Conceptual Incongruence between Prion Disease and Genetic Diversity in Ovine Species within European Union defined by Informational Statistics Terms

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Abstract

Biodiversity and the studies of spongiform encephalopathies in the farm animals are highly topical concerns of the contemporary scientific world. Both themes are very interesting for the life sciences and very important for the application field of animal breeding. The implementation of these two concepts creates an antithetical paradigm: the achievement of genetic prophylaxis joins with the decrease of genetic diversity. The paper examines the genetic diversity and its evolution in sheep livestock from the European space in the context in which the European Community has developed very laborious and costly programs targeted both for conservation and enhancement of biodiversity and to eradicate the scrapie in small ruminants. This paper utilises a precise method to quantify the genetic biodiversity in all sheep populations in Europe by a modern concept derived from informational statistics - *informational energy*. In addition, the paper proposes concrete and viable solutions to achieve these two desiderata at optimal levels in connection with a perfect perspicacity of sheep breeder which consists in accuracy of the reproduction process and correct application of the selection criteria.

Keywords: *biodiversity, genetic diversity, scrapie, sheep.*

INTRODUCTION

Biodiversity or Biological Diversity is a highly topical term based on living individuals and species. From its defining, as a linguistic entity, by Raymond F. Dasmann in 1968, and so far, by various theorists and researchers the biodiversity acquired different interpretations, meanings and reasons to be used, however being close in semantics. But an unitary and essential conceptual acceptance was assimilated by different political, social and scientific authorities after the UN Conference on Environment and Development (Earth Summit) in Rio de Janeiro in 1992, in which the representatives of 170 states have established and ratified the most comprehensive international regulations related to the worldwide biodiversity. Disputed issues at the Rio Convention dedicated to the promotion of biodiversity have focused on

three main objectives: a) preserving, enhancing and protection of genetic resources, b) sustainable development of biodiversity components and c) fair and equitable sharing of benefits arising from the use of genetic resources. The biodiversity was structured on three successive hierarchical levels: genetic diversity, species diversity and ecosystem diversity, but all three levels include elements that are intermingled (UNCED, 1992). The value of biodiversity for humanity is more than academic. Urgent need to conserve and enhance the biodiversity, in all its details, consists in the interests of economic, scientific, cultural, ecological, ethical and social significance. The correct implementation of biodiversity policies brings huge benefits for mankind in the field of agriculture, industrial materials, medicine and pharmacy, cultural values, intellectual exercises,

environmental services, recreation etc. (ECC, 2001; GRFAC, 2011).

In the referred context, the biodiversity has become one of the most modern concepts of life sciences, being sometimes used in excess as a panacea. There are situations when the enhancement of biodiversity could be harmful to humanity. It is the case of animal pathology in which the use to reproduction of some valuable individuals, but also which are carriers of undesirable genes. The excessive use of such genitors would lead to the dissemination of morbid entities and therefore to an increase of pathological biodiversity too. It is well known that susceptibility / resistance to scrapie in sheep is a priority in the breeding and improvement of this species of economic interest.

It is obvious that the domestic sheep is a species with decisive source of the animal transmissible spongiform encephalopathies. Due to its pathogenic connotations, scrapie is approached as an integrated system of intense and profound biomedical, environmental and economic studies. For these reasons, the scrapie has been included in the European Programme of animal and human TSEs surveillance. Currently, scrapie is a disease which is targeted by all Member States of the European Community. It is framed in List B of the International Epizootic Office. The first program of scrapie eradication in sheep has been implemented by Great Britain early as 1996 (Hunter *et al.*, 1996). At European level, in November 1996, the Committee for Health and Food Safety adopted a Communication to the European Council entitled „An European initiative on research on transmissible spongiform encephalopathies”, due to worries regarding the transmission of bovine spongiform encephalopathy (BSE) through the food chain (Will *et al.*, 1996). Also then that, the Commission presented an action plan which identified the most important research priorities and budgetary requirements necessary to investigate the spongiform encephalopathies in farm animals. Since then, several countries have initiated the control on ovine scrapie. Thus an international scientific current of major attitude was created known as the management of small ruminant health on genetic marker bases. Its actions are directed towards the final targets as: resolving all issues of specific sheep pathology,

qualitative improvement of sheep productions, increasing their reproduction performance, reducing the financial costs for breeding of small ruminants (sheep and goats) (ECC, 2001; GRFAC, 2011).

