



Better Training for Safer Food *Initiative*

**Breeding programmes for the diffusion
of the genetic resistance to classical
scrapie in the sheep and goat
population**

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Small ruminants TSE resistance



genetic selection in humans to kuru (a prion disease) resistance; an example of recent selection in humans



Subjects

1. specific EC issues about resistance breeding
2. prion protein (PrP) polymorphisms & "resistance"
3. strains (scrapie types, BSE) and PrP polymorphisms
4. EU regulation in sheep
5. sheep results in application EU rules: country dependent
6. EFSA issues on breeding towards resistance
7. atypical/Nor98 scrapie in sheep
8. goat breeding options (but no formal actions) against classical scrapie and BSE
9. references: legislation/opinions/research



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1. specific EC issues about resistance breeding

Objectives of TSE eradication programs:

to protect

- animal health by reducing and eventually eradicating scrapie and;
- public health from the theoretical risk of BSE (if it is there and being masked by scrapie)

Specification aim

issues:

(how to) enhance the resistance by
breeding

(what needed for) prevention of
culling



TSE roadmap 2 outlines for the future of scrapie eradication measures:

Measures could be brought in line with the latest scientific information, which could mean e.g.

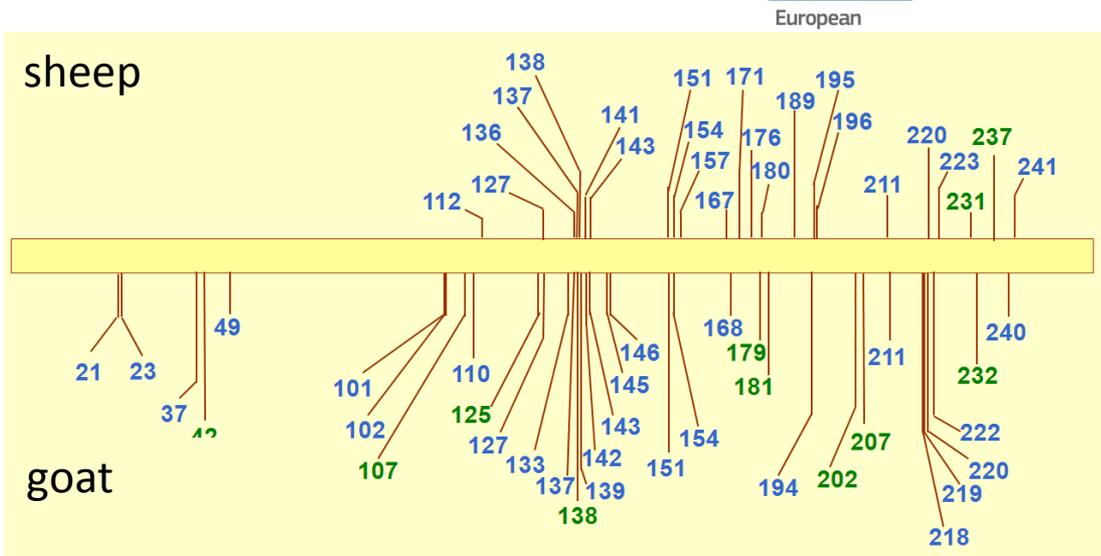
- continuing to encourage genetic control of the disease in sheep through breeding programmes
- adapting measures for atypical scrapie if data confirms that this scrapie strain is not contagious



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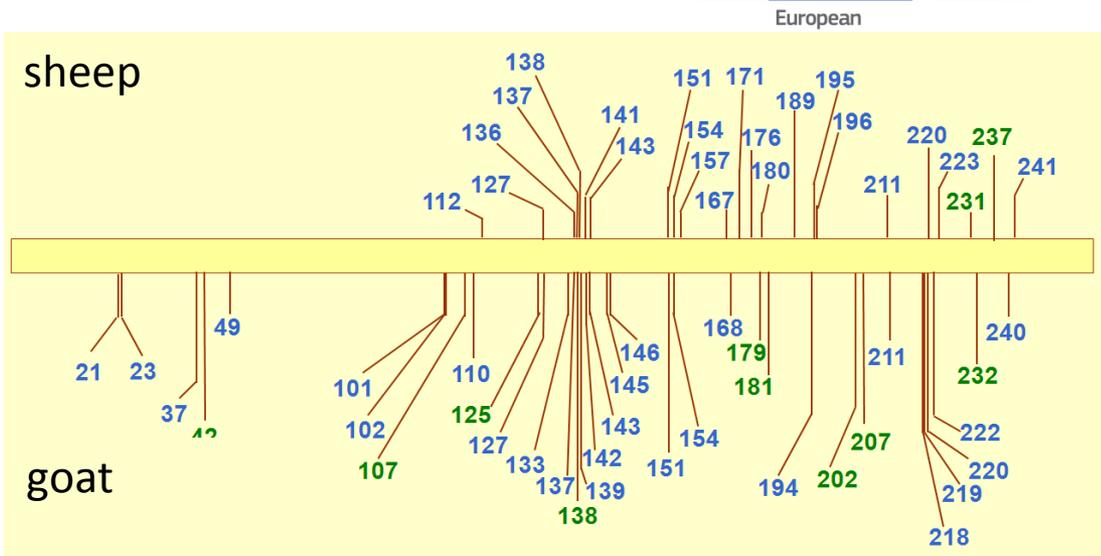
2. prion protein (PrP) polymorphisms & "resistance"

