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ESTIMATION OF GENETIC PARAMETERS FOR SCRAPIE RESISTANCE IN LOCAL BREEDS OF SHEEP RAISED IN ROMANIA

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Abstract

In the European Union, animal breeding programs have been implemented to increase scrapie resistance in sheep. In addition to increasing animal resistance to an infectious disease, selection for pathogen resistance has the potential to lessen the transmission of the pathogen to offspring, particularly when the population under consideration may serve as the primary pathogen reservoir. Several sheep populations from Romania were used in this study: Tsurcana; Tsigae; Merinos; Cap Negru de Teleorman and some imported breeds: Suffolk; Ille de France and Awassi. Sanger sequencing method was used to identify PRNP genetic polymorphism, at 136, 154 and 171 codons. From the analyzed samples, a moderate share of the homozygous ARR allele, responsible for the highest resistance to scrapie, respectively (R1), was observed, and the heterozygous ARR/ARQ (R2) and homozygous ARQ/ARQ (R3) genotypes had an abundance of over 50% of the genotype panel. In practice, the use of phenotype as input to the model is given by numbers (risk classes from 1 to 5). To estimate the heritability of resistance to scrapie, the animal threshold model was used.

Key words*: scrapie, genotype, sanger sequencing, trehold animal model, heritability.*

INTRODUCTION

Sheep and goats are susceptible to the transmissible spongiform encephalopathy (TSE) known as scrapie, which has a protracted incubation period and progressing neurological symptoms like tremors, pruritus, and ataxia (Jeffrey & Gonzalez, 2007). The disease is brought on by an accumulation of improperly folded prion protein in the brains of infected animals, which results in the death of neuronal cells and the development of sponge-like vacuoles (Mabbott, 2020). There is currently no cure or vaccine for scrapie, and the precise mechanisms underlying prion pathogenesis and transmission remain poorly understood.

The potential transmission pathways for scrapie have recently come to light and include direct contact with infected animals, contaminated environments, and vertical transmission from ewes to their offspring (Konold et al., 2008). Additionally, extensive research into the genetics of scrapie susceptibility in sheep led to the identification of a number of prion protein gene (PRNP) polymorphisms that are related to varying degrees of disease susceptibility (White et al., 2008) with the identification of several prion protein gene (PRNP) polymorphisms associated with varying degrees of disease susceptibility, genetic susceptibility to scrapie has been thoroughly investigated (Jeffrey & Gonzalez, 2007). Numerous studies have shown scrapie to be heritable, indicating that genetic factors significantly influence disease susceptibility (White et al., 2008).

According to genetic studies Otelea et al. (2011) consider that PRNP polymorphisms at codons 136, 154, and 171 were strongly associated with classical scrapie. When this polymorphism is combined, it results in five PRNP codon haplotypes or alleles and fifteen PRNP diploid genotype combinations found in sheep. The susceptibility of sheep to scrapie varies greatly between genotypes, ranging from high resistance (ARR/ARR) to severe vulnerability (VRQ/VRQ). The primary goal of this study is to estimate the heritability for scrapie resistance of rams accepted for reproduction and raised on Romanian territory, with a secondary goal of presenting a common method of screening for scrapie resistance.

MATERIALS AND METHODS

The method and materials used for DNA extraction, amplification of the segment of

interest and genomic sequencing, is similar to the methods used by other researchers Otelea et al. (2011). For this study, 3918 samples belonging to different breeds raised in Romania were used. Blood samples were collected in 10 ml tubes containing K3-EDTA anticoagulant and were kept at -20 degrees Celsius until DNA extraction. Thermo Fischer Pure Link Genomic DNA mini Kit was used for DNA extraction. The obtained DNA was quantified to verify the quantity and purity using a Nanodrop One spectophotometer. The clasic PCR technique was used to amplify the DNA sequence,The PCR was set up in a volume of 25 µl with 2 µl DNA solution, the volume of reagents for 1 reaction: 0.125 µl Ampli GoTaq Polymerase-Promega (5U/µl), 1.5 µl MgCl2 (25 mM), 0.5 µl dNTP mix (10 mM), 5 µl buffer 10 x for polymerase, 0.6 µM of forward primer(5'-GGTCAAGGTGGTAGCCACAGTCAGTGG AAC-3') and 0.6µM of reverse primer (5'-ATCACCCAGTACCAGAGAGAATCCCAG GCT-3'). The thermal cycling program included: a denaturation (3 min at 95°C), 40 cycles of amplification (45 s at 95°C, 45 s at 59°C, 60 s at 72°C), and a final extension (7 min at 72°C). It was used an Thermal Cycler from Thermo Scientific. The electrophoresis migration method was used to check the quality and the lenght of the amplified segment. Agarose gel electrophoresis. PCR products (7 µl) were separated on a 10% agarose gel (70V for 45min) containing ethidium bromide in TBE buffer (10 mM Tris, 2.75 g boric acid/l, 1 mM Na2 EDTA). The visualization was performed in UV transilluminator (Bio-Rad) and the images were captured with a software from Bio-Rad (Otelea et al., 2011).