In 2000, the Directorate General for Health and Consumer Protection of the European Commission has launched a widely program of monitoring, control and eradication of scrapie in sheep (EC, 2000). This disorder should be a present concern in the countries of Central and Eastern Europe. Eliminating or even eradication of ovine scrapie (or causative agents) from the populations of domestic small ruminant is a prerequisite for preventing the penetration of pathogenic ovine prions in the food chain of man (Novak *et al.*, 2000).

But with the scrapie eradication measures, also other genotypes associated with higher productivity traits of animals may disappear. Then the dilemma appears justified if the scrapie eradication must be done anyway and by any means or the negative consequences on narrowing of the gene pool with valuable potential and implicitly on drastically biodiversity decreasing must be evaluated too.

In the mentioned thematic framework, this paper makes an assessment of sheep from European space on the incidence of scrapie disease in the context of measures taken to its eradication in sheep populations in parallel with the evolution of genetic diversity at PrP locus in the first 10 years of the IIIrd millennium. The paper proposes a precise quantification of biodiversity in all sheep populations in Europe using a modern concept derived from statistical information - *informational energy* - a more synthetic parameter than those used in the mathematical (classical) statistics (Hrinca, 2015).

MATERIALS AND METHODS

The genotyping works of animals at the determinant locus of scrapie have been performed on numerous groups of sheep which are farmed in Europe without taking into account their breed affiliation. The sample size was large enough that the experimental results to provide reproducibility from statistical viewpoint. The experimental data of this study come from three informational sources:

- the most important source is represented by sheep populations from the 20 countries that acceded to the European Community until 2004

(Fadiaevsky *et al.*, 2008). The sheep livestock of these countries were monitored for 10 years, between 2000 and 2010; the analyzed sample size varied between 4,000 and 13,000 animals, depending on the experimentation year (Fadiaevsky *et al.*, 2008; EC, 2012; Beringue and Andreoletti, 2014; EFSA, 2014);

- a second source comes from the genotyping performed on approximately 1000000 sheep from the UK in the 1998-2006 period because the Great Britain made such analyzes separately too, having the precedent of bovine spongiform encephalopathy (Baylis *et al.*, 2004; DEFRA, 2005-2008; Reed, 2011);
- the last source contains experimental results performed on 2038 sheep belonging to all breeds from Romania after its accession to the EU (EC, 2013;); the experimental works took place between 2011 and 2013, when Romania joined the European Monitoring Program of spongiform encephalopathies in farm animals in accordance with Council Decision 2009/470/EC (Coşier, 2008; Otelea *et al.*, 2011, Hrinică and Vicovan, 2014).

The biological samples for analysis consisted of peripheral blood taken from the jugular vein in vacutainers containing EDTA Na₂ as anticoagulant.

Using the Real-Time PCR RFLP method, the genetic variants of prion protein have been identified in the genotyped animals (Hrinică and Vicovan, 2014).

The frequencies of alleles and genotypes identified at the PrP locus level were calculated. Also the prion genotypes were distributed in risk classes, being calculated their incidences in each class and on whole classes.

The informational energy indicator (*e*) was used to quantify the extent of polymorphism and genetic diversity (*d*) and the heterozygosity status (*Ht*) of sheep examined at the determinant locus of prion disease (Hrinică, 2015).

RESULTS AND DISCUSSION

As a general rule, quantifying the genetic diversity of the taxonomic entities (population, breed, species etc.) using the genetic markers and by informational energy is achieved by means of frequency analysis (qualitative, discontinuous characters) as statistical support. In fact, the sheep genotyping at PrP locus is not a strictly end in itself to analyze the morbid phenomenon, but the

central objective of this paper was quantifying the genetic diversity and its evolution within a certain timeframe in sheep from Europe using the prion structures as operational tools in the statistical calculation algorithm. Measuring the biodiversity sheep by concepts of informational statistics (introduced and promoted in the qualitative genetics by our recent papers) is the major goal of the study that confers it originality and innovative character.