prion protein (PrP): many genetical variants in both sheep and goat



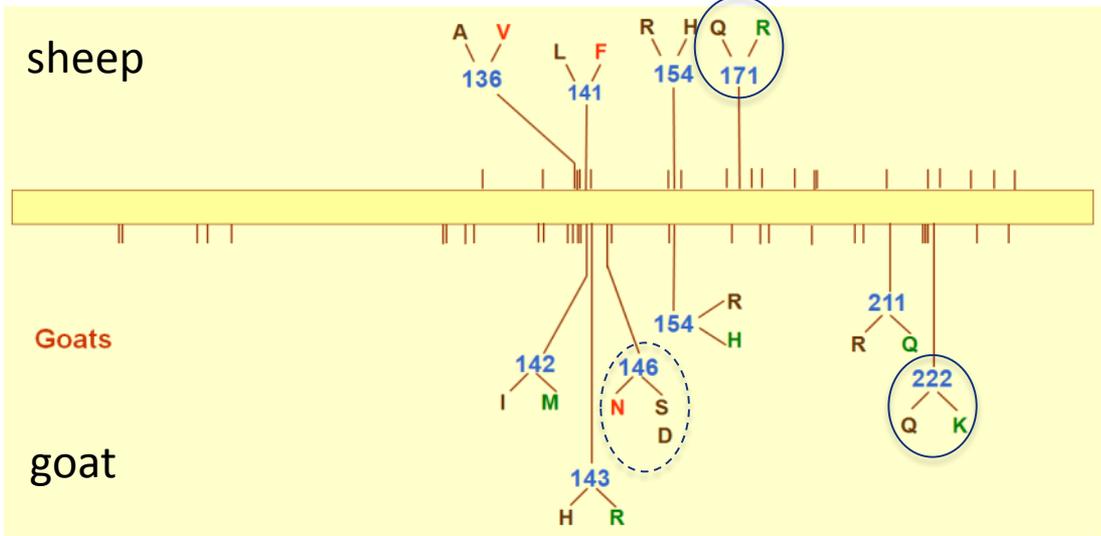
resistance related polymorphisms

prion protein (PrP): many genetical variants in both sheep and goat



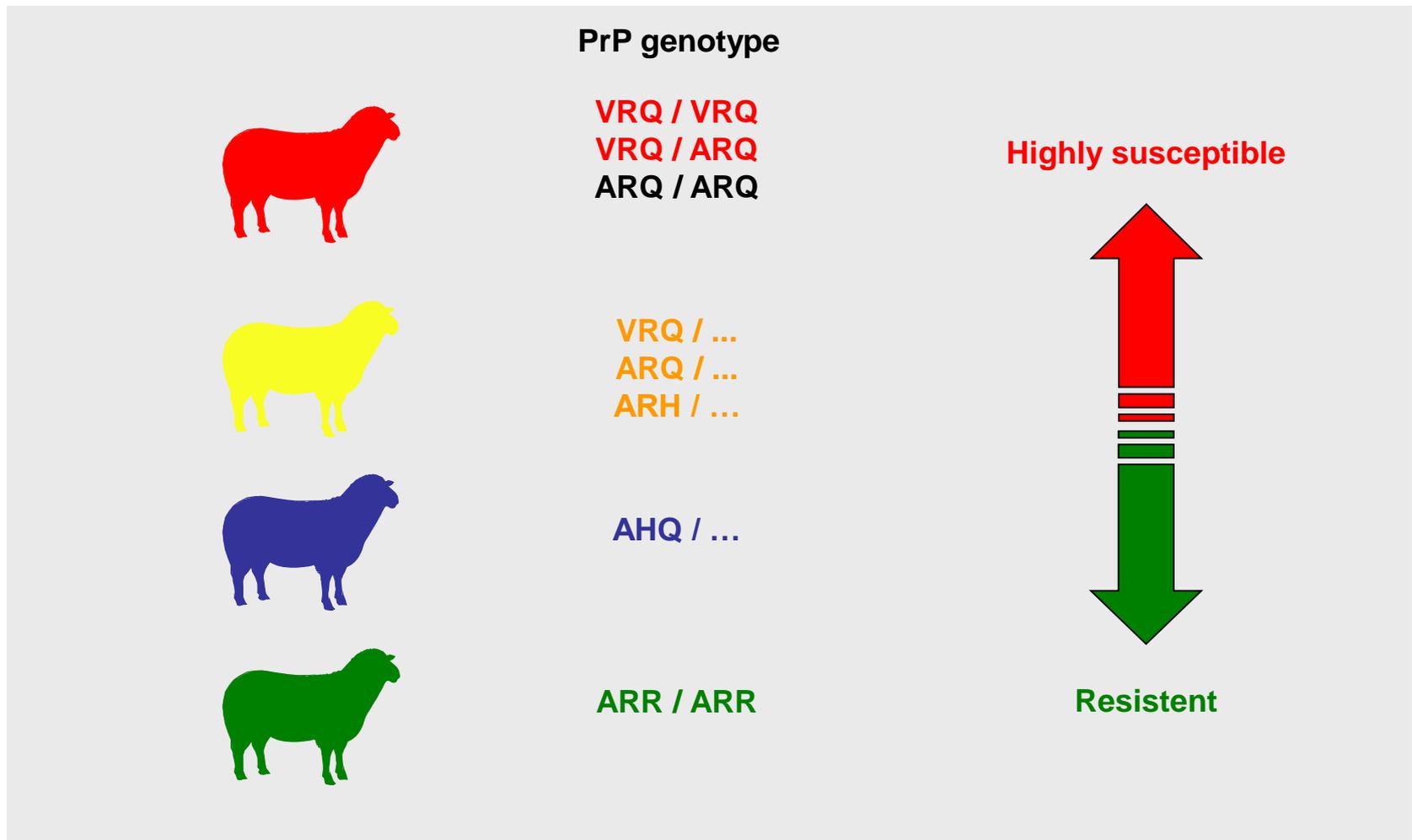
- genotypes in sheep important for directives:
- ARQ (wildtype)
 - VRQ (highly susceptible)
 - ARH
 - AHQ
 - ARR (highly resistant)

for goats: no rules yet but see the two poster hand-outs



resistance related polymorphisms > breeding follows simple Mendel laws

Genetically determined susceptibility of sheep





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3. strains (scrapie types, BSE) and PrP polymorphisms

