INCREASING FOOD CHAIN SECURITY FOR SCRAPIE BY MARKER ASSISTED SELECTION IN SHEEP POPULATIONS

CREŞTEREA SECURITĂŢII LANŢULUI ALIMENTAR PENTRU SCRAPIE PRIN SELECŢIA ASISTATĂ DE MARKERI MOLECULARI ÎN POPULAŢIILE DE OVINE

COŞIER VIORICA*, COROIAN, C.*, DĂRÂBAN, S*, VOIA,S. **
*University of Agriculture Sciences and Veterinary Medicine of Cluj – Napoca - Faculty of Animal Sciences and Biotechnology
**Banat University of Agriculture Sciences and Veterinary Medicine-Timisoara

Romania, by its genetic found made up of over 7.4 millions sheep and 1.8 millions young sheep exported in EU countries, represents an important mark in both European and World sheep breeding sector, in last years marked by many ESST centers. The apparition of this disease and its high risk of danger for reared effective constraint EEC countries to interfere by law regulations concerning scrapie control and stopping disease spreading. In our country, controls identified the presence of scrapie in livestock. The international reference laboratories post mortem certified the presence of disease. Immunohistochemical detection of PrPSc is a standard diagnostic method for sheep scrapie. By immunohistochemistry (IHC), PrPSc is detected in lymphoid tissues during preclinical and clinical disease. After genotyping at PrnP locus, the marker assisted selection is compulsory, and flocks include only genotypes with high disease resistance.

Key words: scrapie, ESST, PrnP, PrpSc, Marker Assisted Selection

Introduction

Prion pathogenesis, diagnostic and selection. The sheep spongiform encephalopathy, also named scrapie, belongs to disease family designated by generic name Transmissible Subacute Spongiform Encephalopathies (ESST), besides bovine spongiform encephalopathy (mad cow disease), feline and mouse spongiform encephalopathy, spongiform encephalopathy in man: Creuzfeld-Jakob disease, Kuru disease, etc. All maladies included in ESST category, including scrapie, are characterized by a general clinic picture with lots of common points: lost equilibrium, uncoordinated movements, trembling, exagerated contact reactions, fear or agresivity. All symptoms are characteristic for nervous system alteration. The disease is lethal and brain examination shows a spongious aspect correspondent to vacuolization of neurons and appearance of amyloide plaques, sign of proteic deposits accumulation in brain (Gu Y.,et al., 2002; Aguzzi, A., et al., 2004).
The presence of sheep spongiform encephalopathy, also named scrapie, was observed beginning with XVIII century, but it did not produce great losses in flocks and it was not transmissible to men. The bovine spongiform encephalopathy identified in 1986 in UK, recorded a fast expansion. Suspicions exist, that disease is transmissible through insufficiently thermal treated meals obtained from meat and bones from infested animals, included in cows' feeding. Presently, disease transmission to man is studied, relaunching the interest for study of spongiform encephalopathy generally, and in ruminants especially.

**Diagnostics.** In our country, controls identified the presence of scrapie in livestock. The national reference laboratories post mortem certified the presence of disease. Due to this reason, it is necessary to perform an active control of disease within presence areas, using immunohistochemical analysis in order to found PrP carriers, in different, even sub clinical stages.

Many species contacted spongiform encephalopathy: sheep, goats, bovine, wild ruminants, mink and felines. If elucidation of the nature of pathogen agent and transmission modalities is not completely, yet, researchers from INRA demonstrated the existence of a genetic control of scrapie resistance. It opens new and interesting perspectives for reducing incidence of disease in sheep flocks.

The molecular analysis of this gene in sheep show the existence of more genetic variants resulted from mutations at 136, 154, and 171 codones. The Prm-P locus is polymorph with known variability: polymorphisms within codones 136, 154, and 171 are associated to different sensibilities to experimental and natural spongiform encephalopathy. The substitutions of nitrogen basis specifying other amino acid determine apparition of the following genetic variants:

- alterations from codone 136, which can specify Alanine (A) or Valine (V);
- alterations from codone 154, which can specify Arginine (R) or Hystidine (H);
- alterations from codone 171, which can specify Arginine (R), Hystidine (H), Glutamine (Q).