In the scrapie case in sheep, the frequencies of the five alleles found at the PrP locus are used. Depending on the resistance downtrend of sheep to this malady the five prion alleles are: ARR> AHQ> ARH> ARQ> VRQ. In its essence, the program for the scrapie eradication in sheep was focused on ever more marked fixing of ARR allele in genotypes which confer resistance to this malady, on the one hand, but also removing as much as possible of prion genotypes containing VRQ allele that is associated with a greater susceptibility of sheep to contaminate with the pathogenic agent of scrapie. Also, the decreasing of the ARQ allele incidence has been aimed which, in its turn, causes a low resistance to disease (Matthews, 2005; De Vries *et al.*, 2005).

During the first years of observations in sheep from the European Community countries, the ARQ allele loaded almost half of the allelic panel. Also the VRQ allele, with a quarter of the allele fund was enough spread and the ARR allele was situated only on the third place (about 14%). The other two alleles, AHQ (9%), but especially ARH (4%), recorded lower incidences. After applying the selection works to increase resistance to scrapie, suddenly there is an increase of the ARR allele frequency to about 50% (in 2006), following the upward trend to over 65% (in 2010). A sudden frequency decline is observed in the VRQ allele case, registering the lowest incidence throughout the program selection, its presence in populations ranging between 1.2% and 2.3%. The ARQ allele achieved a reduction of its contribution to genotypes establishing, but its frequency has remained fairly considerable over the years ranging between 30% and 35%. The other two alleles, AHQ and ARH, reduce their incidences, too, but the declines are not significant (Fig. 1). So, during this implementation period of scrapie eradication program a significant increase frequency of ARR allele occurs, accompanied by

an incidence diminishing of the four other prion alleles.

In Great Britain, even at the beginning of experiments, the ARR allele was very common in sheep populations (approximately 44%). Also, the ARQ allele occupies a third of allelic panel. The VRQ and AHQ alleles, each of them with about 10% have a middle spread and the ARH allele is rarely met (4%). It is a sign that the action to eradicate scrapie in UK started earlier than in countries of the continental European Union. In subsequent years, we are witnessing a strong increase of the ARR allele frequency, so that in 2006 it reaches almost 70 percents. Simultaneously, decreasing the incidence of ARQ allele occurs by about 15%. If the allele AHQ diminishes slightly its presence, then the frequency increase of ARH allele is insignificant. The presence of VRQ allele is becoming more insignificant, decreasing to 3% in 2002 and 1% in 2006 (Fig. 2).

In sheep from Romania, with only one moment of reference (2013), half of allelic panel is occupied by the ARR allele and a quarter by the ARQ allele. The AHQ and ARH alleles are rarely met in

populations (about 2%). The VRQ allele, although it has recorded a relatively low frequency, its spread is significantly higher than in sheep populations of the European Community from the monitoring program termination (Fig. 3). However, the sheep livestock of Romania being at their monitoring beginning have a health status as regards scrapie which does not jeopardize their existence. This proves that in Romania some measures to reduce the incidence of scrapie have been taken from the time when the disease has become a planetary problem, even if these measures were not at the level and accuracy of those within European Union. Great thing is that, once the sheep breeds from Romania were accepted in this monitoring program, there is the sure tendency of alignment to the desiderata of the European Union on the spongiform encephalopathy issue in the farm animals.

The frequency values of PrP alleles are the primary structural elements of the database whereupon the calculation algorithm is designed specifically to statistical monotony for the informational energy (Hrincă, 2015). Doing so,

Fig. 1. Allele frequencies at the PrP locus in sheep livestock from European Union

Fig. 2. Allele frequencies at the PrP locus in sheep livestock from Great Britain

one can quantify the genetic diversity (d) from the PrP locus level of sheep herds, by calculating the difference between the number 1 and the amount of informational energy (e). Depending on the energy information amount, the degree of genetic diversity ranges from 0 to 1, in absolute numbers. An important role to establish the values of the two informational statistics parameters is conferred by the number of alleles and their frequencies. But the combinatorial capacity of these alleles and their arrangement in genotypes, number of genotypes expressed, their frequencies and their configuration in the panel prionic are still more decisive in this context. The polarization degree of alleles and genotypes contribute to the definition of the two indicators of informational statistics. A good uniformity of alleles and genotypes determines low informational energies creating the premises to increase the genetic diversity. On the contrary, the polarization of alleles and genotypes, eventually also accompanied by a reduction of their number, leads to an increase of

informational energy, having the natural effect the narrowing of genetic diversity size (Hrinca, 2015).

