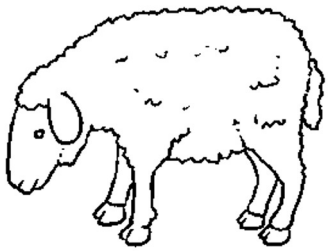
Genetic breeding program

Joint Workshop of the EURLs for TSE and AP Rome, Italy – May 13-15, 2024

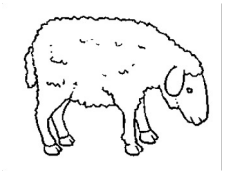


Strategies for breeding against scrapie based on the selection of genetically resistant breeding rams

Gabriele Vaccari

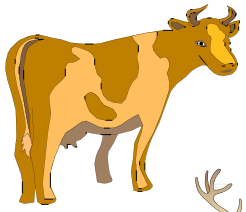
EURL-TSE
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin
Istituto Superiore di Sanità - Rome

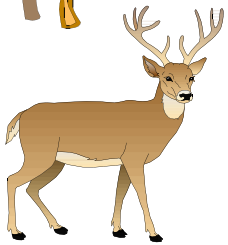
Animals Transmissible Spongiform Encephalopathies



Scrapie (Sheep and Goats)

BSE – Bovine Spongiform Encephalopathy





Chronic wasting disease

Scrapie - Introduction

Written documentation on scrapie dates back to 18th Century

Reported world-wide, endemic in many EU and non-EU countries

Common features of TSEs render scrapie elusive in regard to eradication and control (long, silent preclinical period; lack of ante-mortem tests; no vaccines)

The emergence of BSE spurred efforts to eradicate scrapie (zoonotic potential of TSEs, BSE in small ruminants)

Scrapie

- Neurodegenerative prion disease of sheep and goats with fatal outcome and long incubation times

- Known since the 18th century, widespread throughout the world (not in Australia and New Zealand) and endemic in numerous countries

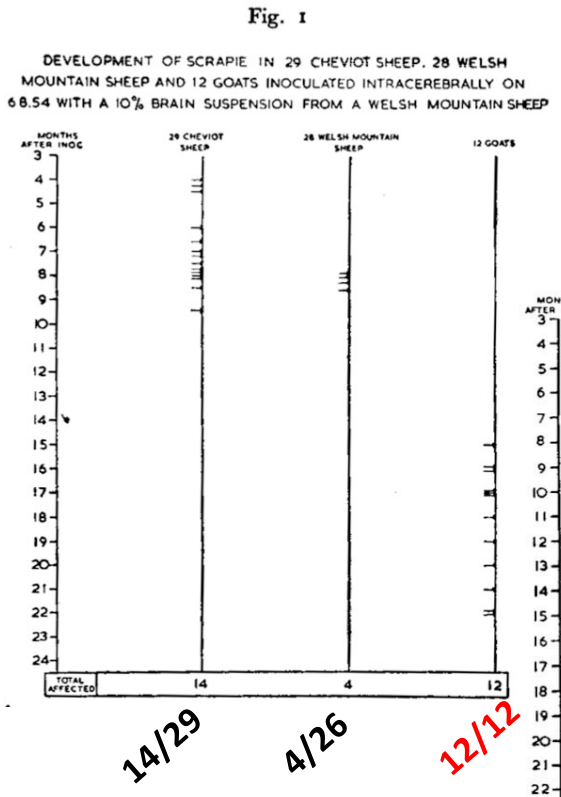
- Characterized by two shapes Classic Scrapie Nor98 (or Atypical Scrapie)

- It has infectious or sporadic characteristics Susceptibility linked to PrP genotype.

