



ScIcE Project

- Classical Scrapie in Iceland, a model for prion diseases worldwide -

Project Lead: Christine Fast

**Vincent Beringue, Juan Carlos Espinosa, Fiona Houston, Gesine Luehken,
Romolo Nonno, John Spiropoulos, Stefania Thorgeirsdottir**

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Classical Scrapie = Nothing new left to explore?

Resistance breeding is already established and ARR genotypes are very successful in the eradication of classical scrapie

But what to do in case you do not have ARR in your sheep and you need / want to preserve them (endangered breeds, special traits etc.)

Classical scrapie is mostly shed with the placenta and horizontal infection is subsequently due to environmental contamination (stables, pastures)

Experimental scientific knowledge exist that scrapie prions reside on fomites, move around with dust even after decontamination of the stables

Single classical scrapie strains may have zoonotic potential and can be transmitted to humanized transgenic mice

It is critical to understand the exact mechanism of Prion Strain Evolution under natural conditions and the factors that determine the zoonotic potential of a Prion Strain





Scrapie in Iceland – Special Features of Scientific Interest

There is only one breed of sheep in Iceland

- ↪ genetically isolated from other breeds since its introduction by settlers from Scandinavia at about 870 to 950 AD
- ↪ partly living in regions with high natural infectious pressure

Scrapie was introduced in the mid-north of Iceland in 1878 by a ram from Denmark

- ↪ all Classical scrapie cases today dating back to the same outbreak

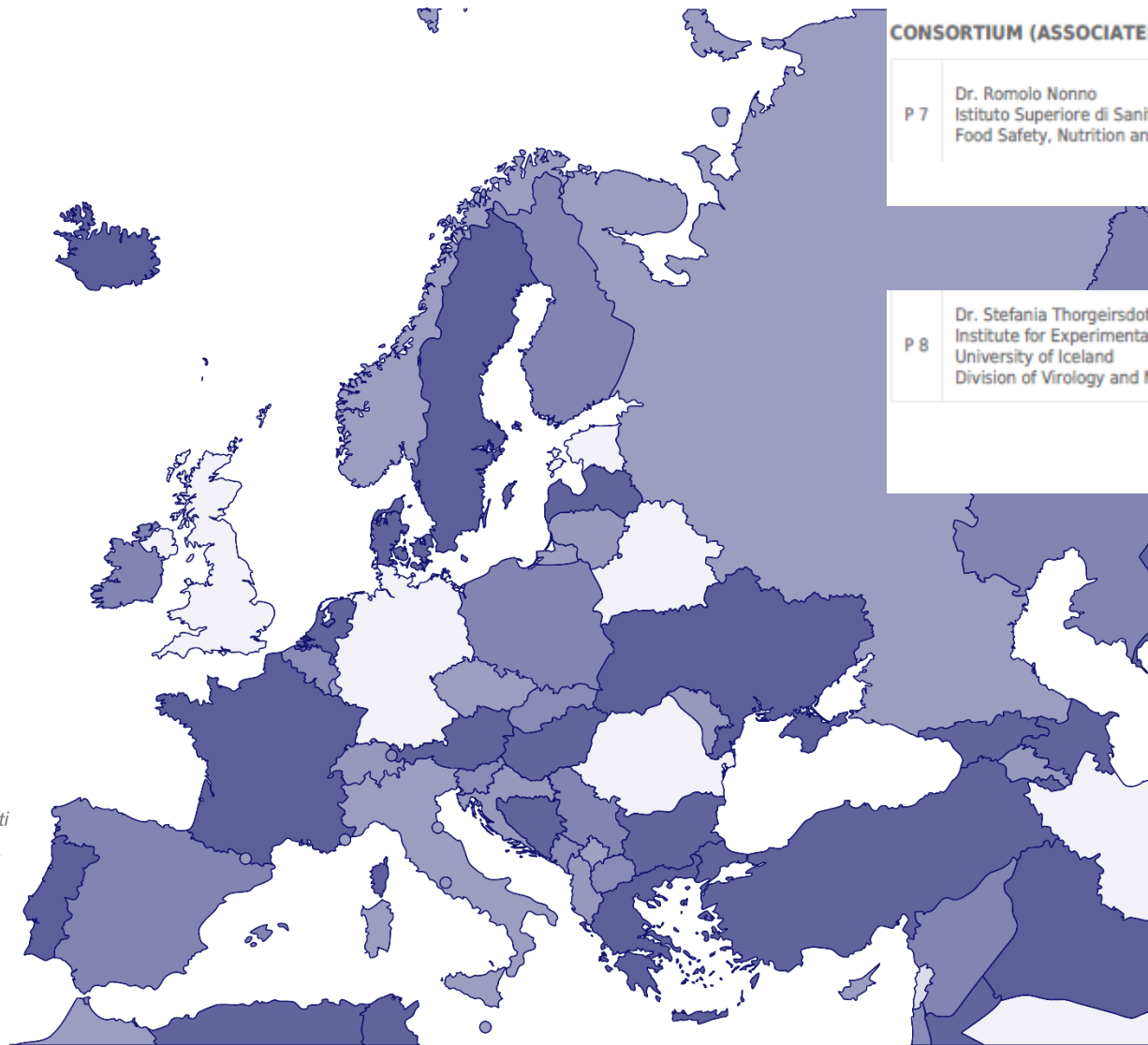
Culled sheep were often buried or burnt close to the stables or meadows.

