Keldur
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Scrapie situation and recent findings from Iceland

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19th Annual Meeting of the EURL for TSE

Torino, Italy

Icelandic sheep – one breed (*Ovis brachyura borealis pall*)

summer

fall

winter

spring

Number of sheep in Iceland 1703-2020

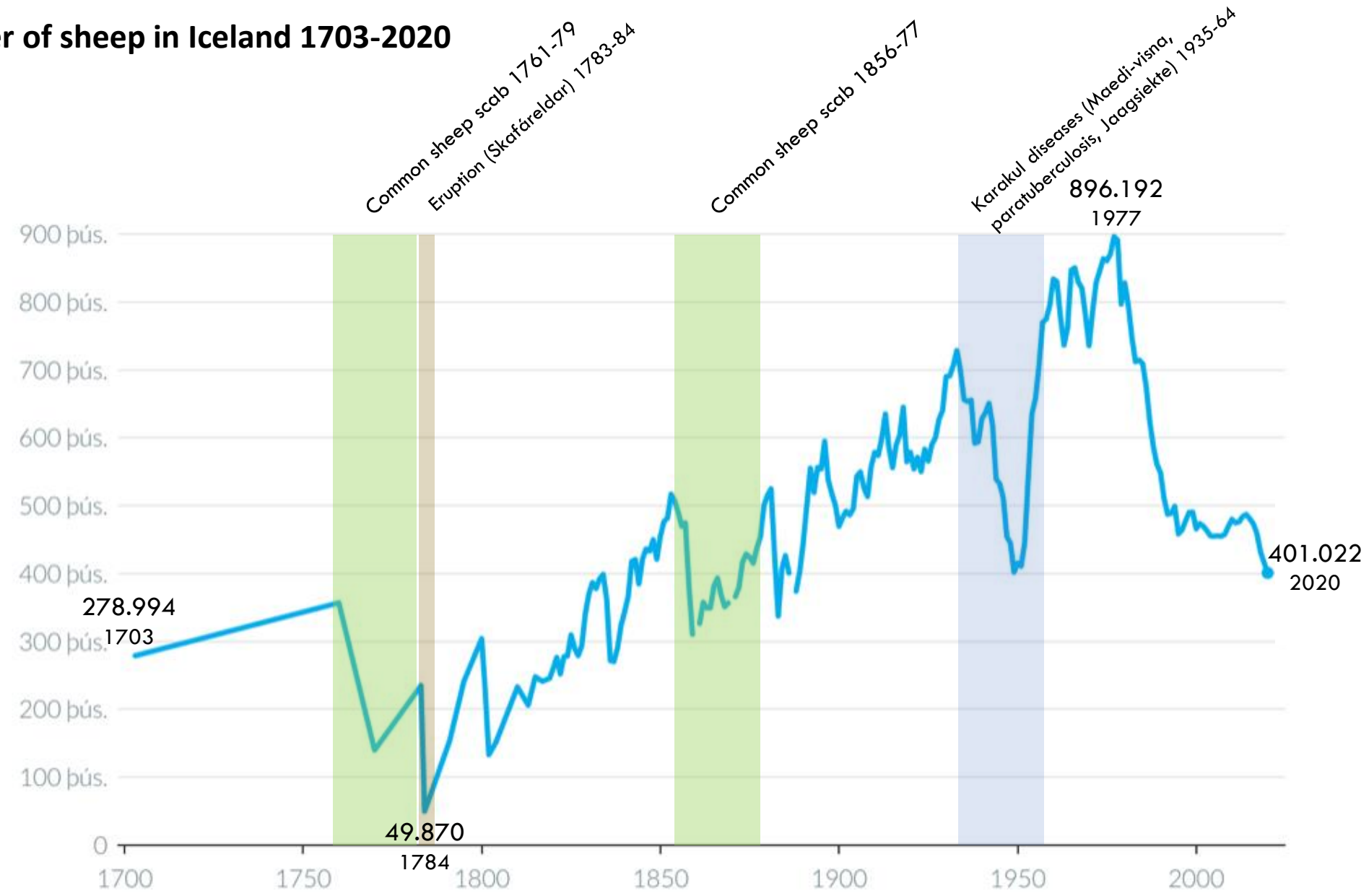
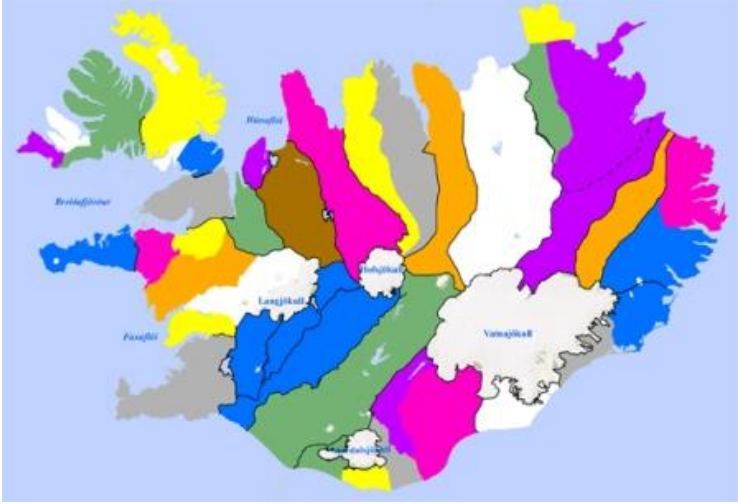