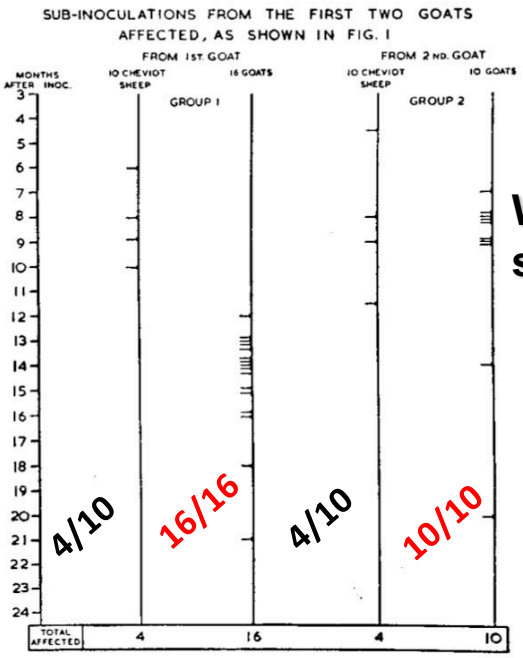
- Control interventions based on the traditional approach to animal infectious diseases are ineffective in eradicating scrapie:

- - long incubation period
- - silent preclinical period
- - absence of a host immune response
- - absence of ante-mortem diagnostic test

Genetic scrapie resistance



During the first experimental transmission studies some sheep was resistante to the development of the disease

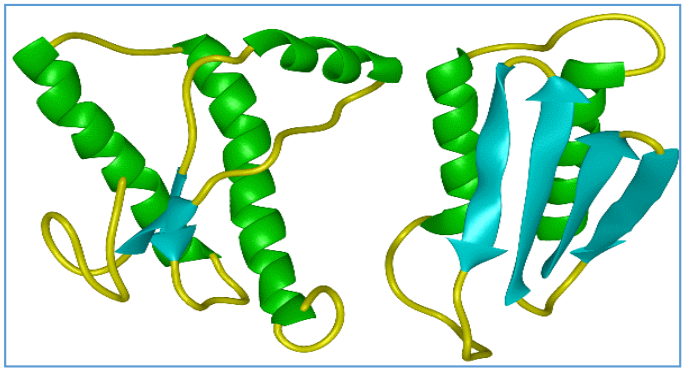


While goats were all susceptible.