In sheep populations from the European Community during the 10 years of scrapie monitoring, the informational energies are low or moderate, which entail the important values of genetic diversity at the PrP locus level. But there is a rectilinear, uniform and constant development of the two informational-statistical parameters as regards their sense and values; from year to year the informational energies increase and the genetic diversity decreases (Fig. 4). Thus, in 2002, due to the relatively uniform distributions of the genetic structures at the PrP locus, the informational energy barely reaches a threshold of 0.15, conferring to populations a great genetic diversity (0.85). In 2006 we are witnessing a very significant increase of informational energy (0.10 points), in order that then, every year, an increase by 0.02 - 0.05 points happens, so that in 2010 it amounts to 0.35. As a result, the genetic diversity has a declining trend with the same order of

Fig. 3. Allele frequencies at the PrP locus in sheep livestock from Romania

Fig. 4. Coefficients of informational energy (e), genetic diversity (d) and heterozygosity (Ht) at the PrP locus in sheep livestock from European Union

magnitude, recording a sharp drop in 2006 (0.75) and then the decreasing curve is slower until 2010. However, the margin narrowing of genetic diversity from 0.85 in 2002 to 0.65 in 2010 is extremely large. This phenomenon happened because of the scrapie eradication measures by ever stronger fixing of the ARR allele, year by year, in genotypes that confer increased resistance to this disease, as well as by diminishing, at the same pace, the frequency of genotypes containing the allele VRQ associated with an increased susceptibility to contamination with the prion protein. In this context, even if the genetic prophylaxis is performed to high standards, the sharp drop of genetic diversity in a short period of time remains worrying.

The same trend of evolution of the two informational statistical parameters occurs in sheep populations from the Great Britain as in those from the continental Europe. Only that in the United Kingdom the time in which this phenomenon occurs is much shorter (Fig. 5). Thus, since 1998, the sheep livestock had the same genetic diversity (0.85) to that found in the sheep livestock on the continent in 2002. Until 2002 there was a quite emphasized selection pressure, causing significant spread of the ARR allele in detriment of the other four alleles. Nevertheless, due to a relatively uniform distribution of prion genotypes an exaggerated increase of energy informational does not occur, thereby contributing to achieving a high level of genetic diversity (0.80). After 2002, the selective pressure for fixing the ARR allele continues with a higher intensity and the other alleles the genotypes containing the ARR allele, an increase of entropy

in the system occurs leading implicitly to increase of informational energy, which reaches a quite high coefficient (0.4). So that in four years of monitoring and selection works of the highest accuracy, the genetic diversity decreases greatly and rapidly, its coefficient reaching the value 0.60 in 2006. The fact is all the more obvious as the genetic diversity of populations in the UK is lower with 0.05 points than that found in populations from the mainland just in 2010 (0.65). The rationale behind this approach of the British veterinary authorities was that the UK had an extremely costly and dangerous precedent on spongiform encephalopathy from cattle and therefore the measures to eradicate scrapie in sheep were much harsher, tougher and implemented even earlier than in the other Community States.

The particularized case of Romania shows that, in terms of both informational-statistical parameters at the PrP locus, sheep populations are at level of those from the UK in 2002 and in the period 2002-2006 of those from European Community, respectively $e = 0.20$ and $d = 0.80$ (Fig. 6). This reality shows that the sheep populations from Romania possess both a good genetic diversity and an acceptable status as regards their resistance / susceptibility to the scrapie malady.