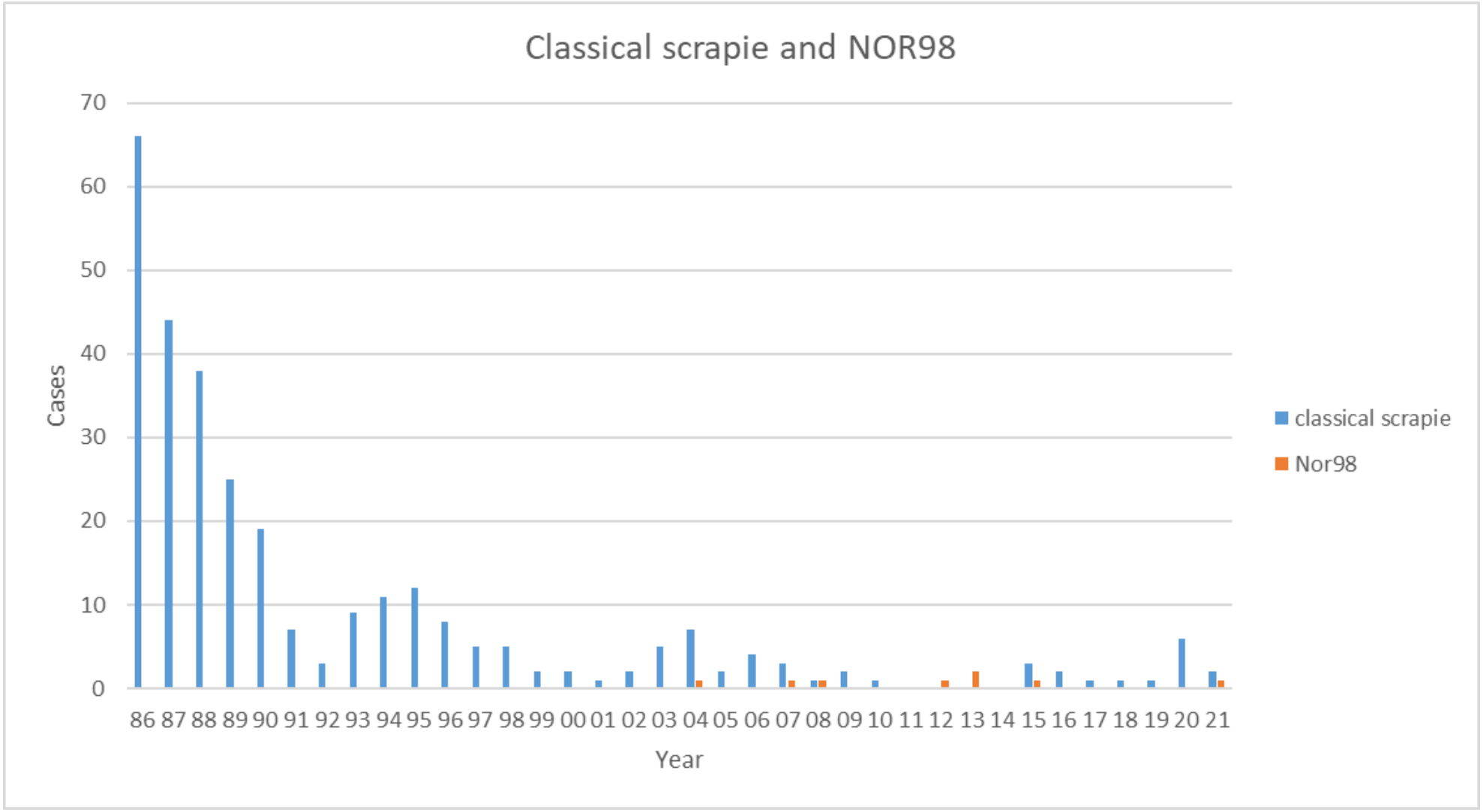
Photo: Vilhjálmur Svansson

Scrapie surveillance in Iceland



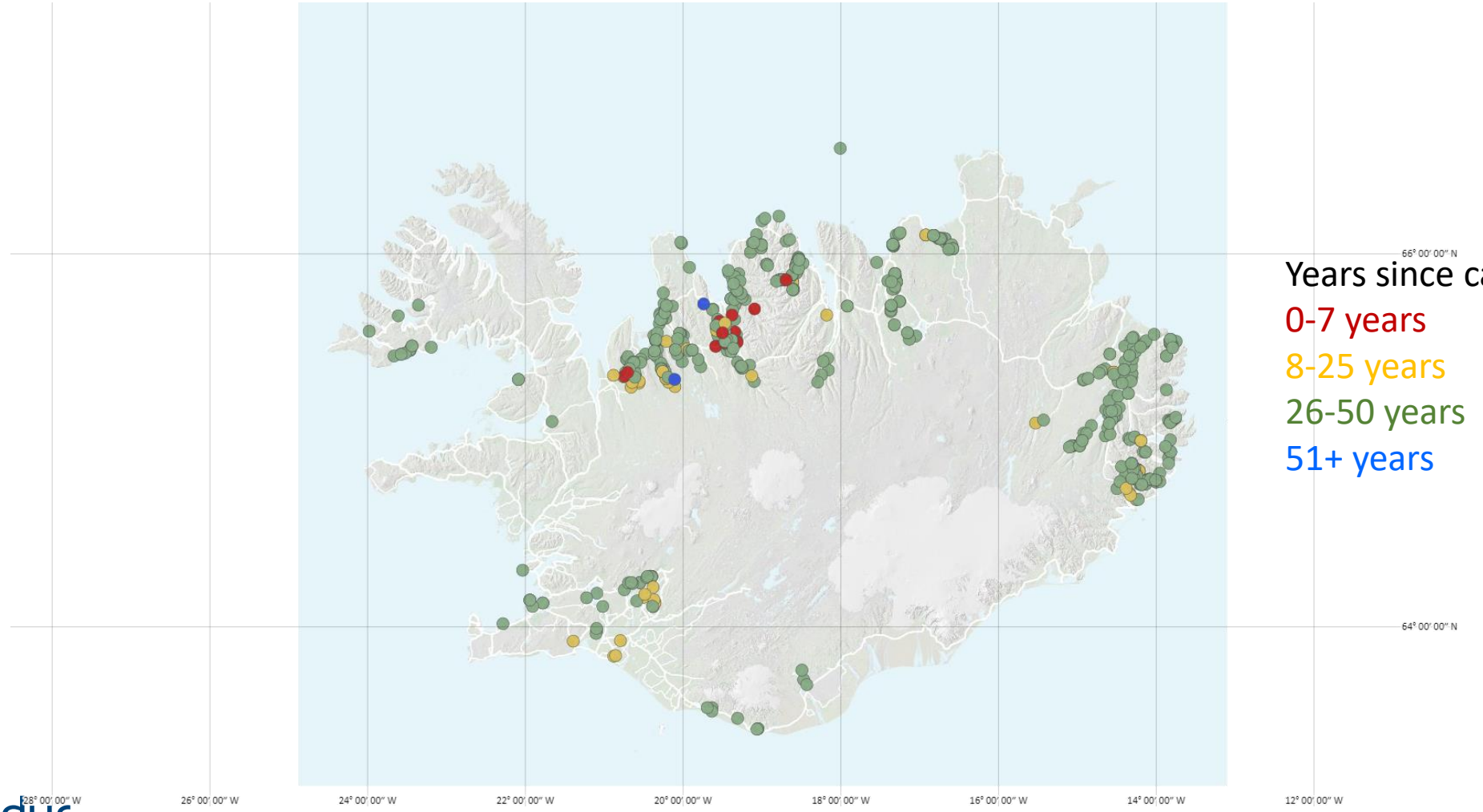
- Scrapie believed to be imported to Iceland in 1878
- Passive surveillance until 1978 (clinical suspects)
  - Notifiable disease
  - 1930-40: Quarantine zones and stamping out (Karakul diseases)
- Active scrapie surveillance since 1978
  - Steps taken towards scrapie eradication
    - 1978: Testing of healthy slaughter by histopathology (HP)
      - culling of scrapie flocks in newly infected areas
    - 1986: Culling of all scrapie flocks and farmers get compensated
    - 1993: Enhancement of eradication program
      - cleaning and disinfection of premices
      - period without sheep (2-3 years)
      - restocking from scrapie-free areas
    - 2012: Atypical/Nor98 scrapie flocks not culled
  - Rapid testing of abattoir samples by elisa since 2004
    - Healthy slaughter (adult sheep)
    - Fallen stock and clinical suspects

