Assessment of genetic susceptibility to classical and atypical scrapie in five Russian locally derived sheep breeds
Tatiana E. Deniskova, Olga V. Kostyunina, Marina I. Selionova, Sergei N. Petrov, Veronika R. Kharzinova, Gottfried Brem, Natalia A. Zinovieva

L.K. Ernst Institute of Animal Husbandry, Dubrovitzy, Moscow, Russia
All-Russian Research Institute of Sheep and Goat, Stavropol, Russia, Institute of Animal Breeding and Genetics, VMU, Vienna, Austria.

E-mail: horarka@yandex.ru

Abstract (Click)
Scrapie is the transmissible spongiform encephalopathy or prion disease that causes fatal degenerative damages in the central nervous system of affected sheep. To-date two forms of scrapie are known: classical, and atypical, or Nor98. Genetic susceptibility to classical scrapie is determined by allele polymorphisms in codons 136, 154 and 171 of the PRNP gene. In this regard, the aim of our research was to analyze PRNP polymorphisms in 136, 141, 154 and 171 codons in five Russian locally derived sheep breeds including Altai Mountain, Kuibyshev, North Caucasian, Russian Longhaired and Tsigai.

Material & Methods (Click)
Identification of the alleles in the codons 136 (A/T/V), 141 (L/F), 154 (R/H) and 171 (Q/R/H/K) of PRNP was performed by PCR and then by pyrosequencing on the PSQ96MA (Qiagen, USA) in five sheep breeds (n=86).

Prion normal: PrP
Prion abnormal: PrPs

Results (Click)
The most frequent genotypes were ARR/ARQ in the Kuibyshev, North Caucasian, Russian Longhaired and ARQ/ARQ in Altai Mountain and in Tsigai. The F141 allele was found in two animals. Sheep of class G1 were detected in all breeds.

Conclusion
Here we demonstrated the necessity of the monitoring for genetic susceptibility for classical and atypical (Nor98) scrapie forms in sheep herds in Russia.

Acknowledgments
The research was performed within the framework of the Federal Agency of Scientific Organizations No. AAAA-A18-118021590138-1

E-mail: horarka@yandex.ru
Genetic monitoring is the only method to prevent the appearance of Scrapie in the sheep flocks.

Scrapie is the transmissible spongiform encephalopathy or prion disease that causes fatal degenerative damages in the central nervous system of affected sheep.

**Scrapie**

**Classical**

**Atypical (Nor98)**

Genetic susceptibility to classical scrapie is determined by allele polymorphisms in codons 136, 154 and 171 of the PRNP gene. Thus, A\textsubscript{136}R\textsubscript{154}R\textsubscript{171} allele is associated with resistance while V\textsubscript{136}R\textsubscript{154}Q\textsubscript{171} is linked with susceptibility. There are five classes of genetic resistance with its decreasing from G1 to G5.

The atypical scrapie was discovered in Norway in 1998. The F allele in 141 codon of PRNP is responsible for susceptibility to Nor98.

<table>
<thead>
<tr>
<th>Classes of genetic resistance to Scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Type 1</td>
</tr>
<tr>
<td>Type 2</td>
</tr>
<tr>
<td>Type 3</td>
</tr>
<tr>
<td>Type 4</td>
</tr>
<tr>
<td>Type 5</td>
</tr>
</tbody>
</table>

The aim of our work was to analyze polymorphisms in 136, 141, 154 and 171 codons of PRNP in five Russian local sheep breeds.

Our research group:

- T. Deniskova
- O. Kostyunina
- M. Selionova
- S. Petrov
- V. Kharzinova
- G. Brem
- N. Zinovieva

The atypical scrapie was discovered in Norway in 1998. The F allele in 141 codon of PRNP is responsible for susceptibility to Nor98.

- No immunity response
- No vaccines or medicine
- No reliable diagnosis in vivo
- Clinical symptoms appear on the final stages of the disease
- 100% lethality

Genetic monitoring is the only method to prevent the appearance of Scrapie in the sheep flocks.
Materials and Methods

Five Russian sheep breeds

- Altai Mountain (n=16)
- Kuibyshev (n=16)
- North Caucasian (n=19)
- Russian Longhaired (n=14)
- Tsigai (n=21)

Workflow

1. DNA extraction
2. PCR
3. Pyrosequencing

Pyrograms of different genotypes in 136/141/154 multiplex

Pyrograms of different genotypes in 171 codon

AG/AG
AG/GG
AG/GT
Results

We found six different alleles (ARR, ARQ, ARH, AHQ, AHR and VRQ) and eight different PRNP genotypes in five Russian breeds (Table 1).

The most frequent genotypes were ARR/ARQ (G2, genetically resistant) in the Kuibyshev (50.0%), North Caucasian (47.4%), Russian Longhaired (42.9%) and ARQ/ARQ (G3, little genetic resistance) in Altai Mountain (43.8%) and in Tsigai (42.9%).

Sheep of the most genetically resistant class G1 were detected in all studied breeds with frequency varying from 4.8% in Tsigai to 35.7% in Russian Longhaired. The allele VRQ was found in all breeds (from 3.6 to 7.1%) except for North Caucasian.

Table 1. Classes of genetic resistance to typical scrapie

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altai Mountain</td>
<td>16</td>
<td>6.25</td>
<td>31.25</td>
<td>50.00</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Kuibyshev</td>
<td>16</td>
<td>18.75</td>
<td>50</td>
<td>18.75</td>
<td>-</td>
<td>12.5</td>
</tr>
<tr>
<td>Russian Longhaired</td>
<td>14</td>
<td>35.71</td>
<td>42.86</td>
<td>14.29</td>
<td>-</td>
<td>7.14</td>
</tr>
<tr>
<td>Tsigai</td>
<td>21</td>
<td>4.76</td>
<td>33.33</td>
<td>47.62</td>
<td>-</td>
<td>14.29</td>
</tr>
<tr>
<td>North Caucasian</td>
<td>19</td>
<td>31.58</td>
<td>47.37</td>
<td>21.05</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1. The frequencies of PRNP genotypes in codons 136, 154 and 171

Figure 2. Atypical Scrapie (Nor 98)

The F_{141} allele presented in two animals of North Caucasian breed that had satisfactory genotypes for classical scrapie (ARQ/ARR and ARQ/ARQ).