Pattison et al., 1959

PrPC -> PrPSc conversion

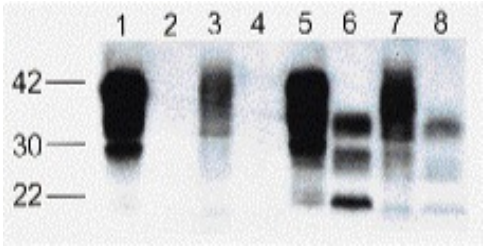
PrP^{C}



PrP^{Sc}

PrP^{C} PrP^{Sc}

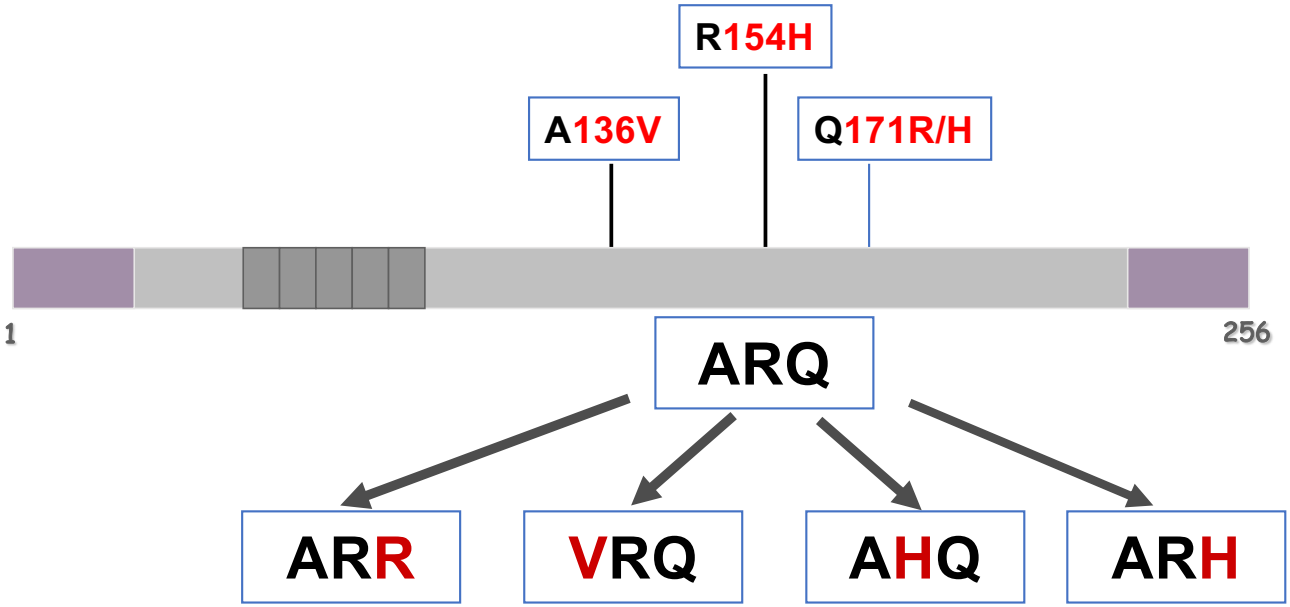
Proteinase K - + - + - + - +



Polymorphisms of the sheep PrP gene

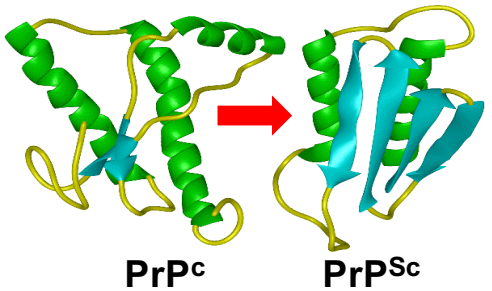
The gene that controls sheep susceptibility has been identified as that coding for the prion protein

5 main alleles has been identified



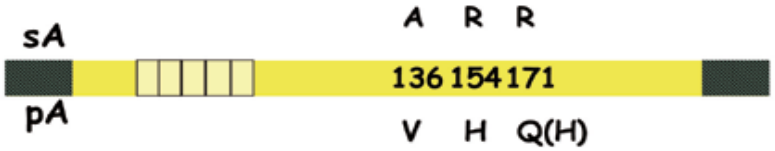
Host scrapie susceptibility

- Key event is the transformation of a protein encoded by the host into a pathological isoform that accumulates in the CNS as insoluble protein deposits



- The clinical manifestation is the result of the interaction between the infecting agent and the host's PrP coding gene

- Host susceptibility is influenced by PrP polymorphisms, mainly at codons 136 (A/V), 154 (R/H) and 171 (R/Q/H)



ARR
ARH
AHQ
ARQ
VRQ

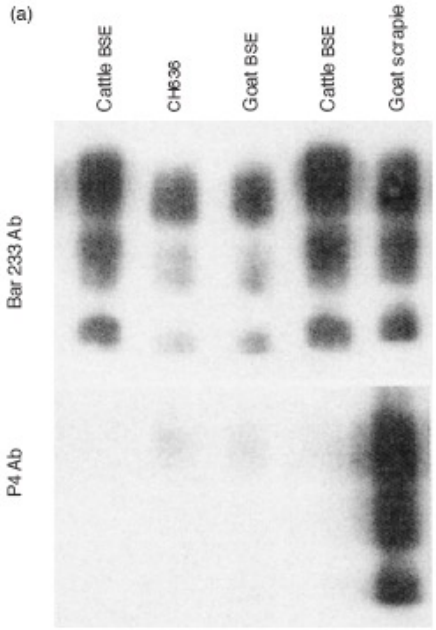
susceptibility

- The role of some additional polymorphisms has been proven, at codons 112 (M/T), 137 (M/T), 141 (L/F), 176 (N/K)

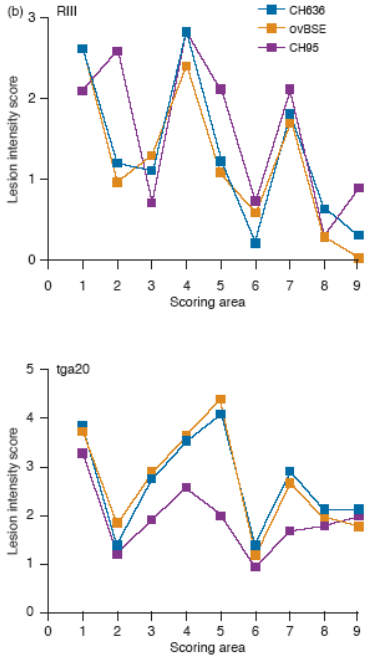
BSE agent signatures in a goat

The **Veterinary Record**, April 16, 2005

Biochemical strain typing



Bioassay



correspondence

Theoretical models of sheep BSE reveal possibilities

But we must remember that these theories are based onspeculation, not on fact.