Number of scrapie cases 1986-2021



Distribution of classical scrapie cases in Iceland

Map: Icelandic Food and Veterinary Authority (https://landupplysingar.mast.is/)



Years since case:

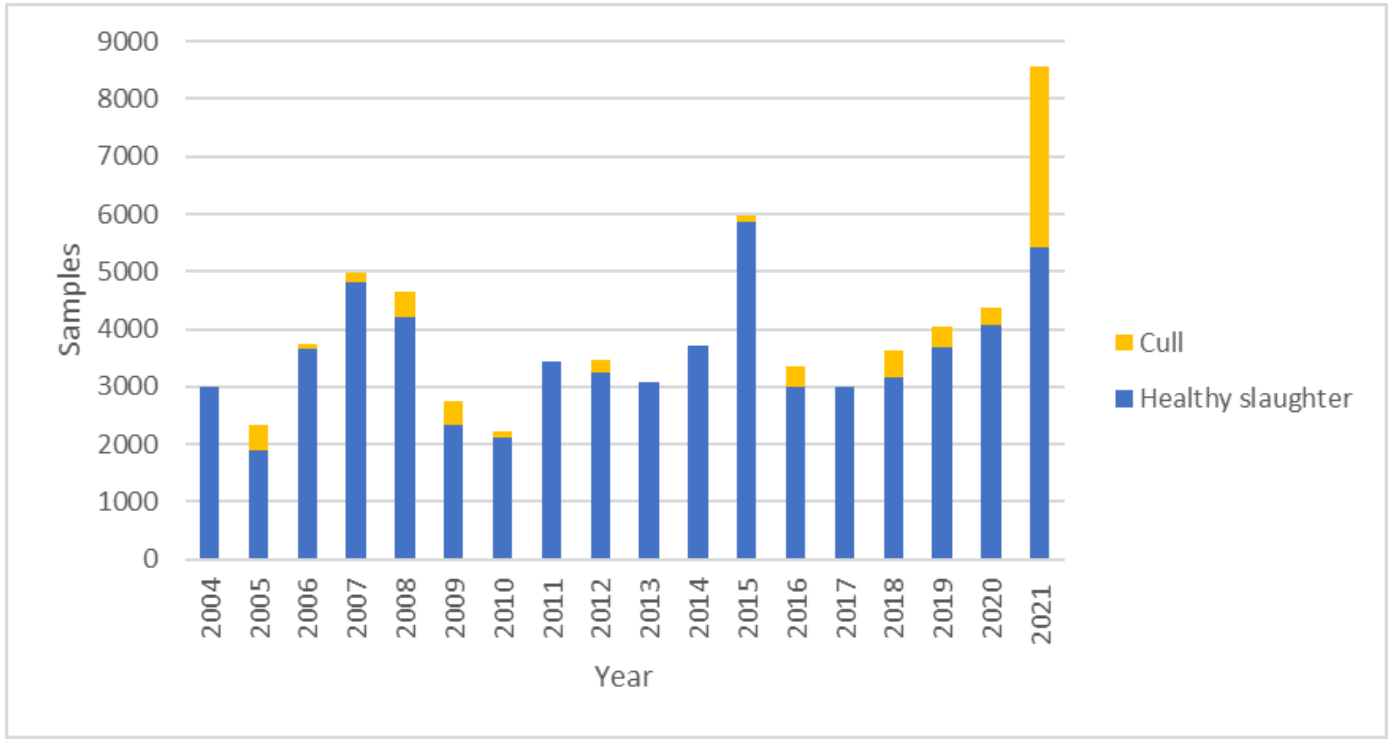
0-7 years

8-25 years

26-50 years

51+ years

Samples screened for scrapie at Keldur 2004-2021



Where are the scrapie cases found?

Testing by elisa 2004-2021

Index samples	Total cases	Classical scrapie	Nor98 scrapie
Clinical suspects (CS)	18	16	2
Fallen stock (FS)	7	6	1
Healthy slaughter (HS)	14	9	5
Total	39	31 (79,5%)	8 (20,5%)

Additional cases	Total samples	Classical scrapie	Nor98 scrapie
Culled flocks	7174	6197 (27 flocks)	977 (4 flocks)
Positive	262	261 (21 flocks)	1

Genotypes - study from 1999

PrP gene polymorphism and natural scrapie in Icelandic sheep

Stefania Thorgeirsdottir, Sigurdur Sigurdarson, Hjalti Mar Thorisson, Guðmundur Georgsson and Astridur Palsdottir
Journal of General Virology (1999) 80:2527-2534

- Breed survey
  - scrapie-free and scrapie regions
- 16 genotypes detected
  - nine genotypes <1%
  - new polymorphism; 138-N and 151-C
  - 137-T at low frequency on five farms
  - 171-R not detected (n=932)
- Scrapie association
  - 136-V strong relation to scrapie risk
  - 154-H possibly protective ?