**Selection for scrapie resistance.** Due to scientific progress recorded in last years, many countries put into practice national selection programs for increasing scrapie resistance in sheep populations. Holland practices these programs from 1999, UK from 2000, and France from 2002. The aim of these programs, developed during 5 years, is to eliminate VRQ alleles from population and increase frequency of ARR allele, delivery of lambs derived from resistant rams, homozygous on ARR allele.

**Materials and Methods**

**Immunohistochemistry.** Tissues sampling and processing: Palatine tonsils, retropharyngeal lymph node, and third-eyelid lymphoid tissue are taken by biopsy and placed in fixative (formalin 10%). The samples embedded according to standard procedures in paraffin. Tissue sections 4 µm thick will be cut on a rotary microtome and mounted on treated glass slides (Vectabond; Vector, Burlingame,
CA) and dried overnight at 37°C. Sections will be dewaxed and hydrated by routine methods before the antigen retrieval procedure. To ensure the specificity of the pathological PrP immunostaining, several pretreatments will be performed. Immersion in 98% formic acid for 15 min, proteinase K treatment for 15 min at 37°C (Roche, Mannheim, Germany; 4 µg/ml), and hydrated autoclaving (immersion in distilled water in a pressure cooker) will be applied.

PrPsc detection are performed using the monoclonal antibody (Mab) P4, L03, L42 (R-Biopharm, Darmstadt, Germany; 1:500 - Hardt M et al., MAb F99/97.6.1 (Valdez RA, et al., 2003), MAb Fab D18 (Peretz D, et al., 2001), MAb F89/160.1.5 (O'Rourke KI, et al., 1998).

The next phases in Immunohistochemistry are Immunostaining Procedures which includes 2 methods: 1. Avidin–Biotin–Peroxidase System and 2. Catalyzed Signal Amplification (CSA) System.

**Genotyping.** The genotypization at Prn-p gene locus starts from the DNA extracted from blood white globules, biopsies from epithelial tissue, sperm, etc. Any age is suitable for performing genotypization, but selection patterns use only rams naturally resistant to scrapie with ARR/ARR genotype, eliminating VRQ/VRQ rams.

Many European countries (Great Britain, Holland, France, Norway, and Belgium) already put into practice selection programs considering sheep genotypes at Prn-p locus (Arnold M et al., 2002).

In overall, the general possible combinations of the 5 amino acids specified by 5 different codones will determine existence of 15 possible genotypes (Table 1). Each locus has two alleles. In both natural and experimental conditions, ARR and AHQ alleles confer a much higher disease resistance; ARQ and ARH determine an intermediary resistance, and VQR allele a maximal sensibility to scrapie. All molecular analyses performed in different sheep breeds show no illness in individuals with ARR/ARR genotype. According to research performed at INRA, ARR/ARR sheep are also resistant for inoculation with bovine spongiform encephalopathy (ESB) pathogenic agent, while sheep with VQR/VQR genotype, ESB experimentally inoculated, developed disease and died. The ARR/ARR sheep are not contamination sources in natural conditions and ESB inoculation. From this, results the conclusion of natural resistance of genotype to scrapie.
The use of rams with ARR/ARR genotype in reproduction represents a privilege for sheep farms, considering the carriers of a single ARR allele have the lowest capacity to develop disease. The table 1 shows the possible genotypes at Prn-p gene locus codifying prionic protein Pr-P, which determine scrapie and type of risk conferred by genotype (according to Renaville et al., 2002).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Risk intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>Sheep genetically very resistant to scrapie</td>
</tr>
<tr>
<td>ARR/AHQ</td>
<td>Sheep genetically resistant to scrapie but need a particular attention to be used in selection programmes</td>
</tr>
<tr>
<td>ARR/ARH</td>
<td>Sheep with low genetic resistance to scrapie, and farmers must avoid their use in selection patterns.</td>
</tr>
<tr>
<td>ARQ/ARH</td>
<td>Sheep sensible to scrapie.</td>
</tr>
<tr>
<td>ARQ/AHQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
<tr>
<td>AHQ/ARH</td>
<td>Sheep with low genetic resistance to scrapie, and farmers must avoid their use in selection patterns.</td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
<tr>
<td>AHQ/VRQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
<tr>
<td>ARH/VRQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
<tr>
<td>ARQ/VRQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
<tr>
<td>VRQ/VRQ</td>
<td>Sheep very sensitive to scrapie. They must be sterilized or slaughtered.</td>
</tr>
</tbody>
</table>