Sequencing: The PCR reaction products were purified with ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™ Foster City, CA, USA). The DNA sequencing reactions were done using BigDye Terminator Kit v3.1 (Applied Biosystems). The precipitation of DNA sequencing product was performed with BigDye XTerminator® Purification Kit, (Applied Biosystems, Foster City, CA, USA). The primers used for sequencing were the same as for the PCR amplification. The sequencing was performed on 3300 Hitachi Genetic Analyzer (Applied Biosystems) (Otelea et al., 2011).

RESULTS AND DISCUSSIONS

After analyzing the samples from 2019 and 2020, it was observed that of all 15 possible combinations of alleles responsible for the degree of susceptibility to scrapie, only 13 were expressed, from the 2019 genotype table, the ARH/ARH homozygous and AHQ/ARH heterozygous genotypes are missing, and in the 2020 population, ARQ/ARH and ARQ/AHQ heterozygous genotypes are missing. The homozygous ARR/ARR allele was observed in 16.8% of the 2019 population with a slight upward trend in the 2020 population, respectively. 17.98%. A considerable similarity between the two populations would be related to the abundance of the heterozygous ARR/ARQ genotype, which occupies approximately half of the genotype panel (38.79% - 2019)/(40.30% - 2020). A considerable frequency of homozygous ARQ/ARQ genotype was also observed. ARR/ARH and ARQ/ARH have a low prevalence, and the other genotypes are found sporadically in populations (Figures1 and 2).

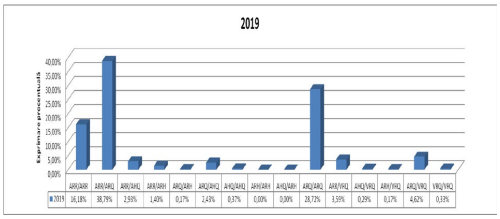

Figure 1. Distribution of genotypes for the breeds tested in 2019

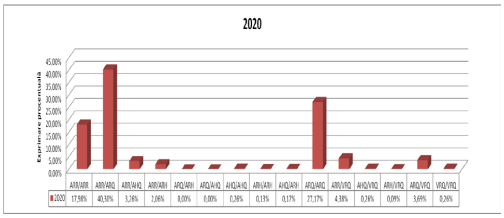

Figure 2. Distribution of genotypes for the breeds tested in 2020

From the samples analyzed in 2021, a moderate distribution of the homozygous ARR allele (R1), responsible for the highest resistance to scrapie, was observed, and the heterozygous ARR/ARQ(R2) and homozygous RQ/ARQ(R3) genotypes had an abundance of over 50% of the panel of genotypes. Although the distribution of

genotype frequencies seems similar to that of 2019 and 2020, in 2021 the share of breeds for testing was very small, implicitly the number of individuals tested was much smaller (Figure 3).

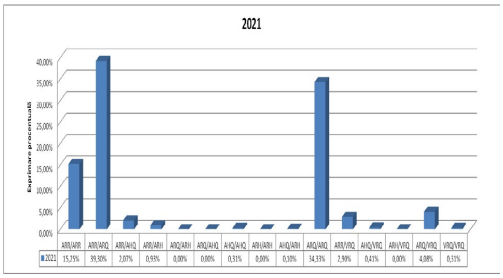

Figure 3. Distribution of genotypes for the breeds tested in 2021

In the context of scrapie susceptibility in sheep,we used the threshold animal modelto estimate the heritability of the trait by analyzing data from genotyping samples and measuring scrapie susceptibility in different animals.

The animal threshold model is a statistical approach used to estimate the heritability of scrapie in sheep, which assumes that there is a continuous underlying liability to the disease, with animals crossing a threshold to become clinically affected. This model estimates the proportion of variance in liability that is due to genetic factors, providing valuable insights into the genetic factors that influence disease susceptibility and resistance. Several studies have used the animal threshold model to estimate the heritability of scrapie in different sheep populations, with heritability estimates ranging from 0.28 to 0.44. While the animal threshold model has some limitations, it remains one of the most widely used methods for estimating the heritability of scrapie in sheep (Houston et al., 2003; Pálsson et al., 2004; Toppinen et al., 2008).