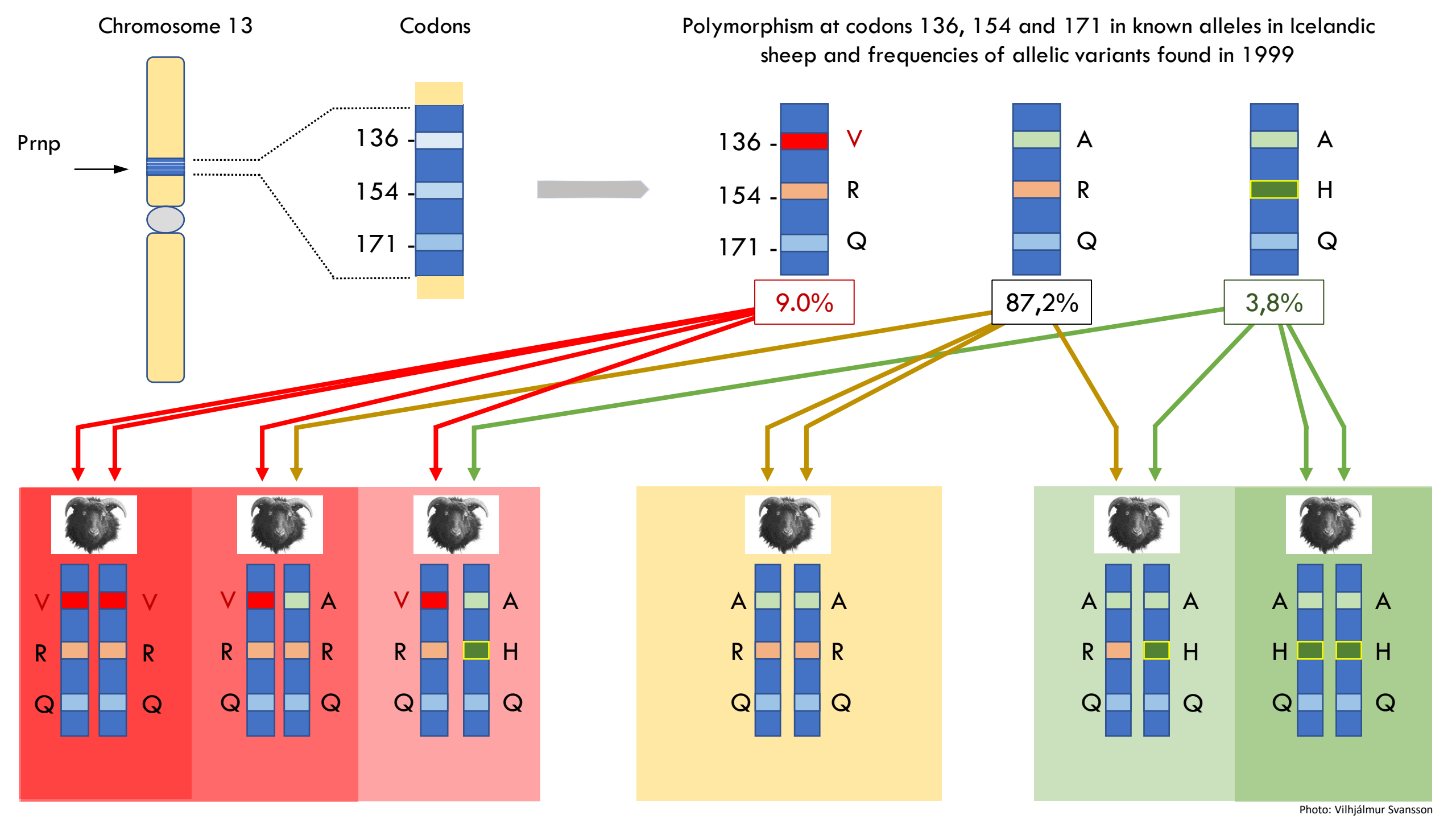


Photo: Vilhjálmur Svansson

Scrapie status 2021

- Incidence of scrapie in Iceland has lowered drastically over time
  - Goal of eradication not been reached
  - Two types of scrapie
    - Classical: infectious, culling of positive flocks
    - Nor98: spontanous, no culling of flocks
  - Testing annually 3-4000 sheep samples
  - Usually few scrapie cases per year
- Scrapie reoccurs on farms despite culling, cleanup and restocking, especially in high-risk areas
  - Scrapie still in the environment, pens and pastures?
  - Breeding for resistance not an option

2021: a new search for ARR and other potentially protective genotypes

- Why now?
- Unusually high number of scrapie cases detected in 2020
  - Six farms, four of them connected
  - Culling of 3000 sheep
- Farmers with no additional scrapie cases in flock opposed to culling of their flocks
- Pressure from farmers for new approach, e.g. import of semen
- Previous search not thorough enough?
- T137 found at several farms in 1999; could possibly be used instead of ARR?
  - Shown to be protective in a study on Sarda sheep (Vaccari *et al.* 2009)

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Widespread search for potentially protective prion protein variants in the Icelandic sheep population delivers promising results