NATURE | VOL 415 | 10 JANUARY 2002 | www.nature.com

news feature

A wolf in sheep's clothing

Is BSE lurking in sheep, but masked by scrapie? Reliable and fast tests that can tell the diseases apart are urgently needed, says Declan Butler.

P. DEAN/AGRIPICTURE

NATURE | VOL 414 | 6 DECEMBER 2001 | www.nature.com

TSE are not genetic but infectious disease.
However animals are susceptible depending on their genotype

EU Member States have implemented sheep breeding programmes to reduce the prevalence of sheep with TSE susceptible prion genotypes

COMMISSION DECISION

of 13 February 2003

laying down minimum requirements for the establishment of breeding programmes for resistance to transmissible spongiform encephalopathies in sheep

(notified under document number C(2003) 498)

(Text with EEA relevance)

(2003/100/EC)

Individual genotypes grouped according to the classification of the English National Scrapie Plan and adopted by the EU

NSP1	**ARR/ARR**	**Genetically most resistant to scrapie**
NSP2	**ARR/ARQ, ARR/ARH, ARR/ AHQ, VRR/ARQ**	**Genetically resistant to scrapie**
NSP3 (ARQ/ARQ)	**ARQ/ARQ**	**Genetically little resistance to scrapie (ARQ/ARQ may be scientifically reviewed)**
NSP3 (others)	**AHQ/AHQ, ARH/ARH, ARH/ ARQ, AHQ/ARH, AHQ/ARQ**	
NSP4	**ARR/VRQ**	**Genetically susceptible to scrapie**
NSP5	**ARQ/VRQ, ARH/VRQ, AHQ/VRQ, VRQ/VRQ**	**Genetically highly susceptible to scrapie**

Most of the national Scrapie Plans are based on the English National Scrapie Plan

Genotyping of male rams and replacements with the aim of creating a breeding stock with greater resistance which subsequently makes it possible to guarantee the spread of the ARR alleles to the entire population.

The scheme is connected to an individual genotype certification system which is fully included in the individual documentation ("passport") that accompanies the animals throughout their production career

CS sheep (2002-2012)

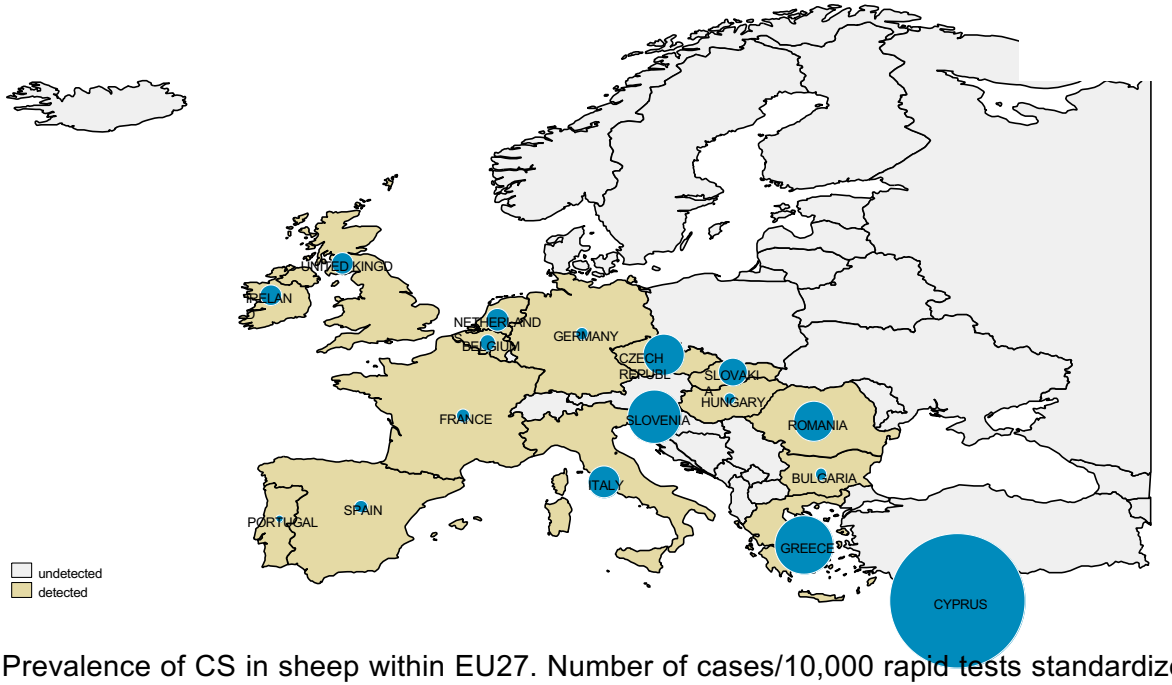
efsa
European Food Safety Authority
EFSA Journal 2014;12(7):3781