Recognized TSE types in small ruminants

- 1. classical scrapie****
- 2. CH1641 scrapie****
- 3. Italian scrapie* (a potential separate cl. scrapie type)***
- 4. atypical/Nor98 scrapie*****
- 5. BSE* (only 2 cases in goats; 1 France, 1 in United Kingdom)***

* 3 band aspect in 15-30 kDa region of western blot, type specific Immunohistochemistry (IHC.)

** 7 kDa Western blot aspect using antibody like 9A2; also multimers present

TSE types in small ruminants

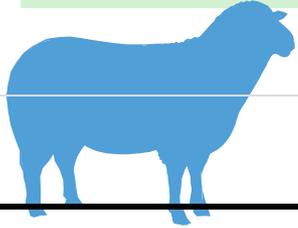
	TSE type	character	sheep
	classical scrapie	world wide; transmitting; lymphotropic ; pruritis; CNS > brainstem; triple PrP ^{res} bands	+
	Nor98/atypical scrapie	world wide; sometimes clinical signs; not contagious; not lymphotropic ; 7 kDa PrP ^{res} band & multimers; cerebellum	+*
	CH1641 scrapie	world wide; rare (?); naturally transmitting (?); ic signs: head tilting & tremors, ataxia; not lymphotropic? ; CNS: brainstem; triple PrP ^{res} bands	+
	Italian scrapie	Italy (France?, UK?); transmitting; lymphotropic ; pruritis; CNS > brainstem; triple PrP ^{res} bands; possible source contaminated vaccine	+
	BSE	extremely rare; lymphotropic ; signs as in scrapie; triple PrP ^{res} bands; brainstem	-***

* in sheep: occurring in 154H, 141F, 171R

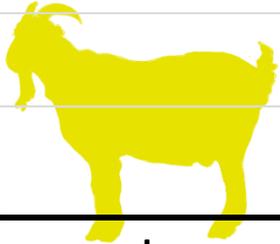
** in goats: Nor98, if occurring, in 154H carriers

*** 2 cases; could be due to BSE epidemic

PrP polymorphisms & TSE types



TSE type	susceptible -----> resistant			
cl. scrapie	136V	wt (ARQ)	154H	171R
cl. scrapie (Italian)	wt	136V, 154H		171R
scrapie (CH1641, exptl)	154H?	wt	136V	?
atypical/Nor98 scrapie	154H	171R, wt		136V
BSE (exptl)	wt		136V	171R



TSE type	susceptible -----> resistant			
cl. scrapie (France)	wt (ARQ)	154H, 142M, 211Q		222K
cl. scrapie (Italian)	wt			222K
scrapie (CH1641, exptl)	wt			?
atypical/Nor98 scrapie	154H		wt	?
BSE (exptl, oral)	wt	211Q	142M, 222K	

Conclusion on susceptibilities/resistance to TSE in small ruminants

*PrP genotype and TSE-type are independent
determinants of susceptibility.*





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4. EU regulation export/import in living materials from sheep (animal, embryo, ova)

REGULATIONS (999/2001):

Animals for breeding intended to other Member States:

*should only be required to come from holdings
with a controlled risk of classical scrapie*

*(based on compliance with a list of requirements
for at least three years)*

REGULATIONS (999/2001): (Chapter C: part 4)

Sheep: framework for recognition TSE-resistant status of certain flocks.

- 1. On at least the following two levels (if participating in the breeding programme):*
 - a) level I flocks composed entirely of ARR/ARR genotype animals;*
 - b) level II flocks whose progeny have been sired exclusively by ARR/ARR genotype rams.*

Member States may decide to grant recognition on further levels to suit national requirements.

- 2. Regular random sampling animals from TSE-resistant flocks shall be carried out: (a) on the holding or at the slaughterhouse to verify their genotype; (b) in the case of level I flocks, in animals over 18 months of age at the slaughterhouse, for TSE testing in accordance with Annex III.*



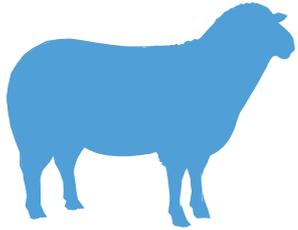
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breeding programs:

compulsory in 2004

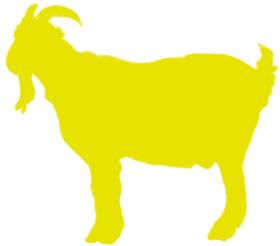
voluntary in 2007

scrapie prevalence in Europe (2002-2012)



classical scrapie: 8.7 / 10,000 tested (17 MS)

atypical scrapie: 5.8 / 10,000 tested (21 MS)



classical scrapie: 2.2 / 10,000 tested (8 MS, excl. CYP)

atypical scrapie: low (5 MS)

sheep

different countries - different strategies

Great Britain: (scrapie has been a notifiable disease since 1993)

