

PEDIGREE ANALYSIS OF THE FORMER VALACHIAN SHEEP

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ABSTRACT

The objective of this study was to assess the genetic variability of the former Valachian sheep breed. The analysis was based on the pedigree information of a single nucleus flock located in Northern Slovakia. The reference population was limited and consisted of 27 individuals (live animals born during the period 2007-2010). The results, therefore, need to be interpreted with caution. The whole pedigree population consisted of 137 individuals. The pedigree completeness, generation intervals, levels of inbreeding and of average relatedness were computed for both populations. The mean inbreeding for the whole population was 1.69% and the mean inbreeding for the reference population was 6.48%, respectively. The mean average relatedness for the whole population was 5.36% and the mean average relatedness for the reference population was 10.55%, respectively. An individual increase in inbreeding, the realized effective population size and the analyses of founders and ancestors were computed for the reference population. The mean individual increase in inbreeding was 2.43% and the realized effective population size was 20.6 animals. The number of founders was 62 animals, the effective number of founders as well as the number of ancestors was 20 animals, the effective number of ancestors contributing to the reference population was 5 animals and the number of ancestors explaining 50% of genetic diversity for the reference population was 2 animals. Although the findings may depend on the chosen reference population and may reflect the completeness of the pedigree, they enable to assess the available genetic variability and may be considered a basis for additional research as well as a proposal for measures aimed at maintaining the breed diversity.

Key words: Valachian sheep, genealogical data, inbreeding, average relatedness, individual increase in inbreeding, realized effective population size, gene origin

INTRODUCTION

The former Valachian sheep flocks can be found in the Central and the Northern regions of Slovakia. According to the phenotypic standard, the actual purebred population is estimated at 150 to 200 breeding individuals (Margetín, personal communication). The crossbred population is estimated as of a larger size. The breed is mainly spread in commercial flocks with no individual pedigree recorded in the flock book. Only one nucleus flock is recognized. History of the breed can be traced back to the 13th-14th century when the move from Balkan region known as Valachian colonization occurred. Crossbreeding aimed at improvement in milk and meat traits, which started in early 1950s, resulted in

recognition of the new Improved Valachian breed in 1982 and in diminishing of the former Valachian breed. Thus, about 150 breeding individuals of both sexes sustained in 1993 (Margetín and Bullová, 2004).

The breed fits low-input production systems and altitudes between 600-1200m above sea and is found in mountainous and submountainous regions. It has low feed requirements, is well adapted to open air husbandry and resists unfavourable climate. The animals can be either horned or pooled; are of small body frame, their live weight is 35 to 40kg in adult females and 45 to 50kg in adult males, the wool is of coarse/carpet type. The litter size is mostly a single lamb. The milk production is about 80 to 100 litres per standard milking period (150d). The weaning period of lambs varies from 40 to 60d which is

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common for sheep in Slovakia regardless of the breed. The former Valachian sheep has the important functions such as maintaining natural landscape and agrotourism and its historical value has been recognized. Although the pedigree analysis is considered an important tool for monitoring the genetic diversity and may be a basis for activities aimed at maintaining small population, it has not been done for the former Valachian sheep until now. Nevertheless, an increasing attention is given to small populations and the research aimed at assessing their genetic diversity is ongoing in Slovakia. The studies are based either on pedigree or molecular analyses. Pedigree analyses were done for Slovak Pinzgau cattle, Arab horse, Slovak Sport pony and Red deer (Kadlečík *et al.*, 2008; Pjontek *et al.*, 2009a,b; Beke and Kadlečík, 2009). Recently, microsatellite markers were used for molecular characterization of Tsigai (Kusza *et al.*, 2009) and the former Valachian sheep (Bauer *et al.*, 2010).

The objective of the present study was to assess the pedigree information of the former Valachian nucleus flock in order to explore the state of its genetic variability. Such parameters as number of generations traced, generation intervals, inbreeding and average relatedness, individual increase in inbreeding, realized effective population size, number of founders, effective number of founders, number of ancestors, effective number of ancestors contributing to the reference population, number of ancestors explaining 50% of genetic diversity for the reference population were analysed and discussed. However, further research is needed and findings gained from both pedigree and molecular analyses should be combined in order to assess the genetic variability of the breed.

MATERIAL AND METHODS

The pedigree analysis of the former Valachian nucleus flock was done using flock book data. To characterize the available genetic variability of the breed, the reference population was formed from 27 animals (23 females and 4 males) born during the period 2007-2010. Taking into account their genetic ties, a size of the whole pedigree population was increased to 137 animals (98 females and 39 males). Most of sires produced 1 to 2 offspring, 1 sire produced 5 offspring, 3 sires produced 7 offspring, 1 sire produced 8 offspring and 1 sire produced 15 offspring. The information was gathered with respect to each path in the animal's ancestry traced until the unknown ancestor was found. The available information was of a limited extent and no data before 1986 were found.