SCIENTIFIC OPINION

Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

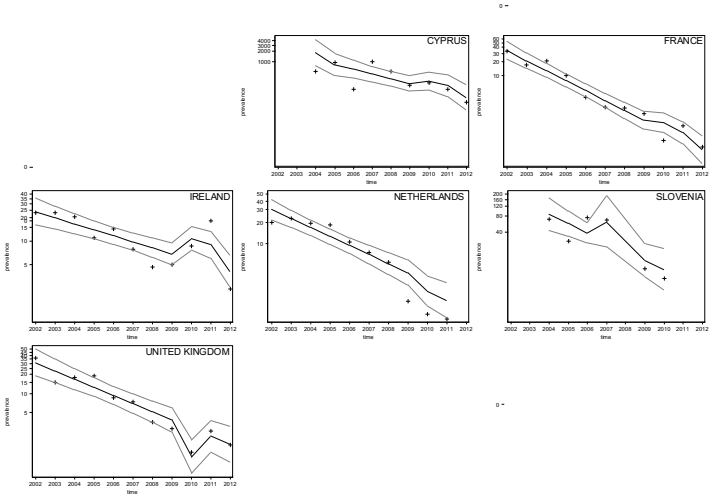


Prevalence of CS in sheep within EU27. Number of cases/10,000 rapid tests standardized by stream, i.e.SHC vs. NSHC over the period 2002-2012.

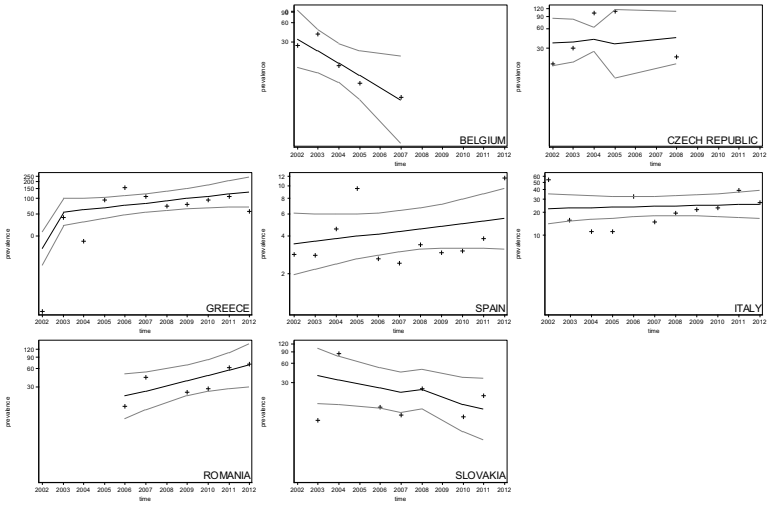
In these 17 countries some 4.7 million sheep were tested leading to the detection of 4 132 CS cases, equal to an overall prevalence of 8.7 cases per 10 000 rapid tests.

At the EU27 level over the period 2002-2012, CS in sheep showed annual stream-adjusted prevalence rates ranging between 5 and 20 cases per 10 000 rapid tests.