NSP – for pure bred breeds
breed with ARR/ARR-rams

Netherlands: (scrapie has been a notifiable disease since 1993)

breed with ARR/ARR-rams
infected flocks: remove susceptible alleles



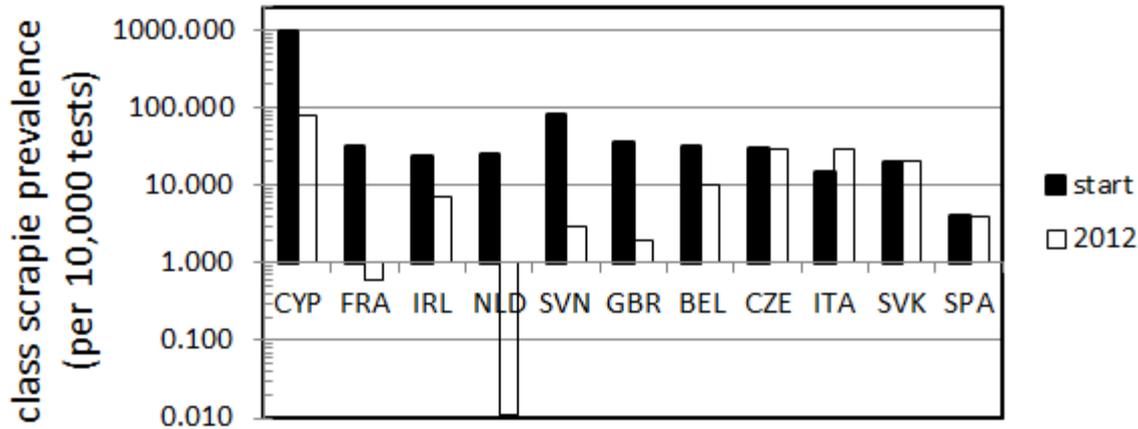
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**5. sheep results after applying EU rules:
country dependent**



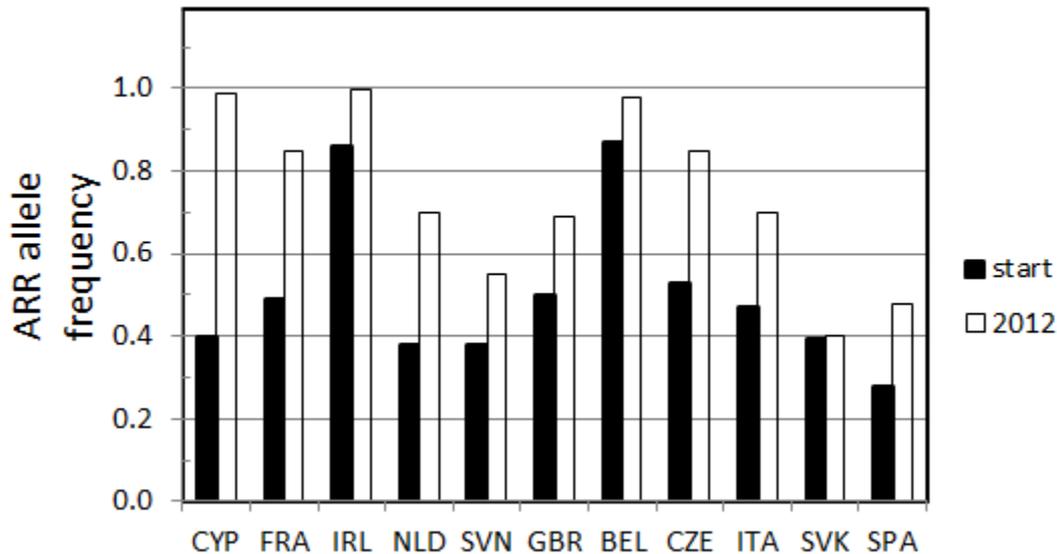
scrapie prevalence trends (\pm 2002-2012)



Scrapie and ARR trends EU member states

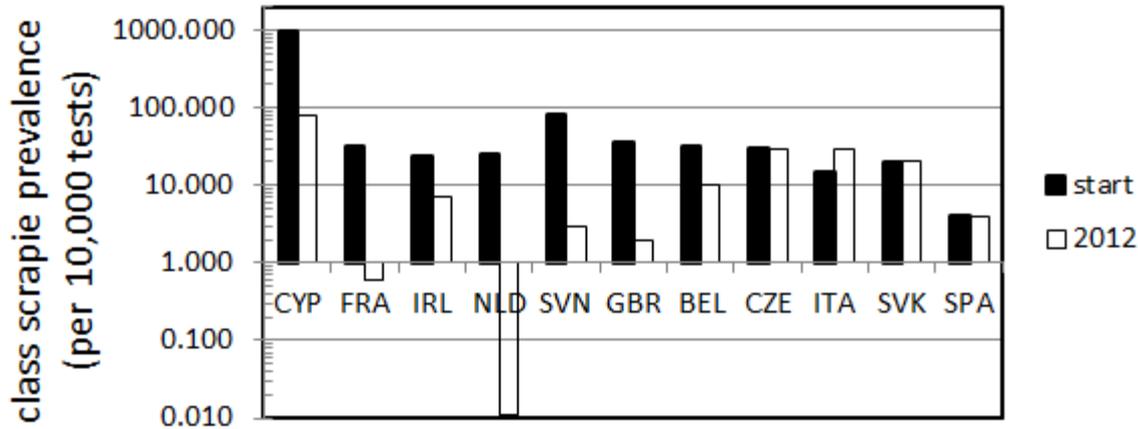
[EFSA J 2014; 12(7) 3781 etc]

ARR frequency trends (\pm 2004-2012)

