Paradox of Genetic Diversity in the Case of Prionic Diseases in Sheep Breeds from Romania

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Abstract
The main target of this debate is the revaluation of the biodiversity concept and especially of its significance in the animal husbandry field. The paper analyzes the genetic diversity at the determinant locus of scrapie (*PrP)* in the sheep breeds from Romania: Palas Merino, Tsigai, Tsurcana, Botosani Karakul, Palas Meat Breed and Palas Milk Breed. The prionic genetic diversity (***d***) has been quantified by means of informational energy (***e***). This study highlights the impact of increasing the genetic diversity from the *PrP* locus level on the health status of ovine species and especially on human food safety. The informational statistics processing shows that the resistance/susceptibility to scrapie is in relation to the degree of prionic genetic diversity. The limitation of genetic diversity by selecting the individuals possessing the ARR allele in both homozygous status and in combination with alleles ARQ, ARH AHQ confers to sheep flocks certain levels of resistance to contamination with scrapie disease. Instead, promoting to reproduction also individuals possessing the VRQ allele in all possible genotypic combinations (including ARR allele) increases genetic diversity but also has as effect increasing the susceptibility of sheep to prion disease onset. From the point of view of morbid phenomenon, the Botosani Karakul breed is clearly advantaged compared to all other indigenous sheep breeds from Romania. For methodological coherency in the interpretative context of this issue, the genetic diversity was analyzed in association with the heterozygosity degree of breeds and their Hardy-Weinberg genetic equilibrium at the *PrP* locus level. Finally, the paper refers to decisions that the improvers must take to achieve the genetic prophylaxis in the scrapie case taking into account the polymorphism degree of prion protein.

Keywords: genetic diversity, informational energy, scrapie, sheep

1. Introduction

The biological diversity or biodiversity is one of the most modern concepts of life sciences. The term of "biological diversity" was invented by Raymond F. Dasmann in 1968 [1], a well-known conservative biologist. But only in 1980, this concept was presented to the scientific community by Lovejoi [2] when it began to be used in science and environmental policies.

Biodiversity is the variety of life and all phenomena that give to it this unique essential attribute. Biodiversity includes all life forms, from simple unicellular structures to complex organisms with superior architectures organized from a biological perspective, their metabolic pathways and cycles that maintain them life, cohabitation way of different populations of plants and animals in the biocenotic context, as well as their relations with the biotope within ecosystems [3].

The definition of biological diversity in its true meaning was adopted by The United Nations Conference on Environment and Development (UNCED), also known as the Earth Summit, that took place in Rio de Janeiro, Brazil, in June 1992, as "*the variability among living organisms from all sources, including terrestrial, marine, and other aquatic ecosystems, and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems*". At

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the same time, the UN Convention on Biological Diversity recognizes the importance of genetic resources of livestock, so that the sure connection of biodiversity with food, agriculture and environment is an institutionalized relationship [4]. Agricultural biodiversity-a subset of global biodiversity-is essential for satisfying the basic human needs regarding food security [5]. The biodiversity of farm animals is a particular biodiversity type created by man by means of artificial reproduction isolation, from which the breeds and breeding lines of domestic animals resulted like populations [6]. The animal genetic resources for food and agriculture are essential components of the biological basis for world food security [7, 8].

Unanimously, most researchers agree the axiom that biodiversity is absolutely beneficial to man and to his activities in agriculture. All of them base their motivation that in genetics the character variability is the action field of selection and represent an essential prerequisite for achieving the genetic progress in animal populations through which morphological and-production types of high economic performance are obtained.

