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

J.G. zn & A. Cuyp, an° ~1630

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

Mead et al., N Engl J Med. 2009. 361;21
genetic selection in humans to kuru resistance: new 127V variant

Mead et al., N Engl J Med 2009. 361;21
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
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
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

- VRQ / VRQ
- VRQ / ARQ
- ARQ / ARQ (Highly susceptible)
- VRQ / ...
- ARQ / ...
- ARH / ...
- AHQ / ...
- ARR / ARR (Resistent)
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

<table>
<thead>
<tr>
<th>TSE type</th>
<th>character</th>
<th>sheep</th>
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<tbody>
<tr>
<td>classical scrapie</td>
<td>world wide; transmitting; <strong>lymphotropic</strong>; pruritis; CNS &gt; brainstem; triple PrP\text{\textsubscript{res}} bands</td>
<td>+</td>
</tr>
<tr>
<td>Nor98/atypical scrapie</td>
<td>world wide; sometimes clinical signs; not contagious; <strong>not lymphotropic</strong>; 7 kDa PrP\text{\textsubscript{res}} band &amp; multimers; cerebellum</td>
<td>+*</td>
</tr>
<tr>
<td>CH1641 scrapie</td>
<td>world wide; rare (?); naturally transmitting (?); ic signs: head tilting &amp; tremors, ataxia; <strong>not lymphotropic</strong>?; CNS: brainstem; triple PrP\text{\textsubscript{res}} bands</td>
<td>+</td>
</tr>
<tr>
<td>Italian scrapie</td>
<td>Italy (France?, UK?); transmitting; <strong>lymphotropic</strong>; pruritis; CNS &gt; brainstem; triple PrP\text{\textsubscript{res}} bands; possible source contaminated vaccine</td>
<td>+</td>
</tr>
<tr>
<td>BSE</td>
<td>extremely rare; <strong>lymphotropic</strong>; signs as in scrapie; triple PrP\text{\textsubscript{res}} bands; brainstem</td>
<td>-***</td>
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* in sheep: occurring in 154H, 141F, 171R

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

*** 2 cases; could be due to BSE epidemic
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<th>susceptible -&gt; resistant</th>
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<tr>
<td>cl. scrapie</td>
<td>136V wt (ARQ) 154H 171R</td>
</tr>
<tr>
<td>cl. scrapie (Italian)</td>
<td>wt 136V 154H 171R</td>
</tr>
<tr>
<td>scrapie (CH1641, exptl)</td>
<td>154H? wt 136V</td>
</tr>
<tr>
<td>atypical/Nor98 scrapie</td>
<td>154H 171R, wt</td>
</tr>
<tr>
<td>BSE (exptl)</td>
<td>wt 136V 171R</td>
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<tr>
<td>cl. scrapie (France)</td>
<td>wt (ARQ) 154H, 142M, 211Q 222K</td>
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<td>154H wt ?</td>
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<tr>
<td>BSE (exptl, oral)</td>
<td>wt 211Q 142M, 222K</td>
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Conclusion on susceptibilities/resistance to TSE in small ruminants

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

[EFSA J 2014; 12(7) 3781- page 2]
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
5. sheep results after applying EU rules: country dependent
Scrapie and ARR trends EU member states
[EFSA J 2014; 12(7) 3781 etc]
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
results of breeding to resistance:
Netherlands
Activities in 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
classical scrapie: results eradication program Netherlands

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

Scrapie prevalence (o/o; n~20,000/yr)

ARR allele frequency per annual selection (~n=500-600/yr)


2014; only 1500 TSE-tests
genotype frequency in a small breed (n=9000) i.e. Fries-Zeeuws milking sheep (1997-2010)
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

[EFSA J 2014; 12(7) 3781 – page 62]
fading out variable aspects

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

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

![Graph showing the prevalence of Nor98/atypical scrapie cases from 2004 to 2014. The graph indicates a change in testing in 2011, with a lower prevalence after that year.](image-url)
atypical scrapie prevalence Netherlands
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

- **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?
- **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.
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