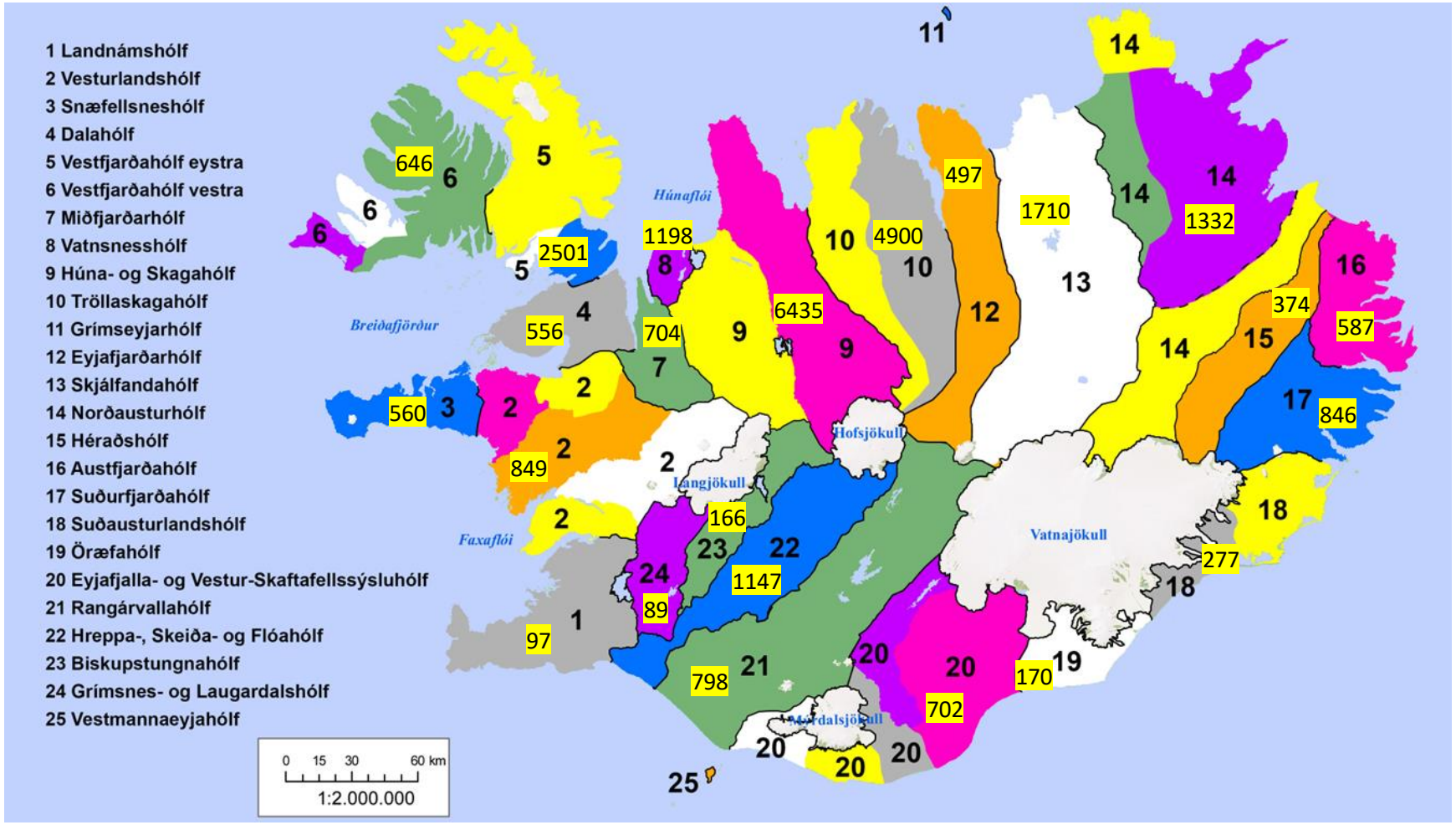
Gesine Lühken[a], Karólína Elísabetardóttir[b], Eyþór Einarsson[c], Vilhjálmur Svansson[d] and Stefanía Thorgeirsdottir[d]

[a]Department of Animal Breeding and Genetics, Justus-Liebig University of Giessen, Giessen, Germany; [b]Hvammshlíð, Skagabyggð, Iceland; [c]Icelandic Agricultural Advisory Centre, Sauðárkrókur, Iceland; [d]Department of Virology and molecular biology, Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavík, Iceland

- Genotyping of 27.959 sheep (July 2022)
  - full coding region sequenced or genotyped at six codons; 136, 137, 138, 151, 154, 171
- Search aimed at farms with their own breeding and regions not affected by extensive culling in the past to fight scrapie and other diseases
  - e.g. lentiviral disease (maedi, visna) and paratuberculosis in the 1930s and common sheep scap (Psoroptic mange) 50 and 150 years earlier
- Voluntary participation by farmers

Distribution of samples in study

612 farms – more than one third of all sheep farms



Results

- ARR
  - 14 adult sheep (0.05%)
  - 55 (0.2%) if including lambs
  - found at only one farm in the far eastern part of Iceland

- T137
  - 41 adult sheep (0.15%)
  - 85 (0.3%) if including lambs
  - from a total of eight farms located in different regions of the country

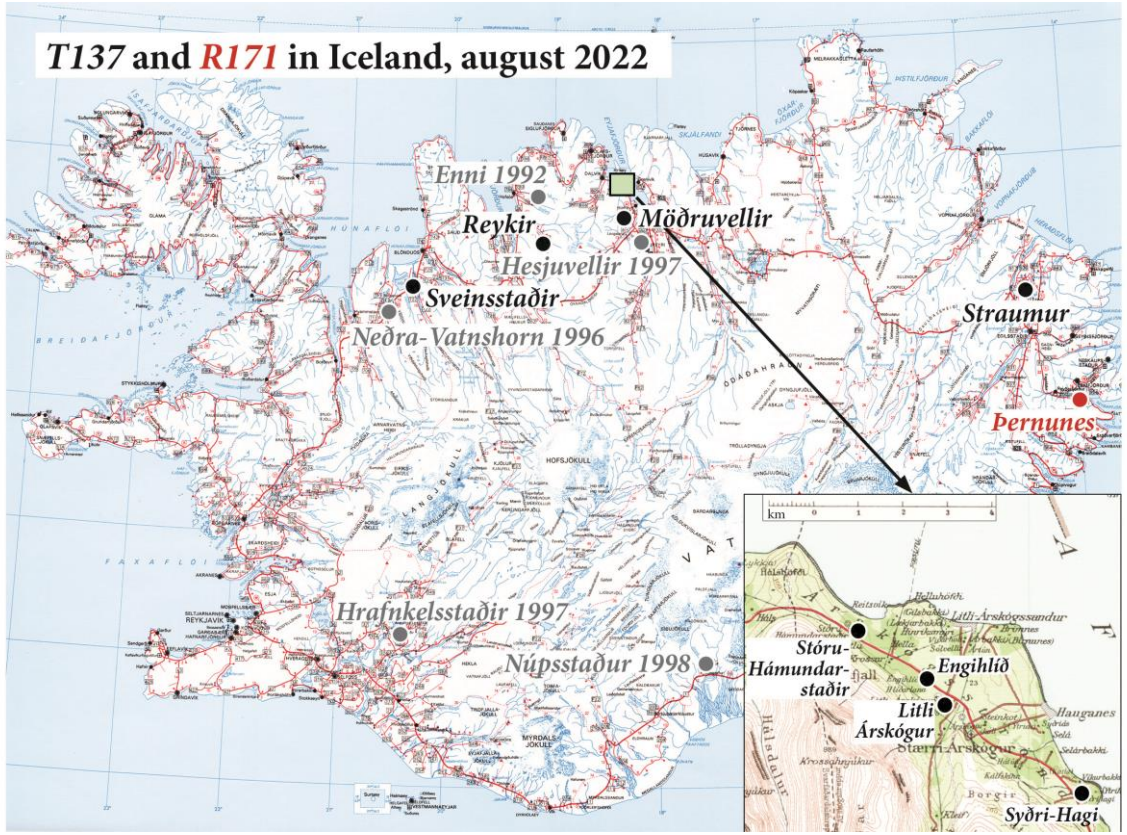
January 2022: ARR finally found in Iceland!

