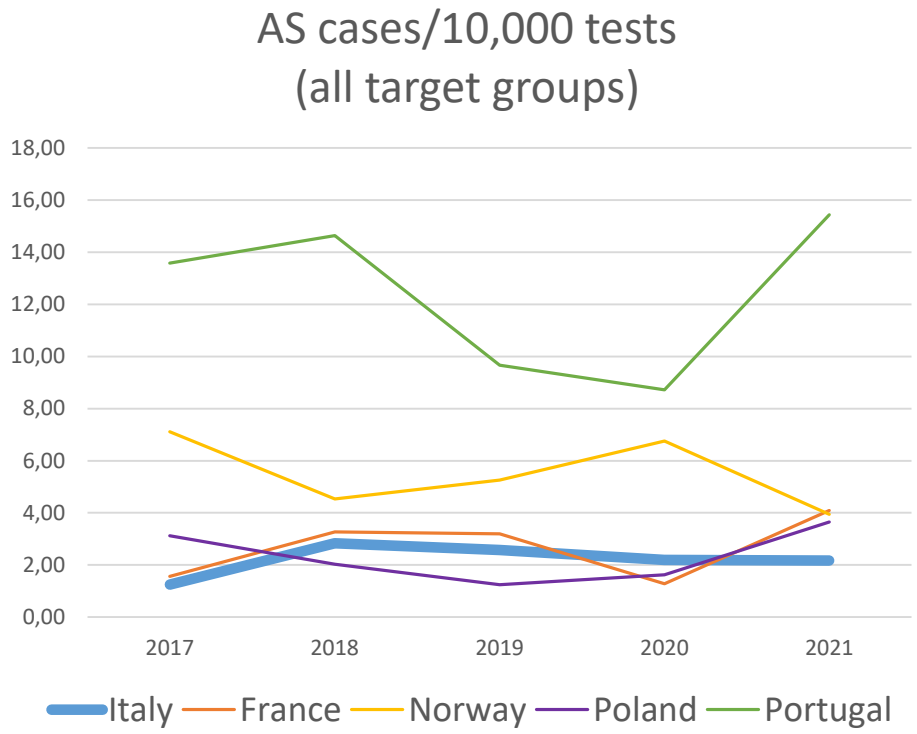
Moum et al. J Gen Virol. 2005, 86, 231–235
Saunders et al. J Gen Virol. 2006;87(pt 11):3141–3149
Moreno et al. Arch Virol (2007) 152: 1229–1232
Luhken et al. Vet Res. 38 (2007) 65–80

Aims of this presentation

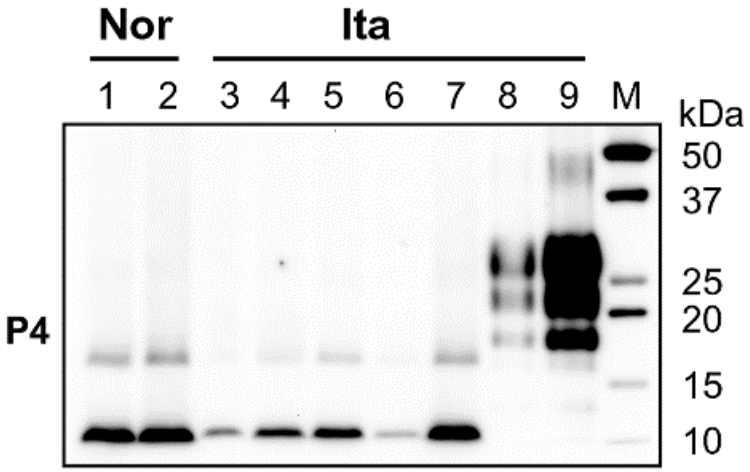
- Share with NRLs some interesting data emerging from AS genotype surveillance in Italy

- **Propose a collaboration to the NRLs in order to falsify the main hypothesis that emerge from the Italian dataset**

AS in Italy



AS prevalence in the range of other EU countries



All tested cases have the same PrP^{Sc} signature and biological properties, similar to the original Nor98 cases

(Pirisinu et al. PLOS ONE 2013; Pirisinu et al., PLOS Pathogens 2022)

4-codon PrP genotype of AS cases in Italy

- 85% (105/123) have at least one allele 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, N172D, E203K)

How can we substantiate quantitatively these findings?