We believe that this exhaustive assessment should be regarded with a certain reserve. We put our supposition in question starting from the circumstances of animal pathology. In conditions of livestock operations both in intensive systems and especially in the current social and economical situation of private farms it must be applied specific programs of continuous improvement of animal populations with the main and immediate purpose to increase the productions on each animal. In order to obtain maximum effect of selection for the features of economic interest, the new progeny generations must come from the most valuable parental couples, the individuals which do not meet minimum improvement requirements to be removed from reproduction. But in these conditions, the use of phenotypically valuable specimens for reproduction, but which are carriers of undesirable genes that are associated with some pathological conditions will cause wide spread of such abnormalities in offspring. In this way, the frequency of these genes will increase in populations in a fast rhythm and with the large number of these gene carriers the mating among them will inevitably occur. The situation is even more alarming if the undesirable gene has lethal effect causing significant mortalities. The more the undesirable gene frequency increases, the more the mortality number also will be higher and consequently the economic losses will be more considerable. In these conditions, when the frequency of genes reach highs, the interventions for detection of carrier individuals and their removing from reproduction involves widely and very expensive actions.

This is the case of scrapie disease in sheep, too. Scrapie belongs to a group of diseases known as transmissible spongiform encephalopathies caused by prion agents which affect both man, but mostly different species of farm animals bearing specific names. Scrapie is a degenerative, chronic and extremely serious disease with a terrible prognosis. The resistance of sheep to scrapie is given by the ARR allele and their susceptibility to this disease is associated with the presence of VRQ allele. In this regard, European and global organizations, involved in animal pathology, have developed programs for scrapie monitoring and for its eradication by increasing the individuals that possess ARR allele and removing the individuals in whose genetic structure the VRQ allele fall in various genotypic combinations [9]. However, through these actions, the genetic variability at PrP locus decreases, locus that can be linked with other loci responsible for productivity traits. So by such actions, the genetic diversity decreases.

Therefore, in this study, we have proposed to quantify the genetic diversity at the PrP locus level in sheep breeds from Romania using modern concepts of informational statistics [10, 11]. We want to demonstrate that the biodiversity should not be always invoked in absolutely favourable terms but it must be interpreted with some caution.

2. Materials and methods

The experiments were performed on sheep populations belonging to breeds reared and exploited in their specific habitats from Romania: Palas Merino, Palas Meat Breed, Palas Milk Breed (in Dobrudja) Tsigai (in northern Wallachia and in central and southern areas of Moldovia), Tsurcana (in south-western Transylvania) and Botosani Karakul (in north-eastern Moldavia). The size of populations analyzed of these breeds is shown in Table 1.

The biological material consisted of blood samples taken from animals into vacutainers containing EDTA as anticoagulant.

Sheep genotyping at the PrP locus to identify the polymorphisms at level of codons 136, 154 and 171 of the prion gene was done by Real-Time PCR technique [12-14].

The degree of *genetic diversity* (***d***) of these sheep breeds at the PrP locus was quantified by means of a concept derived from the informational statistics named the *informational energy* (***e***) [15].

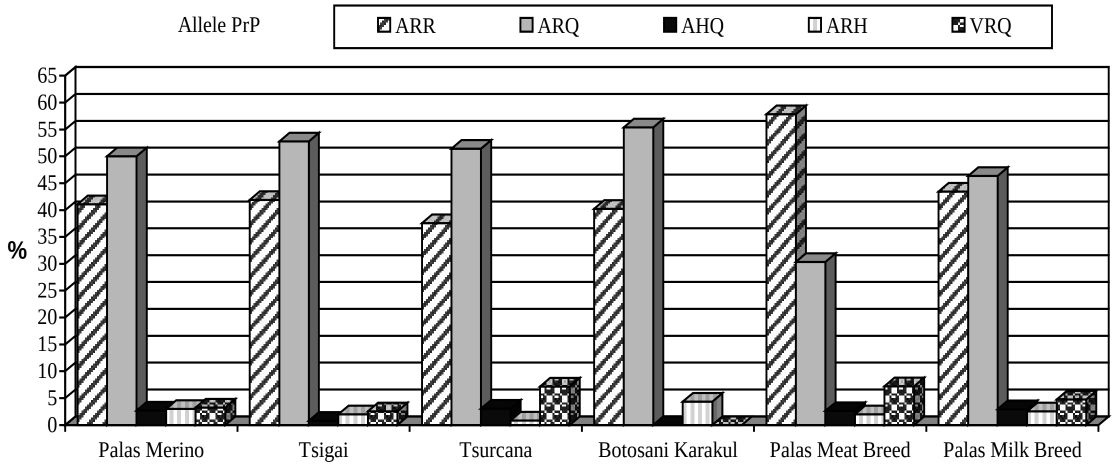
The *heterozygosity degree* of sheep breeds was evaluated also by an informational statistical formula [15].