The model allows researchers to control for factors such as environmental factors that may influence scrapie susceptibility, and to estimate the genetic variance and covariance of the trait.

Since the classification of animals into risk classes - classes from (R1) to (R5) - does not have a normal distribution for estimating the heritability for the 7 sheep breeds, the animal threshold model was used. The model is composed of the incidence matrices and their associated vectors.

The obtained results revealed that sheep resistance to scrapie has a variable genetic determinism, from highly heritable (0.925 in the Tsurcana breed and 0.801 in the Merinos breed) to weakly heritable (0.563 Tsigae, 0.376 Cap Negru de Teleorman; 0.325 Suffolk; 0 .31 Ille de France and 0.194 Awassi) (Table 1).

Other study has investigated the heritability of scrapie susceptibility using genetic approaches such as quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS). These studies aim to identify specific regions of the genome that are associated with scrapie resistance or susceptibility (Houston et al., 2003).

Moreno et al. (2010) observed that heritability of polygenic and QTL effects for log-transformed incubation time in different PrP populations is moderate, ranging from 61% ($p = 0.05$) to 74% ($p = 0.04$) at different significance levels.

$$\searrow_{ijk} = f(t)_i + H_j + a_k + e_{ijk}$$

Figure 4. Animal threshold model vectorial writing

$$\searrow = Ft + Xb + Zu + e$$

$$\begin{bmatrix} Q & L'X & L'Z \\ X'L & X'WX & X'WZ \\ Z'L & Z'WX & Z'WZ + G^{-1} \end{bmatrix} \begin{bmatrix} \Delta t \\ \Delta b \\ \Delta u \end{bmatrix} = \begin{bmatrix} p \\ X'v \\ Z'v - G^{-1}u \end{bmatrix}$$

Figure 5. Animal threshold model in matrix writing

Table 1. Estimated heritability for each breed using the threshold animal model

Breed	Observations	σ_a	σ_e	σ_t	h^2
Awassi	14	1.103	4.583	5.686	0.194
Ille de France	41	1.626	3.615	5.241	0.31
Suffolk	37	1.215	2.514	3.73	0.325
Cap Negru de Teleorman	106	3.679	6.096	9.775	0.376
Tsigaie	242	7.123	5.529	12.652	0.563
Merinos	822	21.873	5.428	27.3	0.801
Tsurcana	2656	68.587	5.552	74.14	0.925

σ_a-additivevarriance
σ_e- error of varriance
σ_t-totalvariance

Following the analysis of the obtained data in Table 1, it was observed that in the imported breeds: Ille de France, Awassi, Suffolk, even if the individuals were classified in resistant classes (R1); (R2), the weaker genetic

determinism was also influenced by the number of genotyped individuals. This is due to the fact that larger populations are more likely to contain a broader range of genotypes, which can aid in capturing more genetic variation in the trait of interest.
If the population is small, individuals may have limited genetic variation, resulting in an underestimation of heritability. In contrast, a large population may have more genetic variation among individuals, resulting in a more accurate estimation of heritability.

CONCLUSIONS

The animal threshold model, by estimating the heritability of scrapie susceptibility, can help identify the genetic factors that contribute to the disease and can inform strategies for controlling and preventing the disease through selective breeding programs.
Overall, the animal threshold model is an effective tool for determining the genetic basis of complex traits like scrapie susceptibility in sheep, and it has been widely used in animal breeding and genetics research.
The animal threshold model has some drawbacks, such as the assumption of ongoing vulnerability to the disease and the requirement for precise clinical case diagnosis. But it is still one of the most popular techniques for determining the heritability of scrapie in sheep, and it has given us important knowledge about the genetics of disease susceptibility and resistance.
Sanger sequencing is a trustworthy method for identifying specific genetic mutations linked to scrapie resistance or susceptibility in sheep and goats. It can determine whether an animal has a genotype associated with a higher or lower risk of developing scrapie by sequencing specific regions of the prion protein gene. This data can be used to make informed breeding decisions and to develop targeted scrapie control programs in livestock populations.
To completely comprehend the intricate genetic and environmental components that contribute to scrapie susceptibility in sheep, more research is required.
Sanger sequencing can be an effective research technique, but to fully understand the underlying mechanisms, it should be used in conjunction with other methods like gene expression analyses and functional studies.
It is important to note that scrapie heritability is only one factor that influences the disease's occurrence. Environmental factors, such as feed contamination, can also have a significant impact.
As a result, both genetic and environmental factors should be considered in flock management and disease control.

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