- ↪ Reoccurrence of scrapie in affected farms indicated that contaminated places might exist



CONSORTIUM

P 1	Dr. Christine Fast Friedrich-Loeffler-Institut Institute of Novel and Emerging Infectious Diseases
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P 3	Prof. Dr. Gesine Luehken Justus-Liebig University of Giessen Institute of Animal Breeding and Genetics
P 4	Dr. Fiona Houston University of Edinburgh The Roslin Institute, R(D)SVS
P 5	Dr. Vincent BERINGUE INRAE <i>*Dr. Olivier Andreoletti</i> <i>*Dr. Jean Luc Villote</i>
P 6	Dr. Juan Carlos Espinosa Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria-Consejo Superior de Investigaciones Científicas (INIA-CSIC) Centro de Investigación en Sanidad Animal (CISA) <i>*Dr. Juan Maria Torres</i>



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P 8	Dr. Stefania Thorgeirsdottir Institute for Experimental Pathology at Keldur, University of Iceland Division of Virology and Molecular Biology <i>*Karólína Elísabetardóttir</i> <i>*Eyþór Einarsson</i> <i>*Dr. Vilhjálmur Svansson</i>



4 fully interactive workpackages:

WP1-3: define the key impacts on this multifactorial disease

WP1: Host Genetics

P3, P4, P5, P6, P7, P8

Survey of PrP Gene Variation

To determine the extent of *PRNP* genetic variability in Icelandic sheep

In vitro and in vivo modelling of protective effect of PRNP variants identified in Icelandic sheep

To predict the effect of certain polymorphisms on susceptibility / resistance to Classical scrapie

WP 2: Prion strain evolution

P1, P2, P4, P5, P6, P7, P8

Analysis of prion strain evolution in Iceland

To analyse the prion biodiversity and zoonotic potential of Icelandic CS strains over time, originating from a single scrapie case 150 years ago

WP 3: Environmental Factors

P2, P4, P8

Analysis of fomites for scrapie containing material

To determine how and where scrapie infectivity remains within the environment and how effective decontamination is in the field





4 fully interactive workpackages:

WP1-3: define the key impacts on this multifactorial disease

WP4: utilise all results for Epidemiological analysis and the development of an economic model

WP1: Host Genetics

P3, P4, P5, P6, P7, P8

Survey of PrP Gene Variation

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***In vitro* and *in vivo* modelling of protective effect of PRNP variants identified in Icelandic sheep**

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WP 4: Epidemiological analysis of scrapie in Iceland and development of economic models

P1, P8

To conduct epidemiological analysis of outbreaks to identify risk factors associated with prolonged exposure to CS and to develop an economic model for CS response



Scrapie in Iceland – Aims of the project

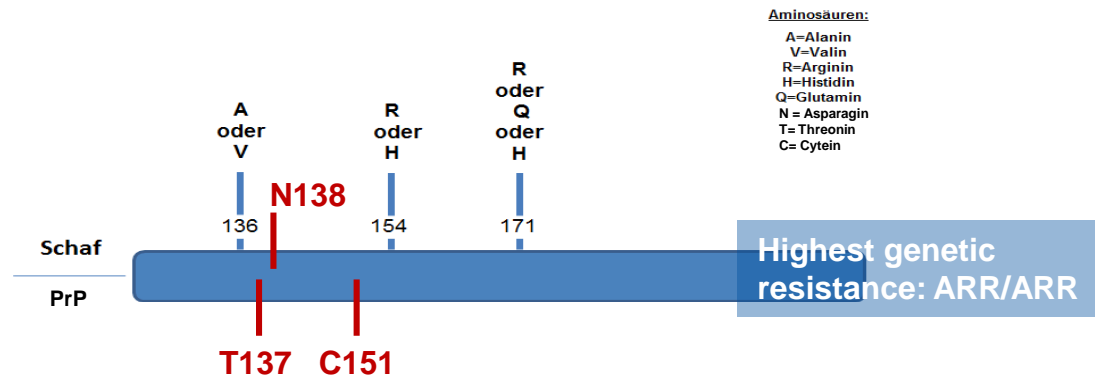
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Survey of PrP Gene Variation