The status of genetic equilibrium at the PrP locus was estimated by means of the χ^2 test (chi square).

3. Results and discussion

The experimental data panel after which the two informational statistics parameters (informational energy and genetic diversity) were calculated was represented by the allele frequencies from the PrP locus of each sheep breed. Usually, in ovine species, there are five alleles at the PrP locus which have implications in the pathogenesis of this spongiform encephalopathy type. These alleles are: ARR, AHQ, ARH, ARQ and VRQ, this being their order in relation to the downward / upward trend of resistance/ susceptibility level to scrapie [13, 14, 16, 17].

All five alleles are found in the sheep breeds from Romania, except the Botosani Karakul in which there are only three alleles, the AHQ and VRQ alleles are missing. The prion alleles have an uneven spread in all breeds. There are two alleles that have a significant representation: ARR and ARQ. Of these, the ARQ allele records the highest frequencies, ranging from 46% (Palas Milk Breed) to 55% (Botosani Karakul). In Tsigai and Tsurcana the incidence of this allele fluctuates around 50%. Palas Meat Breed is the only breed at which the ARQ allele recorded less frequency (30%) than the ARR allele. In fact in this breed, the ARR allele is the most common (58%) compared to all other breeds at which the frequency of this allele varies within a very narrow range, between 40% and 43%. The AHQ and ARH alleles are found in around 2-3% individuals of Palas Merino breed and of the too breeds derived from it. The incidence of AHQ allele is 3% in Tsurcana and of ARH allele is 2% in Tsigai. The alleles AHQ from Tsigai and ARH from Tsurcana recorded the lowest frequencies (0.8%) in almost all breeds. The VRQ allele occurs in all breeds (except for Botosani Karakul) a higher percentage than AHQ and ARH alleles. Worryingly for the presence of this allele and of its incidence is that it is associated in the highest degree with the prion disease. If in the Palas Merino and Tsigai its frequency is 2-3%, it becomes more present in the Palas Milk Breed (5%), exceeding 7% in Tsurcana and Palas Meat Breed (Figure 1).



χ^2=4.93 χ^2=5.12 χ^2=10.29 χ^2=29.74*** χ^2=1.09 χ^2=9.28

Figure 1. Allele frequencies at the PrP locus in Romanian sheep breeds

By the combinatorial capacity of the five alleles there could be expressed 15 possible genotypes classified in five risk classes, demonstrating the emphasized polymorphism at this locus. All these genotypes are found only in Palas Meat Breed. The fewest genotypes (six) are in Botosani

Karakul (ARR/ARR, ARR/ARQ, ARR/ARH, ARQ/ARH, ARH/ARH and ARQ/ARQ). In Tsigai three genotypes are missing: AHQ/AHQ, AHQ/ARH and AHQ/VRQ. In the Palas Merino the genotypes ARH/ARH, AHQ/ARH, AHQ/VRQ, ARH/VRQ and VRQ/VRQ are not expressed. Six genotypes are lacking in the prionic table, ARR/ARH, ARH/ARH, AHQ/ARH, AHQ/VRQ, ARH/VRQ and VRQ/VRQ in Tsurcana breed, respectively AHQ/AHQ, ARH/ARH, AHQ/ARH, AHQ/VRQ, ARH/VRQ and VRQ/VRQ in Palas Milk Breed (Table 1).