The size of genetic diversity and variability of animal populations represents a measure of their heterozygosity degree. The heterozygosity development is a key condition of diversity amplification. A quantification of statistical information shows that there is a biunique relation between genetic diversity and heterozygosity. As a general rule, the heterozygosity records

Fig. 5. Coefficients of informational energy (e) genetic diversity (d) and heterozygosity (Ht) at the PrP locus in sheep livestock from Great Britain

Fig. 6. Coefficients of informational energy (e) genetic diversity (d) and heterozygosity (Ht) at the PrP locus in sheep livestock from Romania

lower values by 0.13-0.16 points than the genetic diversity (fig. 4, 5). The exception occurs in sheep of Romania where the difference between genetic diversity and heterozygosity is approximately 0.27 points (Fig. 6).

The five prion alleles, by their combining, determine the expression of 15 PrP genotypes in the sheep livestock examined in any stage: five homozygous (ARR/ARR, ARQ/ARQ, AHQ/AHQ, ARH/ARH, VRQ/VRQ) and ten heterozygous (ARR/ARQ, ARR/AHQ, ARR/ARH, ARR/VRQ, ARQ/AHQ, ARQ/ARH, ARQ/VRQ, AHQ/VRH, AHQ/VRQ, ARH/VRQ). This state of affairs demonstrates a pronounced polymorphism at the PrP locus level. As in the allele case, there are even large differences of the PrP genotype distributions as regards their contribution at the prion panel. Based on these distributions, many from the increasingly sophisticated analyses can be made on the prion phenomenon. For the ovine pathology, important is framing of all these genotypes in classes of risk as regards the resistance / susceptibility to scrapie of diverse sheep populations. But for this study the association of the risk classes with genetic diversity degree becomes interesting.

According to *Department for Environment, Food and Rural Affairs of the UK Government* (DEFRA, 2005-2008) five classes of risk for resistance / susceptibility to scrapie in sheep have been established. The risk class 1 (R1) includes only individuals with homozygous genotype ARR/ARR, presenting the most increased resistance to scrapie which are recommended to be introduced into the reproduction circuit without conditions. The risk class 2 (R2) has in its composition heterozygous genotypes resulted from the combination of the ARR allele with ARQ, AHQ and ARH alleles. Also the individuals of this class are resistant to scrapie being recommended in the reproduction process, but the establishment of mating scheme should be conceived carefully, especially regarding the individuals containing the ARQ allele. The risk class 3 (R3) comprises the most genotypes (six); the individuals possessing haplotypes for allele AHQ, AHR and ARQ and three heterotypes resulting from reciprocal combinations of these three alleles. The individuals belonging to this class are less resistant to scrapie than from previous classes. They can be used for multiplication of sheep herds, but the breeder must prove perspicacity to conceive the mating

schemes, in particular to avoid the occurrence of ARQ/ARQ homozygotes. In the risk class 4 (R4) there is only one heterozygous genotype, ARR/VRQ. Individuals with this genotype possess a high susceptibility of association with scrapie disease. For this reason, they are not recommended to be promoted to reproduction. However, if they are used to mating, the reproduction process must be subjected to a rigorous control. The explanation of such reasoning resides only in the fixing possibility of the ARR allele in the offspring from this genotype by mating with individuals which possess genotypes with resistance to scrapie. However, it is difficult to explain how a genotype with such a high possibility to develop the disease can result by the combination of the allele with the highest resistance to scrapie with the allele with the highest susceptibility to morbid phenomenon. The last class of risk (R5) includes four genotypes, all containing the VRQ allele both in homozygous status and in heterozygous combinations with the AHQ, ARH and ARQ alleles. The individuals of this class may develop in the highest degree the disease and therefore should not be used, in any case, for reproduction to multiply the sheep herds. The use of such individuals is a reliable source of prion disease dissemination and of its incidence increasing in sheep populations.

In 2002, in the sheep flocks from the European Community, the contamination risk with prion agent was high enough. Around 7% of individuals were involved in class 4, but an overwhelming percentage (37%) belonged to the highest risk class (R5). So that 43.50% of sheep were capable to contract the disease at a high level. Only about 2% of individuals manifested the highest resistance to scrapie. Even the individuals within R2 recorded a moderate frequency (16.65%). The most frequent individuals belonged to the class R3 (37.92%) (Tab. 1).