National CS trends in sheep



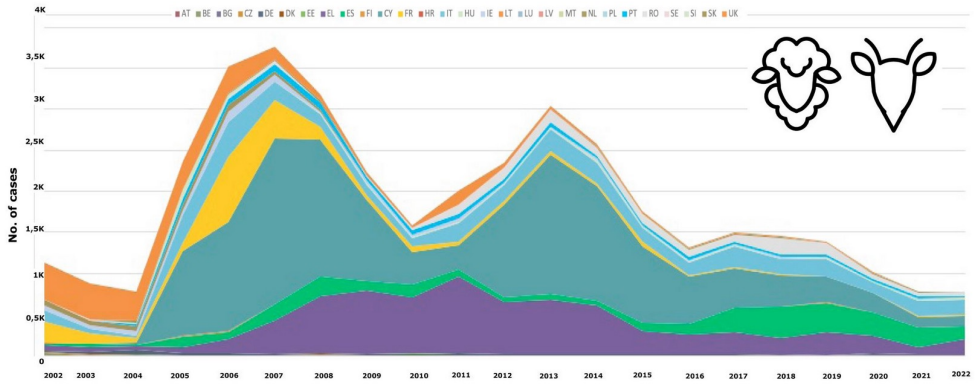
Temporal trend of CS in sheep **in countries where the trend was not statistically different from a flat one**. Crosses (+) indicate the annual stream-adjusted prevalence (cases per 10,000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines). The adjustment on stream was obtained by fitting a negative binomial model (internal reference).



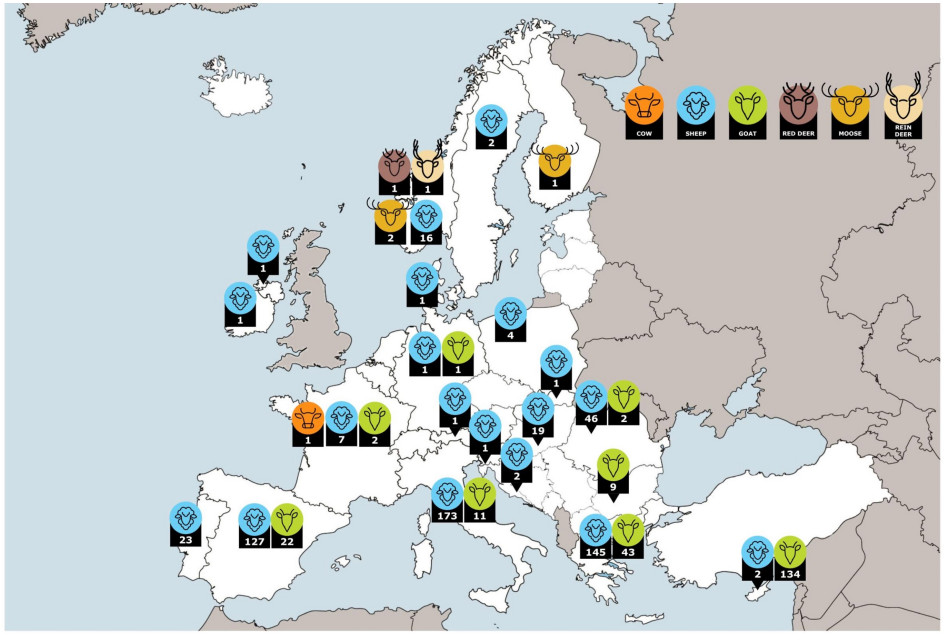
Temporal trend of CS in sheep in **countries where a statistically significant decreasing** trend was confirmed. Crosses (+) indicate the annual stream-adjusted prevalence (cases per 10,000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines). The adjustment on stream was obtained by fitting a negative binomial model (internal reference).

Scrapie Surveillance in EU

Scrapie outbreaks were reported all over Europe (2002-2022)



Scrapie cases (classical and atipycal) in Europe by country (2002-2022).
Source: EFSA (in the chart areas of single countries are stacked)



Animal TSEs cases (classical and atypical) reported in Europe in 2022. Source: EFSA

Polymorphism Q222K has been associated with scrapie resistance

Lysine at codon 222 has been associated with scrapie resistace in two italian case-controls studies.

Several heterozigotes were present but none was scrapie affected.