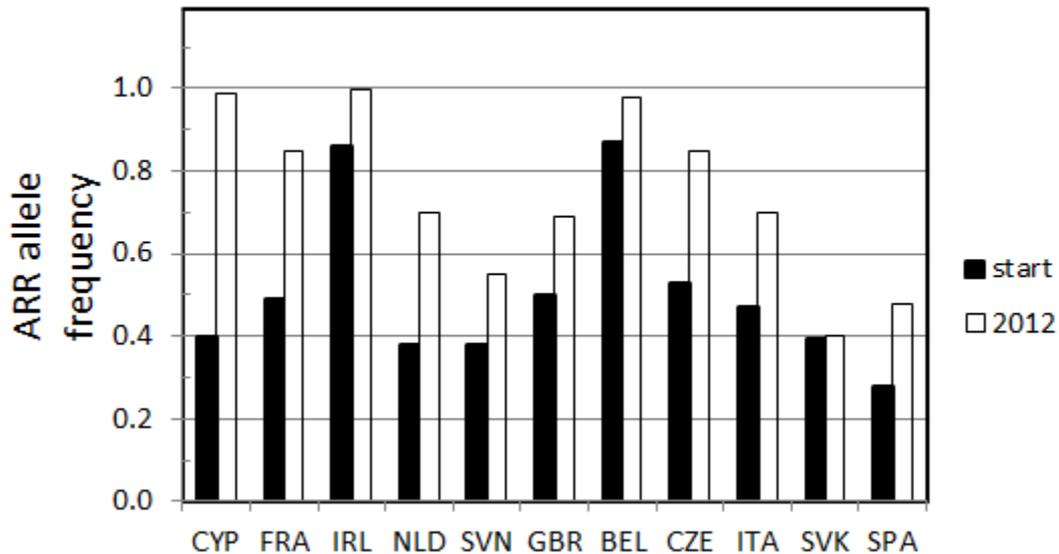




scrapie prevalence trends (\pm 2002-2012)



ARR frequency trends (\pm 2004-2012)



Scrapie and ARR trends EU member states

[EFSA J 2014; **12**(7) 3781. page 15]

Success (only) in CYP, FR, IRL, NLD, SVN, UK.

many countries did not apply in time tight rules and their targets were soft;

EC did change too early to voluntary program



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**results of breeding to resistance:
Netherlands**



- 1998 start breeding with ARR/ARR rams
- 1/2002 scrapie surveillance (limited selection > 18m)
- 2005 compulsory breeding program (EU wide) with ARR/ARR rams
- 2007 cancellation of enforcement scrapie breeding program > voluntary breeding program
- in case of scrapie on farm: compulsory breeding with ARR/ARR rams

Nodelijk et al. 2011: outbreak control possible while non-ARR/ARR animals do not have to be reduced to zero in the flock (but remove VRQ carriers: highly susceptible ones).

Activities in Netherlands



- 2004: obligatory - all Dutch flocks with > 10 ewes use a ram with the ARR/ARR genotype (EU decision 2003/100/EC only required a breeding programme for purebred sheep flocks of high genetic merit, **only voluntary**, until 1 April 2005)
- 2005: all Dutch sheep flocks obliged to use ARR/ARR rams (feasibility ensured by early voluntary start of breeding for scrapie resistance in purebred sheep, which provided for enough breeding ARR/ARR rams)
- June 2007 selection for scrapie resistance became voluntary again