In 2006, when the severe criteria of resistance increasing to scrapie had been implemented, the incidence of individuals involved in these two classes (0.83% in R4 and 1.51% in R5) decreased very much. During the years the frequencies of the two classes were kept almost constant, varying between 1% and 2%, so that in 2010 the two classes recorded an incidence with a little over 3% of individuals susceptible to scrapie (2.17% in R4 and 1% in R5). In contrast, the incidence of individuals within class with ARR/ARR genotype

increased rapidly to 30% in the first three years, then two new emergent peaks are recorded, namely 36% in 2009 and 41.33% in 2010. Also, in a similar rate, the individual frequency of R2 class increased, too, to 45.33% in 2006, with a peak in 2008 (48.17%), being stabilized at about 42% in the remaining years of monitoring. A decrease of individual incidence of the class R3 occurs, but in a slower pace than of those from R4 and R5, recording 22-23% in 2006 and 2007, then 19-20% in 2008 and 2009, in order that in 2010 only 13.65% of individuals to be framed in this class (Tab. 1).

Therefore, the same phenomenon of genetic diversity evolution is noticeable also concerning the sheep framing in risk classes even more obviously than in the case of allelic structure analysis. There is an equally significant downward trend of genetic diversity from 0.85 to 0.65 with the significant decrease of individual incidence to produce the disease in a high degree (classes 4 and

5), with the moderate decreasing of those with lower resistance to scrapie (R3) and with a very significant increase of individual number that are highly resistant (R1) and resistant (R2) to scrapie.

In sheep from the United Kingdom since 1998 the incidence of individuals susceptible to contracting scrapie is incomparably smaller than in herds from the continental European Union. Proof of this are approximately 19 percents of individuals from R4 and R5 classes, each class comprising 9.6 percents of animals of this kind. The existence of individuals from the class R1 (21.3%) and especially those from the R2 class (35.6%) is remarkable, so that at that time one could count on 67% of individuals in selection works. Another 24% of individuals of class R3 could be add to R1 and R2 classes for this purpose. In 2002, the frequency of individuals encompassed within classes R4 and R5 decreases 3 times. The individual incidence within R3 class decreases, too, by four percents. Instead, the sheep share increases within

Tab. 1. Frequencies of PrP genotypes and their framing into risk classes for scrapie in sheep livestock from European Union

Risk class	Genotip PrP	Years					
		2002 n = 1951	2006 n = 5050	2007 n = 4391	2008 n = 11310	2009 n = 13513	2010 n = 8493
Class 1 (R1)	ARR/ARR	1.83	30.33	29.50	30.33	35.67	41.33
	ARR/ARQ	13.08	34.83	36.83	39.00	35.00	34.00
Class 2 (R2)	ARR/AHQ	2.55	8.33	4.18	7.67	5.50	7.00
	ARR/ARH	1.02	2.17	1.17	1.50	1.00	0.83
	Total	16.65	45.33	42.83	48.17	41.50	41.83
Class 3 (R3)	ARQ/ARH	3.66	0.84	0.00	1.00	1.00	0.50
	ARQ/AHQ	9.12	6.33	5.83	4.50	4.33	3.00
	AHQ/AHQ	0.89	2.00	2.00	0.83	1.33	1.00
	ARH/ARH	0.14	0.50	0.00	0.00	0.17	0.00
	AHQ/ARH	0.71	2.33	0.33	0.00	0.50	0.33
	ARQ/ARQ	23.40	10.00	13.17	12.17	12.5	8.84
	Total	37.92	22.00	23.33	18.50	19.83	13.67
Class 4 (R4)	ARR/VRQ	6.73	0.83	2.17	1.00	1.33	2.17
Class 5 (R5)	AHQ/VRQ	4.70	0.17	0.50	0.17	0.00	0.00
	ARH/VRQ	1.89	0.17	0.17	0.33	0.00	0.00
	ARQ/VRQ	24.08	1.17	1.33	1.50	1.50	1.00
	VRQ/VRQ	6.20	0.00	0.17	0.00	0.17	0.00
Total		36.87	1.51	2.17	2.00	1.67	1.00
Genetic diversity		0.8444	0.7534	0.7399	0.7118	0.6973	0.6449

the first two classes: only by 4.5% in class R1, but especially in class R2, with over 10%. In 2006 only individuals with genotype ARR/ARR recorded an extremely important increase (47.33%). All the other four classes of risk show any larger or smaller fallings off. The sheep number within R2 class decreases by 5-6%. Important fact is that the individual number from the class R4 is shrinking to 1.65%, and sheep from R5 class are sporadically met in populations (0.72%). As a result of these actions, the genetic diversity declines even more steeply and in a much shorter time compared to sheep from continental Europe, by 0.25 points, from 0.85 before in 1998 to 0.60 in 2006 (Tab. 2).