Vaccari et al., 2006

Journal of General Virology (2006), **87**, 1395–1402 DOI 10.1099/vir.0.81485-0

Identification of an allelic variant of the goat PrP gene associated with resistance to scrapie

Gabriele Vaccari,[1] Michele A. Di Bari,[1] Luisella Morelli,[1] Romolo Nonno,[1] Barbara Chiappini,[1] Giovanni Antonucci,[1] Stefano Marcon,[1] Elena Esposito,[1] Paola Fazzi,[1] Nadia Palazzini,[1] Pasquale Troiano,[2] Antonio Petrella,[2] Giovanni Di Guardo[3] and Umberto Agrimi[1]

Acutis et al., 2006

Journal of General Virology (2006), **87**, 1029–1033 DOI 10.1099/vir.0.81440-0

Short Communication

Identification of prion protein gene polymorphisms in goats from Italian scrapie outbreaks

P. L. Acutis,[1] A. Bossers,[2] J. Priem,[2] M. V. Riina,[1] S. Peletto,[1] M. Mazza,[1] C. Casalone,[1] G. Forloni,[3] G. Ru[1] and M. Caramelli[1]

Polymorphism Q222K has been associated with scrapie resistance

Barillet et al., 2009

Journal of General Virology (2009), 90, 769–776 DOI 10.1099/vir.0.006114-0

Identification of seven haplotypes of the caprine *PrP* gene at codons 127, 142, 154, 211, 222 and 240 in French Alpine and Saanen breeds and their association with classical scrapie

F. Barillet,[1] D. Mariat,[2] Y. Amigues,[3] R. Faugeras,[3] H. Caillat,[1] K. Moazami-Goudarzi,[2] R. Rupp,[1] J. M. Babilliot,[3] C. Lacroux,[4] S. Lugan,[4] F. Schelcher,[4] C. Chartier,[5] F. Corbière,[4] O. Andréoletti[4] and C. Perrin-Chauvineau[5]

In other studies QK 222 heterozigotes were significantly protected from scrapie, however few animals has been found to be scrapie affected.

Fragkiadaki et al., 2011

Fragkiadaki *et al. Veterinary Research* 2011, **42**:104
http://www.veterinaryresearch.org/content/42/1/104

VETERINARY RESEARCH

SHORT REPORT Open Access

PRNP genetic variability and molecular typing of natural goat scrapie isolates in a high number of infected flocks

Eirini G Fragkiadaki[1*], Gabriele Vaccari[2], Loukia V Ekateriniadou[1], Umberto Agrimi[2], Nektarios D Giadinis[3], Barbara Chiappini[2], Elena Esposito[2], Michela Conte[2] and Romolo Nonno[2]

Polymorphism Q222K has been associated with scrapie resistance

Acutis et al., 2012

Acutis *et al. Veterinary Research* 2012, **43**:8
http://www.veterinaryresearch.org/content/43/1/8

RESEARCH Open Access

Resistance to classical scrapie in experimentally challenged goats carrying mutation K222 of the prion protein gene

Pier Luigi Acutis[1*], Francesca Martucci[1], Antonio D'Angelo[2], Simone Peletto[1], Silvia Colussi[1], Cristiana Maurella[1], Chiara Porcario[1], Barbara Iulini[1], Maria Mazza[1], Luana Dell'Atti[1], Fabio Zuccon[1], Cristiano Corona[1], Nicola Martinelli[3], Cristina Casalone[1], Maria Caramelli[1] and Guerino Lombardi[3]

Incubation time post IC infection

Genotype	Incubation time dpi	Attack rate
QQ 222	569 ± 97	5/5
QK 222	> 1643	0/5

Polymorphism Q222K has been associated with scrapie resistance

JVI Journals.ASM.org

Lacroux et al., et al., 2014

Genetic Resistance to Scrapie Infection in Experimentally Challenged Goats

Caroline Lacroux,[a] Cécile Perrin-Chauvineau,[b] Fabien Corbière,[a] Naima Aron,[a] Patricia Aguilar-Calvo,[c] Juan Maria Torres,[c] Pierrette Costes,[a] Isabelle Brémaud,[b] Séverine Lugan,[a] François Schelcher,[a] Francis Barillet,[d] Olivier Andréoletti[a]

INRA, UMR 1225, Interactions Hôtes Agents Pathogènes, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France[a]; AFSSA-Niort, Laboratoire d'Études et de Recherches Caprines, Niort, France[b]; CISA-INIA, Madrid, Spain[c]; INRA, UR 631, Station d'Amélioration Génétique des Animaux, Castanet-Tolosan, France[d]