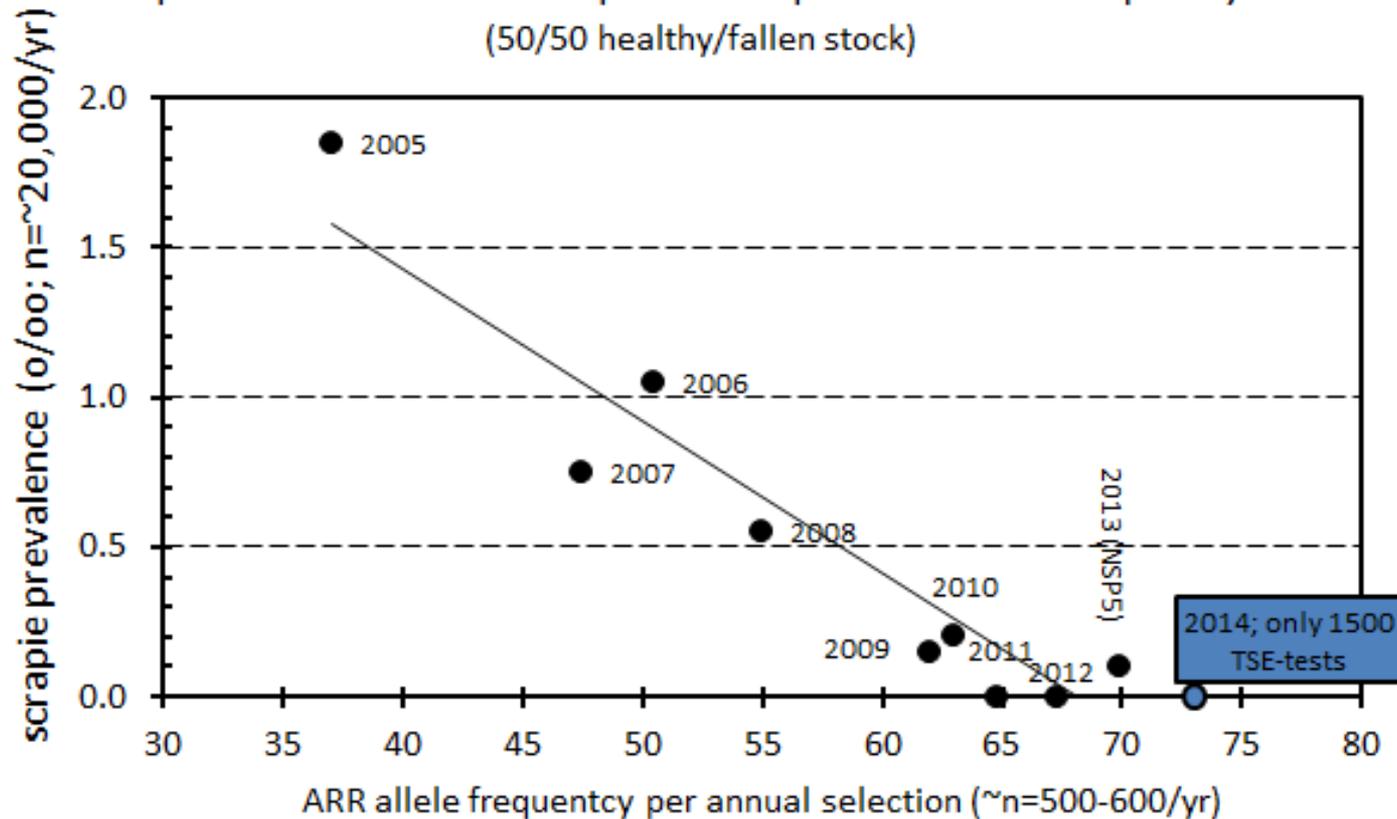
see Melchior et al., 2010, 2011



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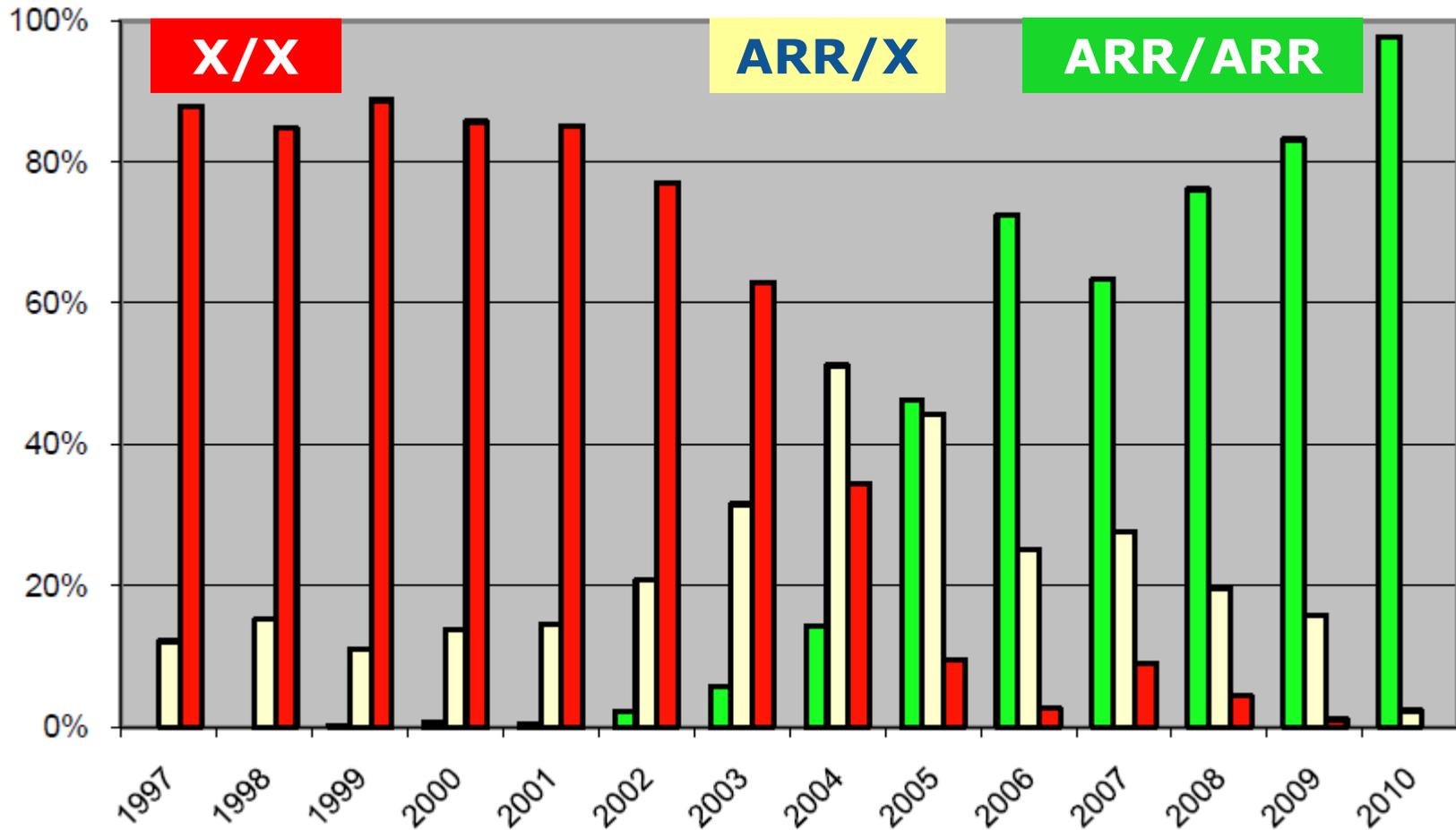
classical scrapie: results eradication program Netherlands

prevalence classical scrapie in sheep & ARR allele frequency
(50/50 healthy/fallen stock)



genotype frequency in a small breed (n=9000) i.e. Fries-Zeeuws milking sheep (1997-2010)