- The first six sheep with ARR in Iceland: five ewes and one ram named Gimsteinn; e. Gem, all found at Þernunes, a farm in the far Eastern part of Iceland.

Sheep detected carrying T137 (41) and R171 (14)

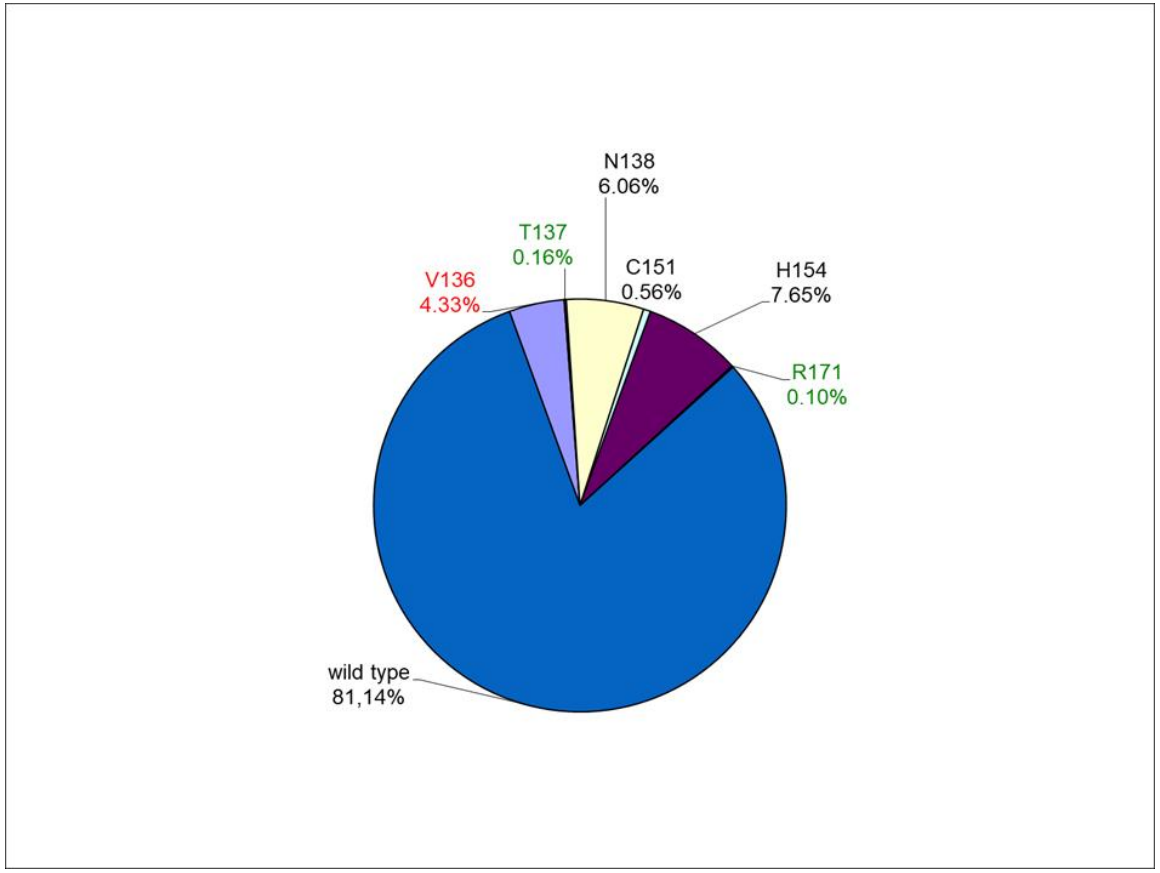
- The Icelandic sheep breed (*Ovis brachyura borealis pall*) shows a great diversity in regard to colour and horns.

Location of farms with ARR and T137 sheep



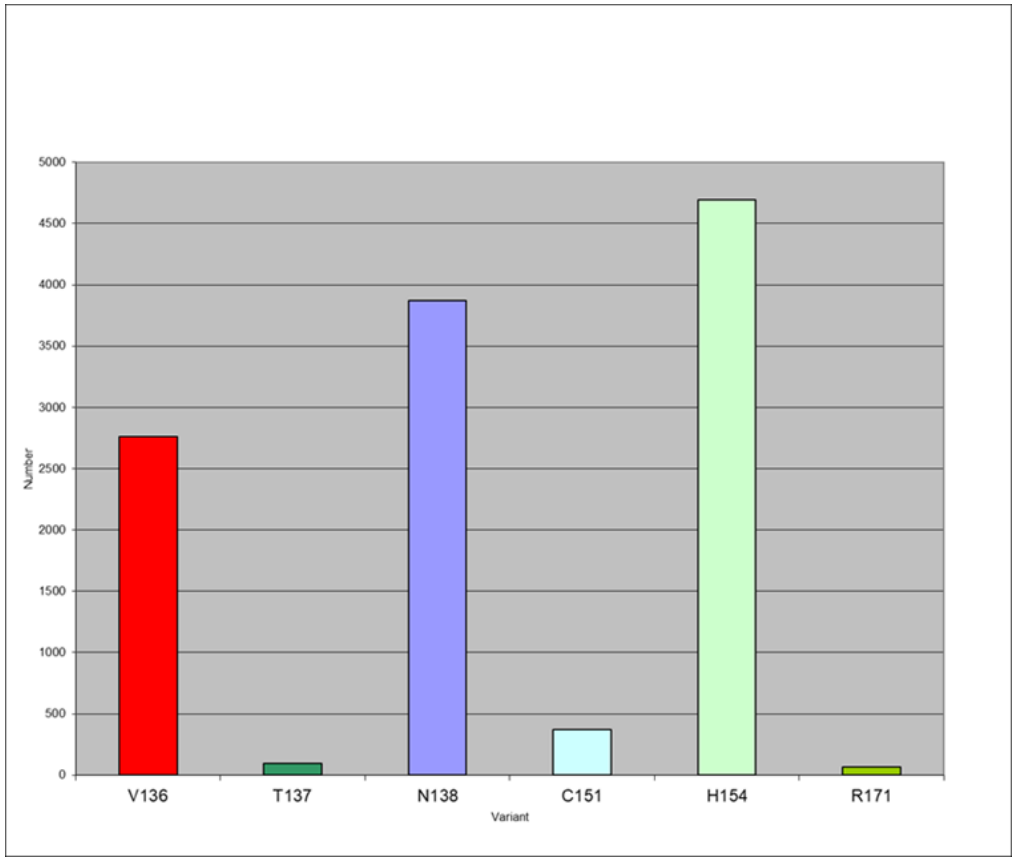
- R171 has only been detected in one farm in the East; Þernunes (red)
- T137 currently found in eight farms, located mostly in the North (black), in addition to five farms, where this polymorphism was found in the past (grey)