In sheep from Romania, the highest frequency is registered by individuals with genotypes of class R2 (40.87%). Half of sheep livestock belong to R1 and R3 classes, each one with about 25%. Individuals with a high morbidity risk comprise 10% of the populations in both classes, R4 and R5,

the frequency of each class oscillating around the 5%. Somewhat more balanced distributions of the 15 prion genotypes determine a rather important genetic diversity (0.80) (Tab. 3).

Therefore, the scrapie casuistry in ovine species from Romania in 2013 was at the 2000 year level from Great Britain or during the years 2002-2006 from the European Community. But it must be underlined that the monitoring and eradication actions of scrapie in Romania are just at the beginning. It is interesting to follow the evolution of genetic diversity in sheep from Romania by applying this program in the mentioned context.

By the impact that the scrapie causes on animal health and food safety for humans, the ideal measure is that the selection to go so far as to be fixed in sheep populations only the ARR allele in homozygous status. In this way, there should be removed from the populations, by artificial selection, all the other alleles that print

Tab. 2. Frequencies of PrP genotypes and their framing into risk classes for scrapie in sheep livestock from Great Britain Britain

Risk class	Genotip PrP	Years		
		1998 n=233	2002 n=13896	2006 n=1000000
Class 1 (R1)	ARR/ARR	21.3	25.40	47.33
	ARR/ARQ	27.9	29.43	26.01
Class 2 (R2)	ARR/AHQ	5.6	7.46	7.57
	ARR/ARH	2.1	9.18	7.71
	Total	35.6	46.87	41.29
Class 3 (R3)	ARQ/ARH	1.6	5.78	2.12
	ARQ/AHQ	6.3	4.32	2.08
	AHQ/AHQ	1.9	0.55	0.30
	ARH/ARH	1.6	0.98	0.31
	AHQ/ARH	0.3	1.47	0.62
	ARQ/ARQ	12.2	8.53	3.58
Class 4 (R4)	Total	23.9	21.63	9.01
	ARR/VRQ	9.6	3.12	1.65
Class 5 (R5)	AHQ/VRQ	2.5	0.46	0.13
	ARH/VRQ	0.3	0.61	0.13
	ARQ/VRQ	5.9	0.81	0.45
	VRQ/VRQ	0.9	0.10	0.01
	Total	9.6	2.98	0.72
Genetic diversity		0.8474	0.8056	0.6056

Tab. 3. Frequencies of PrP genotypes and their framing into risk classes for scrapie in sheep livestock from Romania in 2013

Risk class	Genotip PrP	2013 year n = 2038
Class 1 (R1)	ARR/ARR	24.97
	ARR/ARQ	36.16
Class 2 (R2)	ARR/AHQ	2.26
	ARR/ARH	2.45
	Total	40.87
Class 3 (R3)	ARQ/ARH	1.42
	ARQ/AHQ	1.47
	AHQ/AHQ	0.15
	ARH/ARH	0.15
	AHQ/ARH	0.10
	ARQ/ARQ	20.80
	Total	24.09
Class 4 (R4)	ARR/VRQ	5.74
	AHQ/VRQ	0.10
Class 5 (R5)	ARH/VRQ	0.15
	ARQ/VRQ	3.88
	VRQ/VRQ	0.20
	Total	4.33
Genetic diversity		0.8010

the polymorphic character of the PrP locus. In doing so, many preconditions would be created to reach monotypical breeds at the locus PrP, as it is the case with other loci, particularly those regarding some biochemical-genetic systems (*Alb* locus in most sheep breeds or *Hb* and *Am* loci in some breeds only). But in this case, the genetic information restructuring was carried out normally by natural selection and less through the human intervention, by forced actions. The thorny question that arises through such actions is that with the disappearance of certain genotypes other genes can be removed from populations too which are associated with valuable economic and biological features of animals like production traits, reproduction indices, health status, vigour, ability to adapt to environmental conditions and technological factors, heterosis etc. which may affect the productivity potential of farm animals (Windig *et al.*, 2004; Brandsma *et al.*, 2005). So, by decreasing the variability of the PrP locus, a general narrowing of genetic diversity of this species occurs, leading to genetic depression by installing the emphasized inbreeding (Windig *et al.*, 2004).

