Using Typifix Method for Microsatellite Analysis and SNP Assays with using the Genetic Biodiversity Indicator for Identification in Evves of Romania Genotypes Valuable Resistant in Scrapie

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Abstract: Vulnerability of farm animal breeds is caused by the lack of interest the breeders for one breed. In different region of Romania- the main mean of reducing biodiversity in farm animals is the crossbreeding. The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females. Molecular biology tests based on identification, amplification and characterization of nucleic acid revolutionized the pathology diagnostic, conservation of indigene animal genetic resources, gene assisted selection and food traceability from farm-to-fork and from fork-to-farm. Any replacement females or breeding males should originate from flocks/herds not known to be affected with scrapie and under management practices precluding the introduction of scrapie or, in the case of sheep, should be of resistant PrP genotypes. Susceptible ewes of unknown or questionable disease status should be bred to RR rams or separated from the rest of the flock prior to and following lambing until there is no vaginal discharge to minimize spread to other animals. Another method used by some producers is selective breeding to reduce overall flock susceptibility based on PrP genotype. This method consists of breeding only with rams that are RR or QR. In our study and research, the best genotype class G1 (ARR/ARR) and G2 (ARR/ARH) it was display in the samples of Totesti in percentage of 80%.

Key words: scrapie, genotype, resistance, selection intensity

1 Introduction

Infection with the scrapie agent is determined by the detection of the abnormal prion protein accumulation in nervous tissue and/or lymphoreticular tissues and/or histopathologic lesions in central nervous tissue in susceptible species. The characteristic histopathologic change of nervous tissue is vacuolation of neurons, producing a distinctive appearance of spongiform change. The vacuolar changes may be accompanied by other microscopic features, such as neuronal degeneration, neuronal loss, gliosis, and cerebrovascular amyloidosis. Typically, the histopathologic lesions have bilaterally symmetrical distribution, although the distribution pattern and changes may vary between type of agent and host genetics.

The use of selective breeding and culling to increase genetic resistance to scrapie infection raises concern regarding the practices effect on the genetic diversity of the domestic sheep population and on production traits. A number of studies have been completed evaluating effect of PrP genotype selection and production traits with some studies providing limited evidence of associations between PrP genotype and traits. Overall, when observed, associations between PrP genotype and performance traits tended to be neither strong nor consistent across populations, and there was no tendency for associations between scrapie-resistant PrP alleles and performance traits to be adverse (Dawson et al. 2008; Sweeney and Hanrahan 2008). A study did find that producer perception of animal quality was not influenced by animal susceptibility to scrapie (as determined by PrP genotype).

The rationale for conducting surveillance and scrapie eradication are as follows:

- Economic impact: Scrapie is a non-febrile and insidious disease. Infected flocks with a high percentage of susceptible animals can experience significant production losses. Over several years, the number of infected animals in a flock increases and onset of clinical signs occurs in younger animals, making these flocks economically inviable. Female animals sold from infected flocks can spread scrapie to other flocks.

- Potential public health: The apparent transmission of bovine spongiform encephalopathy (BSE), another TSE, to humans in the United Kingdom has resulted in a call for the eradication of all TSEs in
food-producing animals. Recent research has demonstrated that BSE could be successfully transmitted to sheep and goats orally, and that sheep genotypes traditionally resistant to scrapie were susceptible to BSE.

2 Materials and Methods
For prelevation the samples we use the new method TypiFix™ ear tag system which is a combination of a conventional ear tag with a simultaneous tissue sampling technology. The easy handling of the TypiFix™ ear tag system allows economic sampling of whole populations and is therefore an effective tool for analysis of genetic markers for traceability and breeding traits. The Typi-Fix®-System is a procedure for the collection of DNA containing tissue samples avoiding all these hurdles and problems. DNA purification with DNA FIX columns an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix™ samples. The sorbents retain protein and other contaminants, while the DNA passes the column in the exclusion volume. DNA isolation and purification can be automated through the use of a pipetting robot and a special one-step procedure (Nextec technology). PCR reactions with these samples can also be prepared automatically. The results of the multiplex PCR analyses are linked with the scanned identification number and saved in the animal data bank. This aspect is very important for studying traceability and domestic animal biodiversity. Gel electrophoresis of NCC™ purified DNA from 88 TypiFix™ eartag samples : 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/ EtBr gel. The DNA concentration is about 10 ng/µl or greater = negative control. These higly-performant analyses are conducted at the well-known AGROBIOGEN laboratories in Germany, by Prof. Brem and his team, with their patented methods (method described by Ipate Judith et al. 2008-2010 and A.T. Bogdan et al. 2009-2010).

3 Results and discussion
Determination of genetic diversity of resistance to scrapie
Contact area: state and trends of components of biodiversity
Key Indicator: Trends in genetic diversity of resistance to scrapie
Reason determining indicator: selecting a core of prion disease-resistant animals
Current status: 188 sheep resistant to scrapie
Presentation indicator in the herd studied

The analytical results of samples taken from the project area 41 samples had arginine (R) at codon 171 of prion protein, which confers resistance to structural changes of scrapie. Also present alanine (A) at codon 136 confers resistance to structural changes associated with scrapie. The results of country analysis shows evidence Hateg 102 alanine (A) at codon 136 that confers resistance to scrapie prion structural changes. The presence of glutamine (Q) or histidine at codon 171 may send some characters of resistance to scrapie that was not detected in these samples. The analytical results from 90 samples the presence of glutamine (Q) in codon 171 of prion structural changes that confer resistance to scrapie prion. But classes G5-5 genotype 4 samples were detected with G4 genotype (ARR / VRQ) and 5 evidence-G5 (VRQ / ARQ), which are capable of prion disease. Owners were notified and they took the decision to isolate those animals and not use them for breeding. This way it is possible to make the selection of individuals with the most valuable genotypes resistant to diseases such. In period 2009-2010 were collected other samples, which revealed other valuable genotypes in the Hateg county area. The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. All the probes have in the 136 codon the alanine. The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep. The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep. But for genotype class (genotype classifications by the German Society of Animal breeding-DGfZ in Totesti we detected 12 probes with genotype G4 (ARR/VRQ) and 7 probes with - G5 (VRQ/ARQ) who is susceptibility from scrapie disease. The farmers it was notifie and the farmers
took the decision to isolate those animals and not use them for breeding.

For discussion of these results, with the method shown in figure 3, it can be shown complex relationship between scientific progress of the knowledge society with sustainable development of humanity and the role of international public health organizations (WHO, OIE).

4 Conclusions

3.1. It was analysis the prion protein for scrapie resistance genotyping as codon- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype. The TypiFix™ ear tag system is simple, one-step collection and preservation of tissue samples. The TypiFix™ ear tag system is fast, fully-automated and economical preparation of DNA.

3.2. In Totesti we detected 12 probes with genotype G4 (ARR/VRQ) and 7 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease. The farmers it was notified and the farmers took the decision to isolate those animals and not use them for breeding.

3.3. The best method for preventing scrapie from occurring in a flock or herd is to maintain a closed flock/herd, particularly with regard to breeding females. Any replacement females or breeding males should originate from flocks/herds not known to be affected with scrapie and under management practices precluding the introduction of scrapie or, in the case of sheep, should be of resistant PrP genotypes.

3.4. Were analyzed and identified valuable genotypes, which confer resistance to scrapie. In the future it is possible to create free herds by selecting animals. All genotypes identified will be introduced valuable gene bank.

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References:


Figure 2. Genotypes valuable in Totești

Figure 3. The complex nature of the principles, targets and consequences for the concepts of agrifood independence and sovereignty, based on More animal production