Variants of the prion protein in Icelandic sheep, % of alleles n = 27.959 (sheep), July 2022



- The wild type, ARQ, is the far most common; 81,14%
- All others are less than 10%
- The protective variants, R171 and T137, are very rare; <1%

Variants of the prion protein in Icelandic sheep, number of alleles n= 27.959 (sheep), July 2022



- Comparison of the prion protein variants (except wild type)
- R171 and T137 are the most rare
- H154 is the most common
  - been regarded as potentially protective in the Icelandic sheep breed
- N138 is believed to be neutral
- effect of C151 is unknown

Conclusions

- Although found at a very low frequency, the presence of ARR and T137 offer the possibility of a careful breeding program for scrapie resistance in the Icelandic sheep breed
  - including deep pedigree and genomic data in order not to decrease the diversity of the population
- Use of sheep with T137 is promising, because in contrast to ARR, they are not all from one ancestry
  - ongoing project using PCMA and RT-QuIC tests should prove the protective effect of the identified variants against the Icelandic scrapie strains
- The few scrapie cases detected each year, are located mostly in the North, which should therefore be targeted first for breeding for resistance.

Participants

Gesine Luecken

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Vilhjálmur Svansson
Stefanía Thorgeirsdóttir

Funding: Local fund for development of sheep farming in Iceland.

Breeding for resistance - what has changed?

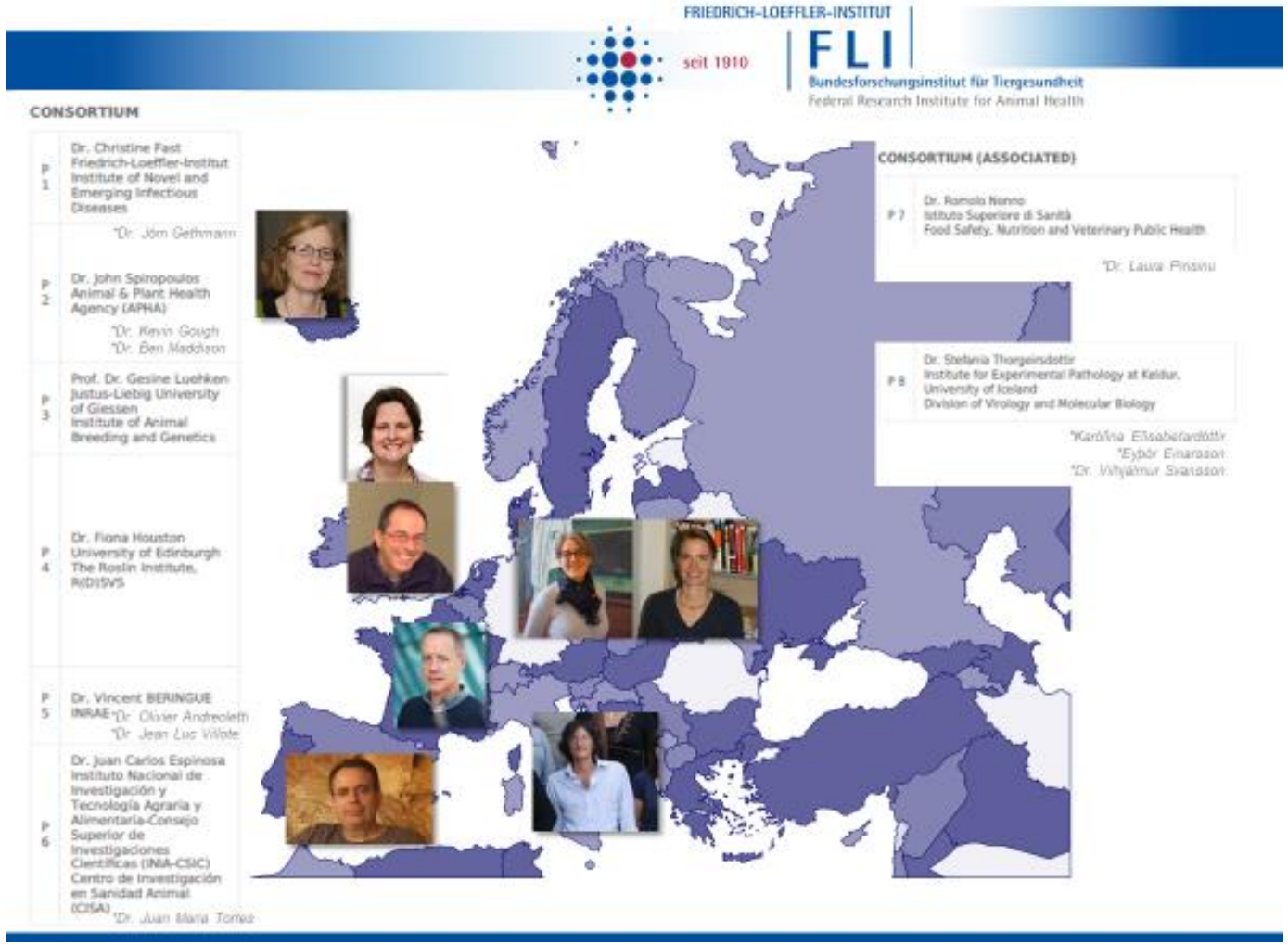
- Breeding centers can offer rams with protective genotypes
  - Three ARR rams (Gem and two lambs)
  - Two T137 rams (still not accepted for breeding for resistance by EU)
  - Farms in high risk areas have priority over low risk or clean areas, but breeding farms in scrapie-free zones will also be quaranteed semen with protective genotypes
- Transport of ARR and T137 sheep between quarantine zones allowed
  - Only between zones of same category in regard to scrapie infectivity or into a zone with higher infectivity
    - All farms where ARR (1) and T137 (8) was detected, are located in scrapie affected areas
  - 12 ARR rams (lambs) have been sold to farms located within the endemic scrapie region in the North, which has priority over other regions with less scrapie