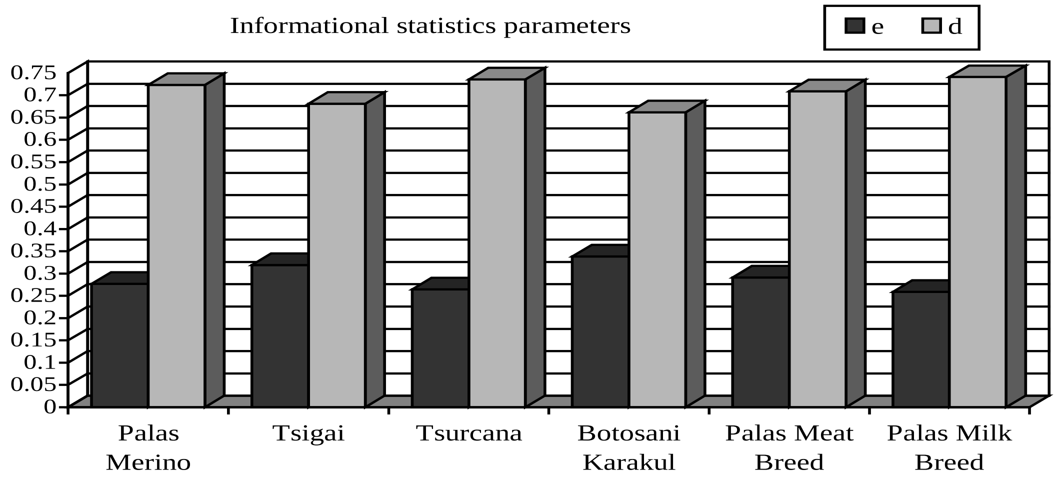
As in the allele case, the PrP genotypes have a considerable distributional variability in the prionic panel of each breed starting from their absence up to value of 50%. However, all breeds have several common characteristics regarding the genotypic ranking. In all breeds the prionic panel is predominated by the genotypes ARR/ARQ, ARQ/ARQ and ARR/ARR. In most breeds the ARR/ARQ genotype is the most common, its presence being between 30% and 50%. By 25-30 percents the ARQ/ARQ genotype takes the second place in this table. The third position is occupied by ARR/ARR genotype with moderate frequencies (15-16%) in Palas Merino, Tsigai and Palas Milk Breed; its frequency is higher in Tsurcana (20%) and becomes significant in Botosani Karakul (27%) and especially in Palas Meat Breed (32.89%). The Botosani Karakul breed differs from the other breeds because within it the most frequent genotype is ARQ/ARQ (44%), followed by ARR/ARR (27%) and the ARR/ARQ genotype occupies the third position (21%). The other genotypes with a few exceptions achieved low frequencies ranging from 0.1% to 4%. The exceptions arise at ARR/VRQ genotype (7%) in Palas Meat Breed and at ARQ/VRQ genotype (10%) in Tsurcana.

Table 1. Frequencies of PrP genotypes and their classification into risk groups for scrapie in Romanian sheep breeds

Risk class	Genotip *PrP*	Breed					
		Palas Merino n=185	Tsigai n=376	Tsurcana n=245	Botosani Karakul n=195	Palas Meat Breed n=900	Palas Milk Breed n=137
Class 1 (R1)	ARR/ARR	16.76	16.22	19.18	27.18	32.89	15.3
Class 2 (R2)	ARR/ARQ	41.08	48.14	29.39	20.51	34.67	40.9
	ARR/AHQ	2.70	0.53	3.27		2.89	3.6
	ARR/ARH	2.70	1.33		5.64	2.55	4.4
	Total	46.48	50.00	32.66	26.15	40.11	48.9
Class 3 (R3)	ARQ/ARH	3.24	1.60	1.63	2.05	*0.89*	0.7
	ARQ/AHQ	1.62	1.06	2.04		1.67	2.2
	AHQ/AHQ	0.54		0.41		0.11	
	ARH/ARH		0.27		0.51	0.11	
	AHQ/ARH					0.22	
	ARQ/ARQ	24.87	26.06	29.80	44.11	9.89	23.4
	Total	30.27	28.99	33.88	46.67	12.89	26.3
Class 4 (R4)	ARR/VRQ	2.16	1.33	4.08	0.00	9.78	7.3
Class 5 (R5)	AHQ/VRQ					0.22	
	ARH/VRQ		0.53			0.11	
	ARQ/VRQ	4.33	2.66	10.20		3.67	2.2
	VRQ/VRQ		0.27			0.33	
	Total	4.33	3.46	10.20	0.00	4.33	2.2