From the retrospect of these reports a few questions can be made: It is well that certain genotypes disappear which are incriminated in prion disease manifestation to achieve a genetic prophylaxis? It is bad that genetic diversity is narrowed by the disappearance of certain genotypes up to creating of monotype breeds at the PrP locus? We believe that to achieve the common desiderates, concerning the biodiversity preserving and reduction of the morbid phenomenon it can be act by lowering or eliminating of genotypes from classes R4 and R5, but that the elaboration of a reproduction process with great discernment is an imperative need to achieve more uniform distributions of genotypes from the risk classes R1 and R2; at the same time, mating schemes of individuals from the class R3 should be judiciously used that to result offspring framed in the first two risk classes. All depends on the skill and flair of breeder. These measures will be beneficial towards reducing the morbid phenomenon, but especially in order not to cause narrowing of genetic pool with some genes associated with superior parameters of animal productivity, thus helping to preserve and enhance the biodiversity in this species. Reaching

concomitant of these targets requires long time and represents key-points for sheep profitability. It is obvious that the selection on the PrP gene is a sound strategy to improve resistance to scrapie in sheep. Therefore, the selection programs on increasing the resistance to scrapie in sheep breeds must define the specific objectives which to be achieved in each sheep breeding scheme. These objectives must be achieved simultaneously, as quickly as possible, both to increase the resistance to scrapie in all populations and to control the disease in the affected flocks. For the objective of selection for ARR allele, the program should stipulate that the PrP genotype is considered as an additional criterion of selection in order to make selection for increased resistance to scrapie, while maintaining the selection for productivity traits and for preservation of genetic variability. Therefore, in this strategy, the breeder must have in view two aspects: from the technical point of view, to manage effectively the production costs and, from the scientific point of view, to be aware that new knowledge is required to be included in selection decisions.

In this study we presented an overview of ovine scrapie on three applicability sections, but this kind of issue should be customized on sheep breeds where different peculiarities of genetic diversity and its evolution can be revealed.

CONCLUSIONS

The paper approaches antithetically two highly topical trends in the sheep breeding field from the European space: the huge benefits brought to humanity by preserving and increasing the genetic diversity and the need for compulsory and unconditional decreasing the prion disease.

The study is based on experimental data derived from the genotyping at the PrP locus of some sheep samples farmed in the European Community which have entered a program for monitoring and eradication of scrapie during a period of 10 years.

In its essence, the program for scrapie eradication in sheep was focused on ever more marked fixing of ARR allele in genotypes which confer resistance to this malady, removing as much as possible of prion genotypes containing VRQ allele that is associated with a greater susceptibility of sheep to contaminate with the pathogenic agent of scrapie and decreasing of the

ARQ allele incidence which, in its turn, causes a low resistance to disease.

The degree of genetic diversity at the PrP locus was quantified by the synthetic indicator named informational energy.

The consequence of scrapie eradication measures led to a emphasized decline of genetic diversity at the PrP locus from 0.85 to 0.60 that has happened progressively over the years to all sheep livestock from Europe.

The drastic narrowing of genetic diversity at PrP locus by applying the genetic prophylaxis measures on prion disease could negatively affect other genetic loci which are correlated or associated with production and reproduction features of sheep.

The study recommends that breeders who deal with selection for the PrP gene to develop sound strategies both to improve the scrapie resistance in sheep and to maintain and enhance the genetic diversity for all traits which express the productivity of this species.

The interpretation of biodiversity in the animal pathology case must be done with prudence and with certain semantic nuances and not in absolutely advantageous terms with exhaustive content.

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