Sequence	n°	%
ALRQ/ALRQ	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%

Full sequencing of PrP in the Italian sheep population
(EC 999/2001 «random genotyping»; 2009-2022; n=9035)

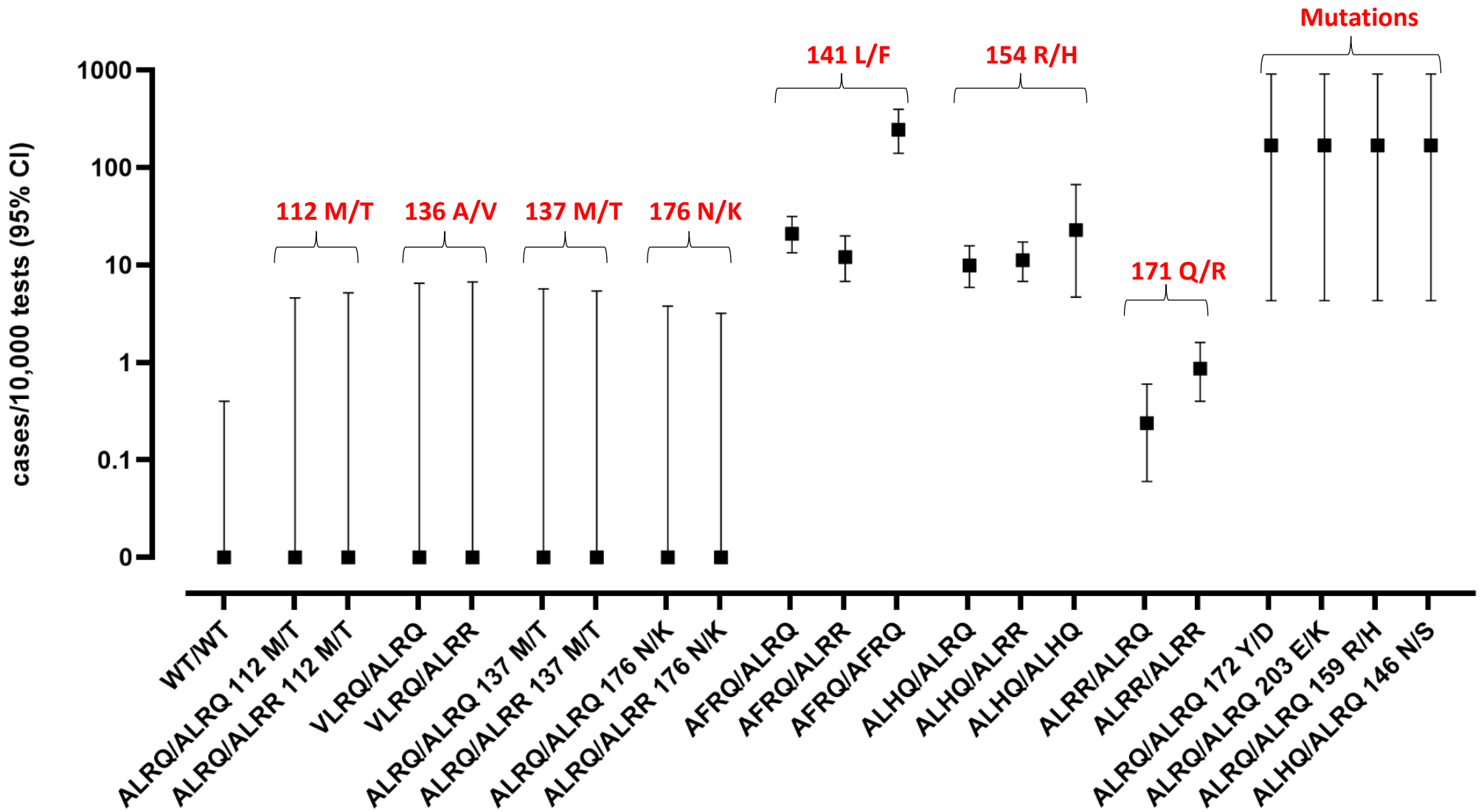
- 42 alleles that combines into >100 genotypes!
- Polymorphisms *vs* mutations (alleles >1% freq are polymorhisms)
- Wt + 8 polymorphisms account for 98% of all alleles
- 33 mutations account for the remaining 2%