The number of PrP alleles, the value of their frequencies and especially their combinatorial manner to express the prion genotypes are decisive elements for the definition of informational statistical coefficient size. The informational energy (***e***) and genetic diversity (***d***) are complementary operational concepts, their values being inversely proportional sizes. The more the allele number is, the allele distributions are more balanced and their combinatorial capacity is greater (by expressing the multiple genotypes), the more the informational energy decreases and genetic diversity increases and vice versa, the two parameters values complementarily ranging between 0 and 1 [10, 11, 15]. The genetic diversity assessed in this way shows, in a quantified expression, the variability degree of prionic system in sheep. In all Romanian sheep breeds the informational energy coefficients have moderate values (below 0.35) showing that these breeds possess a considerable variability of the genetic structure at the PrP locus level (Figure 2). In informational statistical terms the variability margin of these breeds at this locus is 0.65-0.75. The aspect that differentiates these breeds is the prionic variability degree.

The statistical processing indicate that in the Botosani Karakul breed there is the highest value of informational energy which determines the lowest genetic diversity at this locus. Conversely, the lowest informational energy recorded in the Palas Meat Breed makes from this the most variable Romanian sheep breed in the respect of this structure. The other breeds have a high genetic diversity degree (over 0.7); only in Tsigai breed the genetic diversity decreases slightly below this threshold (0.68). In this context, the genetic diversity coefficient, calculated by means of informational energy, reflects the polymorphism degree of prionin system in each breed.



$$\mathbf{e}^{\text{Botosani Karakul}} > \mathbf{e}^{\text{Tsigai}} > \mathbf{e}^{\text{Palas Milk Breed}} > \mathbf{e}^{\text{PalasMerino}} > \mathbf{e}^{\text{Tsurcana}} > \mathbf{e}^{\text{Palas Meat Breed}}; \Rightarrow$$

$$\mathbf{d}^{\text{Botosani Karakul}} < \mathbf{d}^{\text{Tsigai}} < \mathbf{d}^{\text{Palas Milk Breed}} < \mathbf{d}^{\text{PalasMerino}} < \mathbf{d}^{\text{Tsurcana}} < \mathbf{d}^{\text{Palas Meat Breed}}$$

Figure 2. Coefficients of informational energy (**e**) and genetic diversity (**d**) at the PrP locus in Romanian sheep breeds

On the other hand, the size of the two indicators of informational statistics shows the systemic organization degree of autochthonous sheep breeds, size which is inversely proportional to the polymorphism degree of prionic system. According to those mentioned, the Botosani Karakulul has the highest degree of systemic organization, closely followed by Tsigai, while the other breeds present a higher degree of systemic entropy (disorganization).

In the same context, the genetic diversity degree is in agreement with the heterozygosity status of breeds at the PrP locus. Thus, the Botosani Karakul which has the lowest genetic diversity records the lowest heterozygosity degree, and in the Palas Milk Breed with the most obvious genetic diversity the highest heterozygosity status is met. A graphical representation of the two genetic parameters in all sheep breeds would look distributional curves with identical trajectories (Figure3, 4). On the other hand, the Hardy-Weinberg genetic equilibrium at the PrP locus is largely influenced by the genetic diversity degree. In the Botosani Karakul breed, with the lowest diversity, the genetic disequilibrium is very significant, while in all the other Romanian breeds there is an obvious concordance between the empirical and theoretical distributions of prionic genotypes, the χ^2 test having low and without statistical signification values (Figure 1).

In the context of these investigations, the main purpose of our scientific approach is the relevance of genetic diversity at the PrP gene regarding the prion pathology in local breeds from Romania. The disease is triggered by converting the normal protein (PrP^c) from brain in an isomorphic mutant (PrP^{Sc}) which determines disorders with prionic symptomatology [18, 19].

Resistance/susceptibility to scrapie in sheep are given by the risk class in which their prion genotypes are framed (Table 1).