Results and Discussions

In 1996, research performed at INRA, on 600 Romanov sheep, demonstrated the role of different alleles in genetic determinism of resistance/sensitivity to scrapie. During 3 years, the analyses revealed 250 cases of scrapie. The study led to the following results:
- animals with codone 154 codifying Hystidine (H) and codone 171 codifying Arginine (R) on Prn-p gene, are resistant to scrapie;
- animals with codone 154 for Arginine (H) and codone 171 for Glutamine (Q) on Prn-p gene, are sensitive in homozygous state, but this sensitivity increases if codone 136 codifies Valine (V), not Alanine (A).

In the same year, INRA (Sarradin P. et al., 1997) put the basis of a prototype genetic selection pattern for scrapie resistance. During 5 years (1996-2001) only rams of at least one copy of ARR allele carriers were used for reproduction. The share of ARQ/ARQ sensitive rams decreases from 24% to 7%, while share of entirely scrapie resistant ARR/ARR rams increases from 28% to 46%. The rams carriers of hypersensitive scrapie alleles were totally eliminated.

Within a recent epidemiologic study, 1,587 cases of sheep with scrapie (Le Dur A., 2005) confirmed by histopathologic examination, were analyzed. No carrier of ARR/ARR genotype was found, while 13.7% of individuals with scrapie were carriers of VRQ/VRQ genotype.
Conclusions

In our country disease was recorded, based on immunohystochemical analyzes performed on animals after slaughter within effectives from Fetesti, county of Ialomita, and Chirnogi, county of Calarasi. Reference International Laboratory VL confirmed the results of analyses. Weybridge from UK with analyze bulletin no. B-160 from 09.12.2002, (MAAP Communicate from 13.12.2002). National Institute of Diagnosis and Animal Health, Bucuresti coordinate the national control performed using immunohystochemical analyses.

The selection programme for scrapie eradication in sheep and goats in EU country adopted the following positions:

A. Agrees with elimination of animals with genotypes sensitive at Prn-p locus, from flocks;
B. Agrees the reconstruction of flocks starting from resistant animals;
C. Does not agree with introduction in feeding chain of sheep, whatever their age, with sensitive and very sensitive genotypes from flocks with at least one ESST case;
D. Does not agree with introduction in feeding chain of animals more than one year older, from flocks with disease;
E. Does not agree with interdiction of introducing in flocks with disease, scrapie resistant females.

Bibliography

CREȘTEREA SECURITĂȚII LANȚULUI ALIMENTAR PENTRU ESST PRIN SELECȚIA ASISTĂTĂ DE MARKERI MOLECULARI ÎN POPULAȚIILE DE OVINE

COȘIER VIORICA*, COROIAN, C.*, DĂRĂBAN, S*, VOIA, S. **
* Faculty of Animal Husbandry and Biotechnology, Cluj-Napoca, Romania
**Banat University of Agriculture Sciences and Veterinary Medicine-Timisoara

Romania prin genofondul ovin de peste 7,4 milioane capete și prin cele 1,80 milioane capete tineret ovin exportate în țările UE, constituie un pion important în ovicultura europeană și mondială, care în ultimii ani a fost marcată de numeroase semnări ale unor focare de EST ovină (Encefalopatia Spongiformă Transmisibilă). Apariția acestei maladii și gradul ei crescut de periculozitate pentru efectivele exploatare a făcut ca în țările din CE să se intervină prin reglementări legislative pentru controlul bolii și oprirea răspândirii ei. La noi în țară au fost depistate animale bolnave de scrapie postmortem, lucru certificat și de laboratoare de Referință Internaționale. Detectarea PrpSC prin metoda imunohistochimică este o metodă standard pentru diagnosticarea scrapiei la oi. În stadiile preclinice și clinice ale bolii, proteina PrPSC este detectată imunohistochimic în tesuturi limfatice. După genotipizare animalelor la locusul PrnP se poate apela la selecția asistată de markeri moleculari (MAS), fiind reținute în exploatare doar genotipurile care prezintă un grad crescut de rezistență la boală.

Cuvinte cheie: scrapie, ESST, PrnP, PrpSC, selecție asistată de markeri