Breeding for resistance – future plans

- Sheep farmers will have to rely on insemination or buying rams to a much greater extent than before as well as genotyping of flocks
- Concentrate on increasing the number of sheep with protective genotypes in high-risk scrapie areas, while other areas will do it more slowly to prevent too much inbreeding
- Future breeding plan will depend on results from current research projects, e.g. comparison of different PRNP variants by PMCA and RT-Quic

ICRAD proposal – project: SciCe
Classical Scrapie in Iceland, a model for prion diseases worldwide

- Consortium
  - Christine Fast, Germany - coordinator
  - John Spiropoulos, UK
  - Gesine Luehken, Germany
  - Fiona Houston, UK
  - Vincent Beringue, France
  - Juan Carlos Espinosa, Spain
- Associated
  - Romolo Nonno, Italy
  - Stefania Thorgeirsdottir, Iceland



ICRAD proposal – project: ScIce Work-Packages

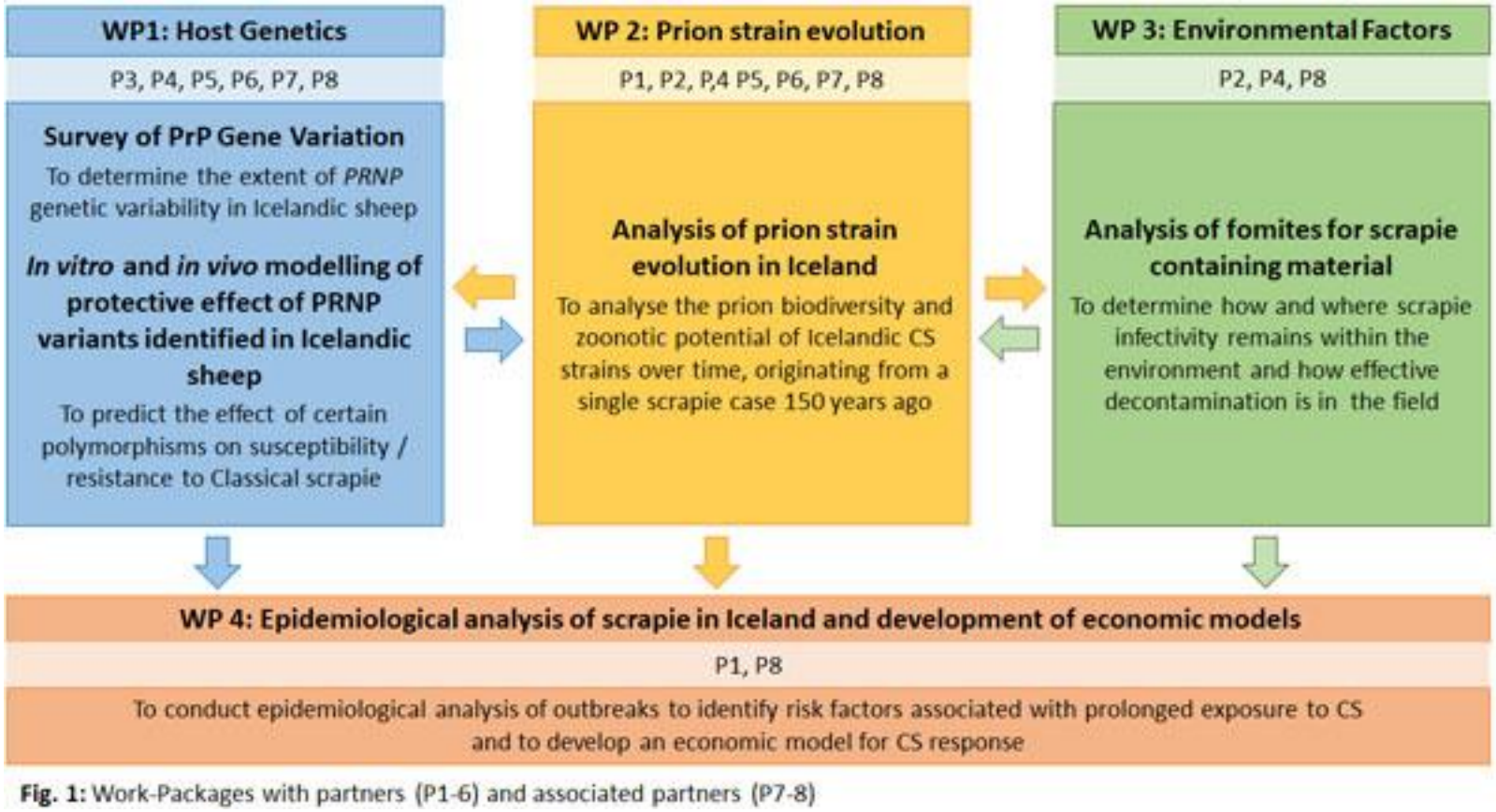


Fig. 1: Work-Packages with partners (P1-6) and associated partners (P7-8)

WP1b: in vitro and in vivo modelling of protective effect of PRNP variants detected in Icelandic sheep

- PMCA and RT-Quic will be applied to test the impact of certain PRNP variants identified in WP1 on scrapie amplification
  - Healthy sheep brains collected in Iceland and Switzerland
    - Polymorphism at codons 136, 137, 138, 151, 154, 171
    - Special emphasis on T137
  - Classical scrapie isolates from Iceland
    - Representing different times, regions and genotypes

WP1b: Testing different PRNP variants with Icelandic scrapie isolates using PMCA

- Fall 2022: fresh brain samples, with variations of T137, N138, C151, H154, collected at Keldur from nine healthy sheep donated by Icelandic farmers.
- Samples will be sent to Vincent Beringue for PMCA testing.

WP3b: Environmental factors

- Analysis of fomites for scrapie containing material
  - To determine where scrapie infectivity remains on farms
  - To determine how effective the decontamination is in the field
- Three categories of farms
  - Group 1. Scrapie within 12 months, not decontaminated
  - Group 2. Scrapie in the last 3-5 years, decontaminated but not restocked
  - Group 3. Farms that have had no recorded incidence of scrapie, preferably in scrapie-free zones
- Ten sampling sites per farm, depending on pen size and situation
  - one bulk sample (5 swabs) in duplicate per site

WP3b: Environmental factors

2022: Sampling fomites on three scrapie farms; two categories

Group 1. Before cleaning of pens

Group 2. After cleaning and rebuilding, but before restocking

Summer 2022: three scrapie farms visited to collect samples; one right before cleaning, two already cleaned and ready for restocking 440 samples ready at -20 °C for PMCA/RT-Quic testing

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Thanks for your attention!