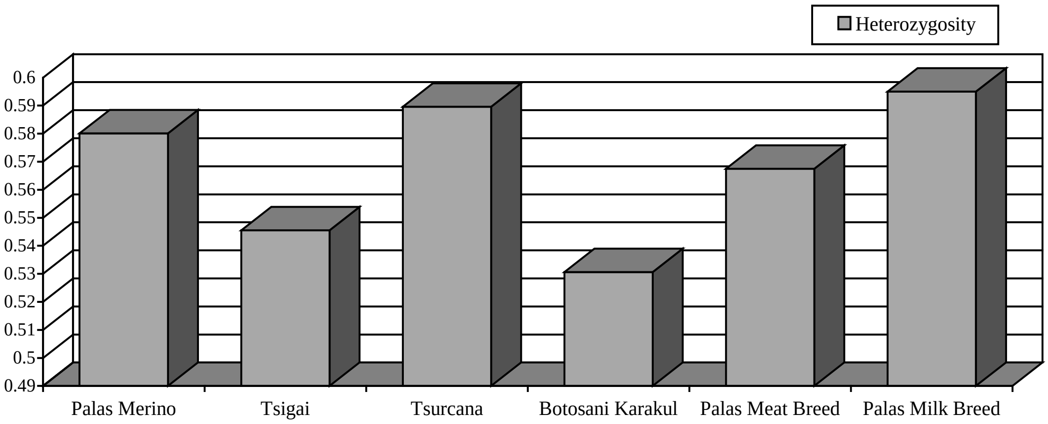


Figure 3. Heterozygosity degree (**Ht**) at the PrP locus in Romanian sheep breeds

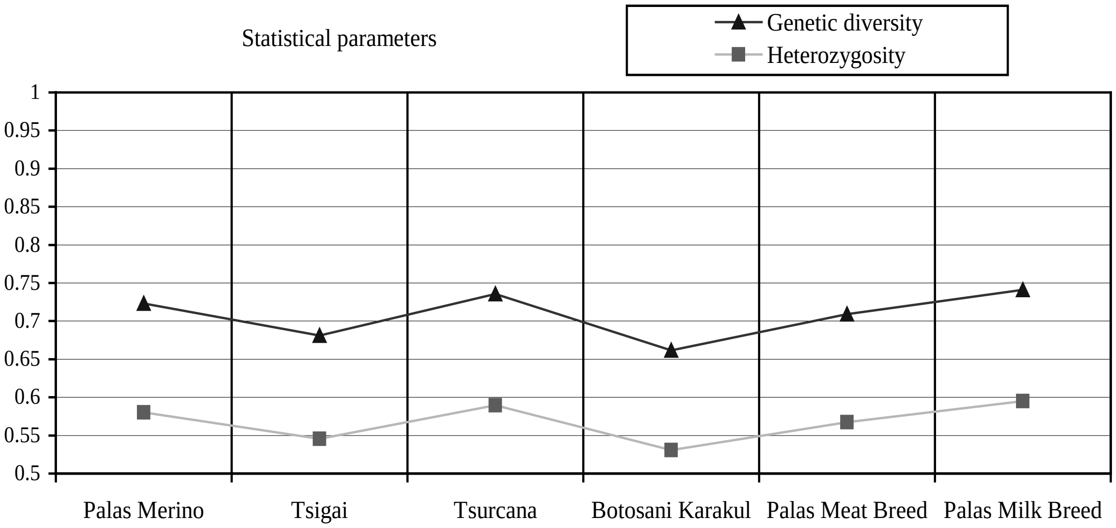


Figure 4. Distributional curves of genetic diversity (**d**) and heterozygosity (**Ht**) in Romanian sheep breeds

The most resistant sheep to scrapie are those possessing the ARR/ARR genotype constituting the first risk class (R1). Also, sheep with genotypes of the second risk class (R2) show resistance to contracting the scrapie but mating scheme of couple partners must be done carefully. The third risk class (R3) comprises less resistant sheep to scrapie but they can be used for reproduction, the main condition being that the mating of partners to be made with great accuracy. The following two risk classes become problematic on sheep promoting for reproduction. The fourth risk class (R4) includes sheep susceptible to scrapie and sheep belonging to the fifth risk class (R5) are the most susceptible to the disease manifestation. Individuals from the last two classes should not be used for reproduction in order not to disseminate the genotypes associated with scrapie in the highest degree. Some authors argue that adopting a tightly controlled breeding program there could be used for herd multiplying also sheep with genotypes of the fourth risk class [20, 21]; but we believe that, according to the implications of these genotypes in the scrapie spreading, this recommendation has no logical support.