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6. EFSA issues on breeding towards resistance

how to breed

1. phenotyping step: **identification**, recording traits, pedigree knowledge, PrP genotype
2. selection step: use reproducers that comply to step 1
3. dissemination step: resistant reproducers to non-participating flocks (if steps 1 and 2 were only applied to part of population)

basic reproduction number R_0

- R_0 : $R_0 < 1$, then scrapie fading out at minimum level ARR allele frequency
- the R_0 is dependent on many variables and therefore region dependent
- estimated ARR frequency for $R_0 < 1$ in different countries: 53% GBR, 70% NLD, 100% CYP. Influenced by many aspects (see also next slide).
 - In GBR – started with a lower VRQ level and contact rates than NLD at similar scrapie prevalence;
 - CYP contact rates are very high between flocks

fading out variable aspects

1. *lambing hygiene*
2. *contact rates*
3. *overall ARR frequency*
4. *frequency of other alleles (i.c. VRQ allele frequency)*
5. *susceptibility pattern across non-ARR alleles*

(TSE strains differ in infectivity towards different PrP alleles – ARQ, VRQ, AHQ, ARH, ARR)

EFSA opinion information

alternative classification system (instead of NSP): number of years of use of homozygous ARR/ARR rams

duration breeding towards resistance

*to reach the minimum ARR level:
continuing for 4-5 years genotyping
activity, use homozygous rams*

7. atypical/Nor98 scrapie in sheep and goats

atypical scrapie can be considered as non-spreading form of an infectious prion disease

sheep: atypical (AS) and classical (CS) scrapie and different alleles

(Fediaevski et al., 2008): PrP allele carriers
among 1258 AS and CS cases detected
between 2002 and 2006 in 18 EU countries.

Allele	Healthy slaughter		Fallen stock	
	AS	CS	AS	CS
ARR	132 (32.3)	48 (9.5)	65 (28.4)	19 (2.3)
ARQ	170 (41.6)	220 (43.7)	98 (42.8)	456 (56.3)
VRQ	3 (0.7)	196 (39.0)	2 (0.9)	285 (35.2)
ARH	5 (1.2)	24 (4.8)	6 (2.6)	38 (4.7)
AHQ	99 (24.2)	15 (3.0)	58 (25.3)	12 (1.5)

atypical scrapie in sheep

most susceptible alleles for atypical scrapie:

ARQ with 141F polymorphism > ARR & AHQ > ARH & ARQ

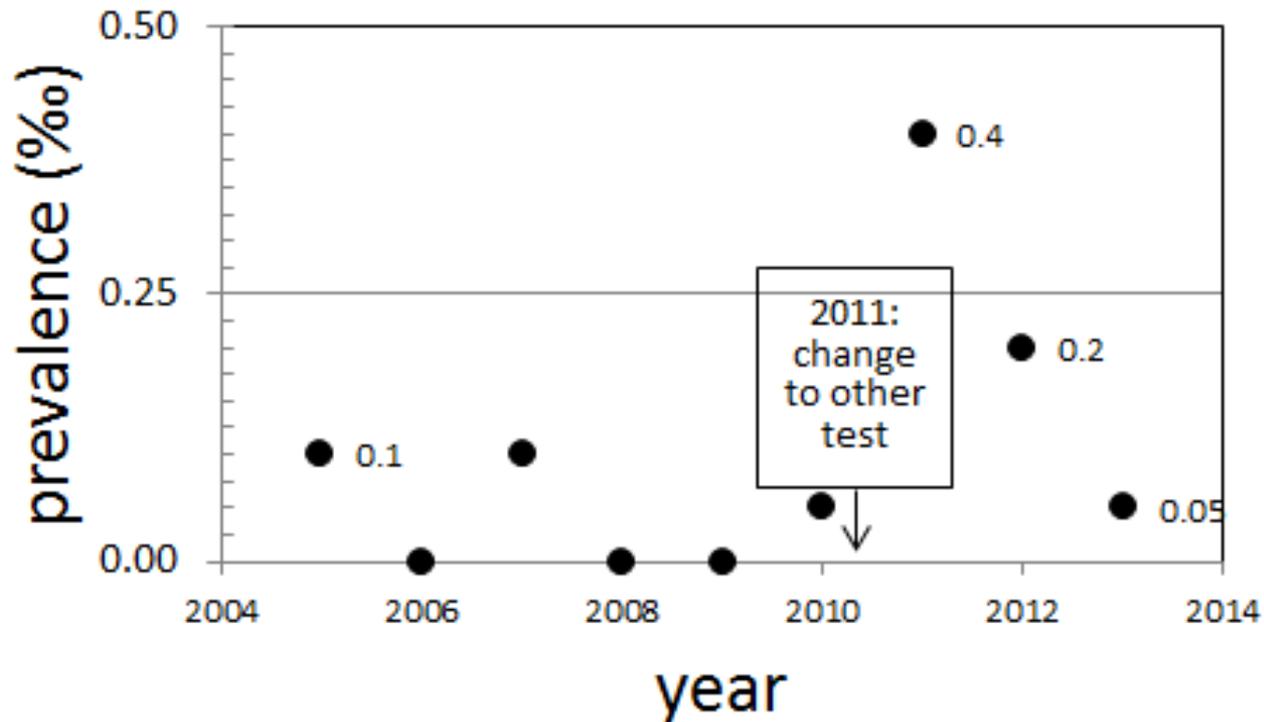
VRQ sheep appear not susceptible for developing atypical scrapie

atypical scrapie is not transmitting in the field; is a sporadic disease with potentially a spontaneous origin