To determine the extent of *PRNP* genetic variability in Icelandic sheep

Prof. Dr. Gesine Luehken, Germany



Variability of the Prion Protein Gene

Identification of additional polymorphism associated with scrapie resistance would

- offer more selective breeding options
 - maintain the genetic diversity
 - maintain specific production trait

One breed under moderate/high infectious pressure
Infected and non-infected sheep/flocks



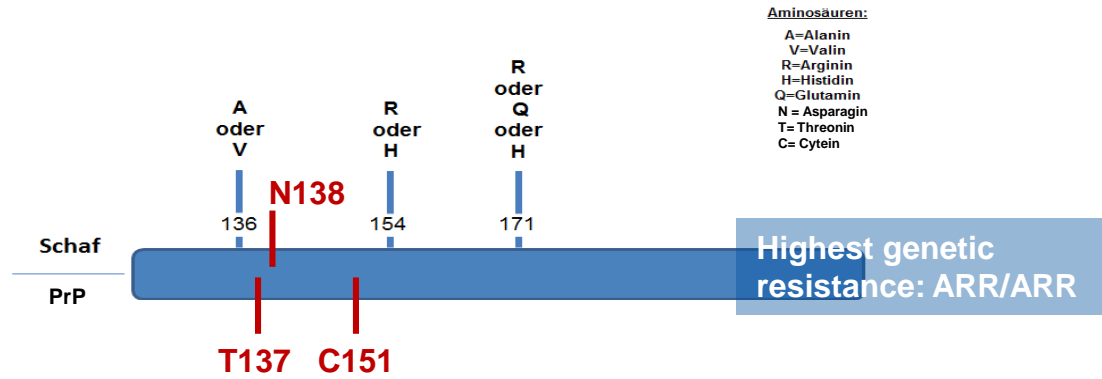
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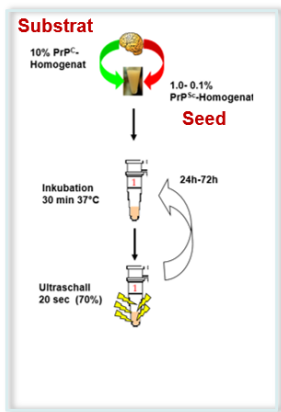
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- Survey of PrP Gene Variation**
To determine the extent of *PRNP* genetic variability in Icelandic sheep
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To predict the effect of certain polymorphisms on susceptibility / resistance to Classical scrapie

Dr. Vincent Beringue, France
Dr. Fiona Houston, UK



In vitro replication assays (PMCA, RT-QuIC)



Certain polymorphism/combination of polymorphism identified in Iceland



Challenged with different prion isolates from Iceland and Europe



Efficiency of resistance can be determined



Scrapie in Iceland – Aims of the project

WP1: Host Genetics

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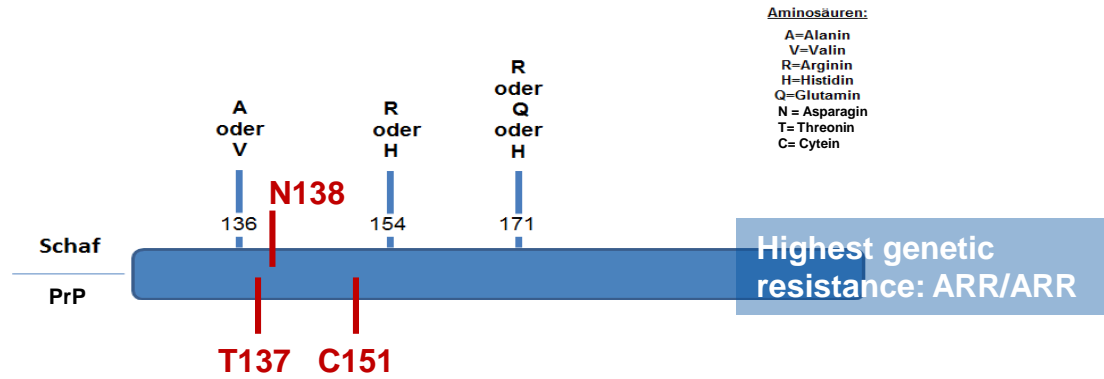
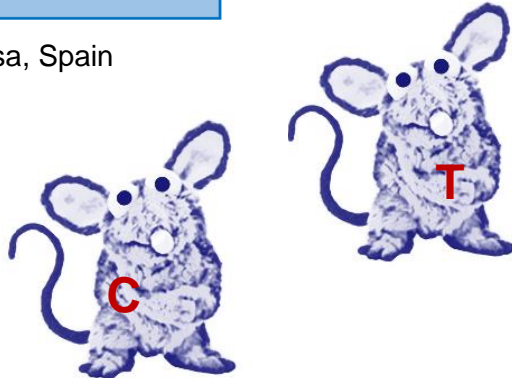
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Dr. Juan Carlos Espinosa, Spain