Having regard to genetic structure of indigenous sheep breeds, it becomes evident that, except the Botosani Karakul, all the other breeds include individuals classified in all risk classes. The most frequent individuals belong to R2 class, their incidence ranging between 30% and 50%. Also, individuals in R3 class are common enough (25-30%). Individuals in R3 class record a significant percentage, too (25-30%). The most resistant genotype to scrapie, ARR/ARR (R1), has a moderate spreading (15-20%); only the individuals of this type belonging to Palas Meat Breed were most numerous (33%). Among these breeds the Tsigai is the most advantaged having the fewest individuals in these two classes (5%), but two thirds of them fall within R5 class. Tsurcana is the most exposed breed at risk of contamination with the disease by the presence of individuals in R4 class (4%), but especially of those in R5 class (over 10%). Palas Merino Breed comprises a slightly higher percentage of such individuals compared to Tsigai breed (6.5%); as in Tsigai, in Palas Merino, too, the individuals of R5 type are more frequently encountered (twice) than those of R4 type. In the biological creations that derived from Palas Merino the incidence of individuals predisposed to contract the prion disease is quite significant: 14% in Palas Meat Breed and 10% in Palas Milk Breed. Unlike Tsurcana, in these last two breeds a large part of individuals are enrolled in the R4 class (10% in Palas Meat Breed and 7% in Palas Milk Breed). The Palas Meat Breed, although comprises a substantial proportion of individuals endowed with the most resistant genotype to scrapie, also possesses individuals with high predisposition to contracting this disease.

The Botosani Karakul sheep is the only Romanian breed which is exposed to the lowest risk to get sick from scrapie; in this breed the individuals at high or maximum risk (4 and 5 levels) are missing, being an exceptional advantage for the genetic prophylaxis of this breed. Less than half (almost 47%) of individuals of the breed have genotypes belonging to the R3class. Approximately one quarter of individuals fall into the R2class. A very important aspect of the Botosani Karakul is that the ARR/ARR genotype (R1 class), with the highest resistance to scrapie, is quite often (a little over 27%).

So, the ARR allele in homozygous status confers maximum resistance to scrapie. But the ARR allele in heterozygous combination with the other alleles, excepting the VRQ allele, helps to achieve a certain degree of more or less increased resistance to installing this disease. Instead, the VRQ allele in both homozygous status and in combination with the other alleles, including the ARR allele, determines the expression of those genotypes which are associated to the highest susceptibility degree of sheep to scrapie (VRQ/VRQ, AHQ/VRQ, ARH/VRQ, ARQ/VRQ) or to a significant extent (ARR/VRQ), having a very low resistance to installing this disease.

These being the problem data, we can say that the possibility (probability) of scrapie installation in sheep from Romania is a variable size in directly proportional relation to the genetic diversity degree at the PrP locus level. If the diversity is low the sheep are more resistant to scrapie installation, as is the case of the Karakul breed. If the diversity increases, then also the pathological manifesting potential of prion protein increases, situation encountered in all the other breeds. Of these, the Tsigai breed possesses a lower diversity, being closer to the Botosani Karakul, this situation being in accordance with lower frequencies of individuals belonging to R4 and R5 classes. But the diversity increasing at the PrP locus is performed by phenotyping of those genotypes containing in their structure the VRQ allele associated with the morbid phenomenon.