See papers of Fediaevski 2008 and 2010

atypical scrapie prevalence Netherlands

Nor98/atypical scrapie cases

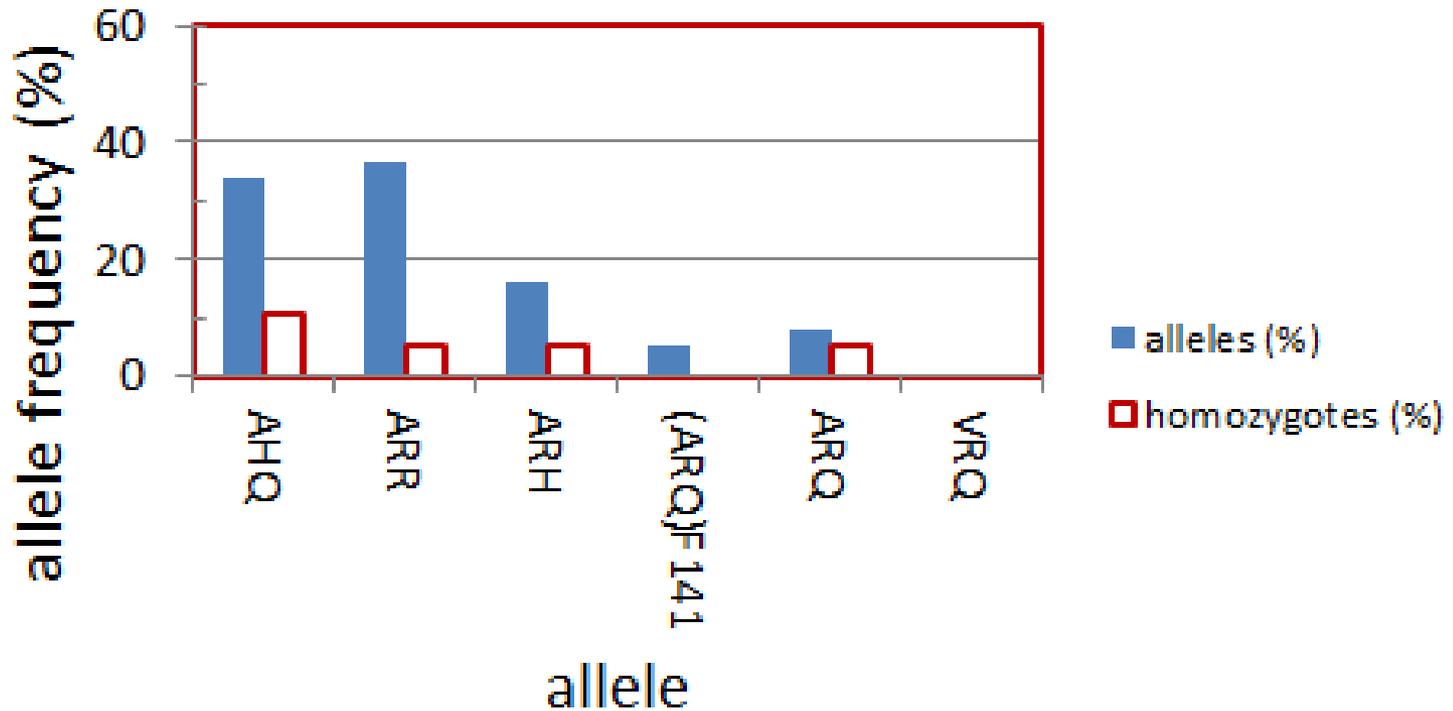




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atypical scrapie prevalence Netherlands

atypical scrapie cases in Netherlands
allele frequencies (2005-2013; n=19)



8. goat

breeding options against classical scrapie and BSE (but no formal actions)

The EFSA opinion indicates that in goats 222K as an excellent resistance allele for breeding as is the 171R (ARR in usual terms) allele. Based on sufficient scientific data.

The frequency of 222K is between 0-17%, depending on the breed and region.

The 146S and 146D allele is being applied in Cyprus with success, but no 222K is present there.

There is as yet no legislation for this genetics-based selective culling in goats.

9. references: legislation/opinions/research

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- **EFSA Journal 2014;12(7):3781** - Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats
- **Nodelijk et al. 2011.** Veterinary Research 2011, 42:5. Breeding with resistant rams leads to rapid control of classical scrapie in affected sheep flocks.
- **Melchior et al. 2010.** BMC Veterinary Research 2010, 6:24. Eradication of scrapie with selective breeding: are we nearly there?
- **Melchior et al. 2011.** Tijdschr Diergeneeskd 2011: 84-93. Acht jaar actieve scrapiesurveillance in Nederland: het effect van het fokprogramma op de prevalentie van scrapie bij het schaap (2002-2010). Dutch with English summary. Active surveillance for scrapie in the Netherlands: effect of a breeding programme on the prevalence of scrapie in sheep (2002-2010).
- **Fediaevsky et al. 2008.** BMC Veterinary Research 2008, 4:19. A descriptive study of the prevalence of atypical and classical scrapie in sheep in 20 European countries.
- **Fediaevsky et al. 2008.** BMC Veterinary Research 2010, 6:9. The prevalence of atypical scrapie in sheep from positive flocks is not higher than in the general sheep population in 11 European countries.
- **Cassard et al., 2014.** Nature Communications. DOI:10.1038/ncomms6821. Evidence for zoonotic potential of ovine scrapie prions.
- **Simmons et al., 2011.** DOI: 10.3201/eid1705.101654. Emerging Inf. Dis. 17:848- 854. Experimental oral transmission of atypical Scrapie to sheep.
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- **The TSE Road map 2.** A Strategy paper on Transmissible Spongiform Encephalopathies for 2010-2015. European Commission. Brussels, 16.7.2010. COM(2010)384 final



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