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%**

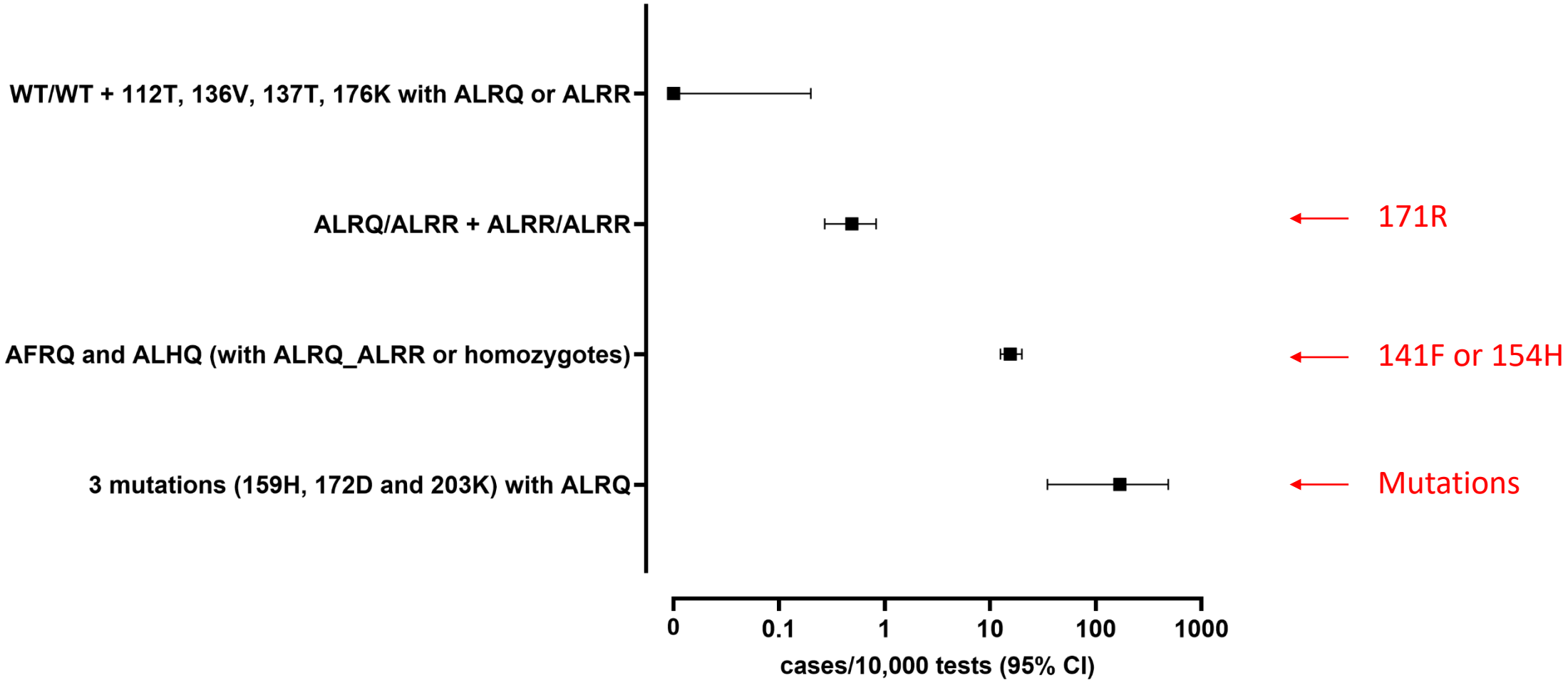
- Population data derive from national random sampling
  - annually (2008-2022; ~650/year)
  - representative sample of the national sheep population
  - stratified by region and breed

- Bias (as a control of TSE cases)
  - Diagnostic tests are not on a representative sample of the national population (breeds might be mis-represented for several reasons: scrapie prevalence, geographic, zootechnic…)
  - Age and sex bias (e.g. slaughtered and tested sheep *vs* national population)
  - …..

AS prevalence in sheep with different genotypes



AS prevalence in sheep with different genotypes



In summary

✓ No AS in wt/wt sheep!

- the same applies to goats in Italy
- AS linked to PrP variations?

✓ Rare mutations associated to AS

- R159H, N172D, E203K (equivalent to E200K in human gCJD)
- R159H and E203K not previously described in any breed
- E224K associated to AS in Romanov sheep, Poland *(Piestrzyn´ska-Kajtoch et al., Mol Biol Rep 2012)*

Do wt/wt sheep develop AS?

- If not, then it could be hypothesized that the necessary condition to develop AS is PrP variation (mutations/polymorphisms)

- AS would resemble a genetic prion disease
  - 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%
  - never described in animal TSEs

- This would clearly support non-contagious etiology for AS

Do wt/wt sheep develop AS?

- We can’t exclude that AS was not detected in wt/wt italian sheep just by chance (low numbers) or because of other «local» factors (specific genetic factor in italian breeds, age of tested sheep in Italy….)

- In Europe? No fully sequenced wt/wt AS case in literature (for example, no wt/wt sheep with AS over 69 sequenced AS cases in UK, Saunders et al. 2006), but few studies with low numbers of cases

- Based on 4-codons studies we can estimate that ~5% AS cases are ALRQ/ALRQ (~100-200, over 3118 AS cases in EU 2002-2022)

Collaboration proposal

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, to add to the Italian dataset

Collaboration proposal

How:

- NRLs willing to partecipate in this study could share:
  - 4 codon data of AS cases (requirement by EC 999/2001)
  - when ALRQ/ALRQ are present
    - full sequence data of ALRQ/ALRQ cases, if already available (published or unpublished)
    - available tissue or DNA of ALRQ/ALRQ cases

- The EURL will
  - gather 4-codon and sequence data provided by the NRLs
  - receive tissue/DNA of ALRQ/ALRQ AS cases and produce sequence data

Collaboration proposal

Why?

- Add value to 20 years of surveillance in EU (increase the scientific content)
- Unrprecedented chance to gain insight into an animal disease so rare and difficult to study
- Contribute to understand the etiology of AS, in order to better address future efforts

Aknowledgments

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