Given the danger that the scrapie represents for human health and food security, most countries have developed programs to eradicate this disease. However, this goal can be achieved only through elimination from flocks of all individuals with genotypes containing VRQ allele and promoting for reproduction of the individuals with genotypes containing the ARR allele (but without ARR/VRQ individuals). But reaching this target means reducing the genetic diversity at PrP locus. From this point of view the Botosani Karakul meets all conditions for achieving the genetic prophylaxis. This condition of things is due to more intense selection pressure to which the Botosani Karakul breed is subject compared to the other breeds. More rigorous selection criteria applied to the Botosani Karakul sheep are mainly a consequence of specialization of this breed for lamb pelt production in six colour varieties, besides other production traits (meat, wool, milk) found in the other breeds, too. A probable condition to prevent expansion of this disease is the animal breeding in pure breed. The cross-breeding process used to create new hybrid entities of sheep determine the polymorphism increasing at the PrP locus, which entails the increasing of occurrence and spread possibility of prion disease. This happens in the Palas Meat Breed and Palas Milk Breed compared to the Palas Merino from which the first two breeds have derived.

So the question that arises is natural, not rhetoric. Biodiversity is good as an axiomatic principle?

Biodiversity is bad in certain situations? At least, a part of the answer can be detached from this study. Genetic diversity is a decisive factor for quality assurance in the animal husbandry field because it is the basis of improvement and evolution of animal breeds of economic and scientific interest. Therefore, not only the conservation of existing genetic resources but also the increasing of their biodiversity are essential attributes taken into account in animal science to the extent that these properties of living matter allows, by selection, a productivity performance gain, higher resistance to diseases and greater capacity to adapt by harnessing the heterosis. On the other hand, it is not the case for increasing the biodiversity that involve the genetic loci responsible for the manifestation of genetic diseases and those with hereditary predisposition, as in the scrapie case. The diseases with genetic substrate can spread rapidly in animal populations causing large zoo-economic losses. Estimating the spreading degree of any undesirable genes and ultimately assessing the probability of a reproduction animal to be the carrier of such genes are fundamental elements in the animal husbandry practice to achieve the genetic and veterinary prophylaxis. This situation requires restricting the variability at respective loci and application of those selection criteria to minimize or eliminate the negative impact on health status of animals. Such action should be done with pinpoint accuracy because the genetic diversity limitation for the morbid phenomenon might cause the genetic pool narrowing with some genes associated with superior parameters of animal productivity. In addition, a genetic depression could occur by installing the emphasized inbreeding. In the few studies on this issue, loss of genetic diversity for PrP gene as a result of directional selection for scrapie eradication in sheep has been observed in some European breeds, too. Some observations indicate that the diversity loss had a banal effect on other more distant loci from the PrP locus [21]. Other opinions reveal that the programs to stop the scrapie spreading can have negative consequences on the genetic diversity with hypothetical repercussions on production traits, reproduction indices, endogamy increasing etc. [22]. In order for the genetic diversity not to be irretrievably lost, some authors recommended that all individuals at high risk of contamination with prion protein should be excluded suddenly from the reproduction circuit so that the economic losses not to be too large [20].

4. Conclusions

Genetic diversity of sheep breeds from Romania at the determining locus of scrapie disease was quantified by means of informational energy concept.

Moderate values of informational energy coefficients (below 0.35) configures a considerable prionic genetic diversity in the autochthonous sheep breeds in the range 0.65-0.75 depending on the number of alleles and genotypes expressed at the PrP locus and on their distributions in the prionic panel.

The Botosani Karakul breed records the most restricted genetic diversity at the PrP locus compared to the other Romanian breeds which have a wider variability of this parameter.

Resistance/susceptibility to scrapie are related to the genetic diversity degree: the Botosani Karakul breed has the highest resistance to scrapie malady, while the other indigenous breeds are more susceptible to contamination with prionic agent especially sheep belonging to Tsurcana, Palas Milk Breed and Palas Meat Breed.

The association of the genetic diversity at the PrP locus with resistance / susceptibility to scrapie in Romanian sheep breeds indicates that the interpretation of biodiversity, of its conservation status and especially of its enhancing need to be nuanced and made with a certain caution.

Improvers must adopt the most correct selection criteria and apply the most appropriate mating schemes to eradicate scrapie, on the one hand, and in order not to restrict drastically the genetic diversity, on the other hand.

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