Incubation time post oral infection

No QK 222 animals was found to be scrapie affected

Genotype	No. of scrapie-affected animals/total no. of animals	Scrapie incubation period (dpi) (mean ± SD)
IRRQS/IRRQS (wild type)	9/9	1,141 ± 93
$M_{142}RQP_{240}$/IRRQS	4/4	1,490 ± 126
$IH_{154}RQS$/IRRQS	0/6	
$IRQ_{211}QS$/IRRQS	0/5	
$IRRK_{222}S$/IRRQS	0/5	

[a] Animals that were still alive were at <2,500 days postinoculation at the time of writing.
[b] Tonsils, prescapular lymph nodes, ileal/jejunal Peyer's patches, and mesenteric lymph nodes.

Polymorphism Q222K has been associated with scrapie resistance

JVI Journals.ASM.org

Lacroux et al., et al., 2014

Genetic Resistance to Scrapie Infection in Experimentally Challenged Goats

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INRA, UMR 1225, Interactions Hôtes Agents Pathogènes, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France[a]; AFSSA-Niort, Laboratoire d'Études et de Recherches Caprines, Niort, France[b]; CISA-INIA, Madrid, Spain[c]; INRA, UR 631, Station d'Amélioration Génétique des Animaux, Castanet-Tolosan, France[d]

Incubation time post IC infection

QK and KK 222 animals showed low attach rate only after IC infection

Genotype	No. of clinically TSE-affected animals/total no. of animals	Scrapie incubation period (dpi) (mean ± SD)
$I_{142}R_{154}R_{211}Q_{222}$/IRRQ (wild type)	5/5	486 ± 21
M_{142}RQ/IRRQ	5/5	788 ± 99
IH_{154}RQ/IRRQ	5/5	624 ± 148
IRQ_{211}Q/IRRQ	5/5	1,291 ± 325
IRQ_{211}Q/IRRQ$_{211}$Q	10/10	770 ± 139
IRRK$_{222}$/IRRQ	2/5	1,900, 2,174
IRRK$_{222}$/IRRK$_{222}$	1/5	2,101

[a] Groups of five goats were intracerebrally challenged in the temporal cortex with the same classical scrap
>2,400 days postinoculation at the time of writing.
[b] Tonsils, prescapular lymph nodes, ileal/jejunal Peyer's patches, and mesenteric lymph nodes.
[c] The two PrP^{Sc}-positive animals were clinically affected.

Polymorphism N146S/D has been associated with scrapie resistance

Papasavva-Stylianou et al., 2007

Short Communication

Novel polymorphisms at codons 146 and 151 in the prion protein gene of Cyprus goats, and their association with natural scrapie

Penelope Papasavva-Stylianou [a,*], Marina Kleanthous [b], Pavlos Toumazos [c], Petroula Mavrikiou [d], Phedias Loucaides [e]

Papasavva-Stylianou et al., 2010

PrP gene polymorphisms in Cyprus goats and their association with resistance or susceptibility to natural scrapie

Penelope Papasavva-Stylianou [a,*], Otto Windl [b], Ginny Saunders [b], Petroula Mavrikiou [c], Pavlos Toumazos [d], Charalambos Kakoyiannis [e]

Serine or aspartic acid at codon 146 has been associated with scrapie resistance in case-control studies in Cyprus althougt some heterozigotes aniamls has been found scrapie affected

In the control group several NS and ND 146 heterozigotes aniamls but limited homozigous

In goats the K 222 allele, but also S 146 or D 146 can protect animals from scrapie

Breeding program in goats can be implemented

The frequency of resistant alleles is however low and or absent in some breed, so particular attention should be use to design the genetic selection


תודה
Dankie
Gracias
Спасибо
شكراً
Merci
Takk
Köszönjük
Terima kasih
Grazie
Dziękujemy
Dėkojame
Ďakujeme
Vielen Dank
Paldies
Kiitos
Täname teid
谢谢
Thank You
Tak
感謝您
Obrigado
Teşekkür Ederiz
감사합니다
Σας ευχαριστούμε
ขอบคุณ
Bedankt
Děkujeme vám
ありがとうございます
Tack