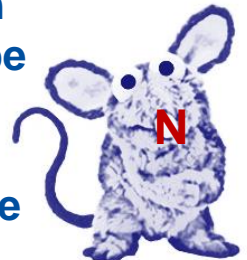


In vivo modelling

New transgenic mouse models with T137 polymorphism identified in Iceland

Challenged with different prion isolates from Iceland and Europe

Efficiency of resistance can be determined in living animals





Scrapie in Iceland – Aims of the project

WP 2: Prion strain evolution

P1, P2, P4 P5, P6, P7, P8

Analysis of prion strain evolution in Iceland

To analyse the prion biodiversity and zoonotic potential of Icelandic CS strains over time, originating from a single scrapie case 150 years ago

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P7: Romolo Nonno, Dr. Laura Pirisinu, Italy
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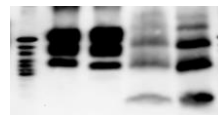


The impact of selective pressure i.e. environmental factors or host genetic is vital for prion strain evolution.

Analysis of Classical Scrapie Biodiversity in Iceland

time, environment, host

Isolates from different regions and years, from individual farms from different years, from different sheep genotypes



Biochemical Examination



In vitro examination (PMCA, RT-QuIC)



Transgenic mouse models, bank voles

Prion Strain characterization allows analysis how they evolve over time, and their zoonotic risk.



Scrapie in Iceland – Aims of the project

WP 3: Environmental Factors

P2, P4, P8

Analysis of fomites for scrapie containing material

To determine how and where scrapie infectivity remains within the environment and how effective decontamination is in the field

Dr. Kevin Gough, UK
Dr. Ben Maddison, UK

CI. Scrapie Experimental studies:

PrP^{Sc} are on farm fomites and dust
Effective decontamination of barns was followed by recontamination



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Field CWD:

PrP^{Sc} is present in environmental areas of frequent use

Identifying PrP^{Sc} under field condition in Iceland by PMCA and RT-QuIC

PrP^{Sc} may reside on fomites, move around with dust, contaminate water running-off from old burial sites



Defining reservoirs under field conditions is an important model for long-term prion infection dynamics worldwide



Scrapie in Iceland – Aims of the project

Dr. Jörn Gethmann, Germany

WP 4: Epidemiological analysis of scrapie in Iceland and development of economic models

P1, P8

To conduct epidemiological analysis of outbreaks to identify risk factors associated with prolonged exposure to CS and to develop an economic model for CS response

Known risk factors of Cl. scrapie:

- PrP^{Sc} infectivity cannot be easily inactivated
- Transmission from ewe to lamb, but horizontal transmission and environmental contamination are also possible
- The genotype of the host has a major impact on susceptibility
- Different Prion Strains might have altered dissemination dynamics

Different sources
of infectivity

Routes of
transmission

Relative
contribution
?

Spread/maintenance of disease in given
field populations

Development of flexible epidemiological and
economic models

Tools for scrapie
eradication
programs in Iceland

Risk assessment and
cost / benefit analysis
of future outbreaks



What`s in ...

...for Iceland?

New breeding strategies could help preserve unique Icelandic sheep breed

New insights in Prion strain evolution and environmental PrP^{Sc} distribution are useful knowledge for the eradication of Icelandic Cl. scrapie

Based on new models, control programs could be further developed and adapted to the Icelandic situation

... for Science?

Hypotheses about Prion strain evolution can be tested, how they evolve over time, the genetic and environmental factors involved

Insights into the role of environmental PrP^{Sc} in disease outbreak persistence.

Knowledge of multifactorial processes triggering the occurrence of zoonotic traits.

But also ...

The expected results can be considered as a blueprint for outbreaks of scrapie and other prion diseases occurring worldwide.

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John Spiropoulos



INRAE, France

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**Many Thanks for your
attention!**