The parameters computed for the whole and the reference population were as follows: maximum number

of generations traced, number of full generations traced, number of equivalent generations traced, generation interval defined as the mean age of parent when its offspring was born (in four possible pathways: father-son, father-daughter, mother-son, mother-daughter), mean inbreeding coefficients and mean average relatedness coefficients. The following parameters were computed for the reference population: mean value of individual increase in inbreeding, realized effective population size, number of founders, effective number of founders, number of ancestors, effective number of ancestors contributing to the reference population, number of ancestors explaining 50% of genetic diversity for the reference population. The parameters were computed using the ENDOG v.4.8 programme (Gutiérrez and Goyache, 2005a). The basic statistics and additional calculations were done using MEANS, FREQ and SQL procedures (SAS/STAT v.9.2, 2002-2008).

The parameters taken into consideration have been explained in detail by a number of authors (Goyache *et al.*, 2003; Gutiérrez *et al.*, 2005b and Royo *et al.*, 2007). The detailed description of computation procedures can be found in ENDOG User's Guide (http://www.ucm.es/info/prodanim/html/JP_Web.htm). Moreover, the detailed description of specific procedures used for computing individual increase in inbreeding and realized effective population size can be found in studies by Gutiérrez and colleagues (2008, 2009). The calculation of the realized effective population size is based on averaging the individual increase in inbreeding of all animals included in the reference population regardless of the presence of animals which would be assigned to different discrete generations according to their pedigree depth. The realized effective population size is thus understood as the global effective size over time which has led to the present inbreeding level in the reference population from the founder population (Cervantes *et al.*, 2008).

RESULTS AND DISCUSSION

There were 45% animals detected with unknown parents when the whole pedigree population was analysed. Twenty four percent of these were males and 76% of these were females. With the reference population, all parents were known. Planinc *et al.* (2010) reported known parents in 97.5% animals of the reference population of Posavje horse (based on genealogical data of 810 individuals born between 2004 and 2008).

Numbers of generations traced for the whole population and for the reference population are given in Table 1. The mean values computed for the reference population were about 2.4 to 3.2 times higher than those for the whole population. Compared to the pedigree

analyses of horse populations in Slovakia (Pjontek *et al.*, 2009a,b), the parameters partly accord with those obtained for Slovak Sport pony, which reference population consisted of 42 individuals. The maximum number of generations traced was 5.76 for the reference population of this horse breed. The similar values in this parameter found for Slovak Sport pony and Valachian sheep were probably a result of pedigree limitations which occurred in both populations and indicated either short history of the breed (Pjontek *et al.*, 2009b) or underlined the fact that pedigree information was available only for certain history of the breed (as seen in this study). Goyache *et al.* (2003) reported the number of equivalent generations traced at 1.09 for the analysed population of Xalda sheep with pedigree of limited depth. When various subpopulations were formed, the number of equivalent generations ranged from 0.87 to 2.50. At individual level, Álvarez *et al.* (2008) reported roughly no more than 4 equivalent generations were found when pedigree of this sheep breed was investigated. In our study, 13 animals appeared to be of 3 to 3.97 equivalent generations traced and 6 animals to be of 4 to 4.84 equivalent generations traced. Danchin-Burge *et al.* (2010) reported a higher number of equivalent generations (ranging from 4.6 to 10.5) for some small populations of sheep breeds in France.

Generation intervals (Table 2) were expressed as the mean age of parents when offspring was born (whether it was used for reproduction or not). Except for the father-daughter pathway with almost the same generation intervals (a difference was less than 2%), the mean values were found higher for the reference population (by 22 to 32%). When generation intervals were computed regardless of the sex of parents and offspring, a difference between the whole population and the reference population did not exceed 17%. The mean generation interval for the whole population was $4.07 \pm 0.157y$ and that for the reference population was $4.94 \pm 0.339y$. Mostly, the lower generation intervals were found in literature. Goyache *et al.* (2003) reported generation intervals ranging from 2.67 to 2.87y on the father side and from 3.11 to 3.23y on the mother side for Xalda sheep (genealogical data of 805 individuals). Prod'homme and Lauvergne (1993) reported generation intervals ranging from 2.2 to 4.1y on the father side and from 3.9 to 5.6y on the mother side for a closed Merino Rambouillet flock over 50 generations of inbreeding. Li *et al.* (2009) reported lower generation intervals for Finnsheep in terms of all possible pathways: father-son 2.96y, father-daughter 3.15y, mother-son 2.65y, mother-daughter 2.49y. Danchin-Burge *et al.* (2010) reported a mean generation interval between 2.9 and 4.1y for analysed sheep breeds in France.

Mean inbreeding and mean average relatedness for the whole population and for the reference population

are given in Table 3. The mean inbreeding for the reference population was almost 4 times higher and the mean average relatedness was almost a double of that for the whole population. A difference in the mean inbreeding values could be because of the effect of cumulated generations occurred (1.16 vs. 3.71 equivalent generations). This effect was also observed in pedigree of Mallorquí horse population (Álvarez *et al.*, 2010). Pedrosa *et al.* (2010) found the population average inbreeding of 2.33% for Santa Inés sheep. The authors recommended the utilization of programme of directed mating to be an appropriate alternative to keep the level of inbreeding under control. Van Wyk *et al.* (1993) found a higher mean value of inbreeding (15%) for Elsenburg Dormer sheep which was kept as a close herd over 40y (1941-1980). Similarly, Lamberson *et al.* (1982) reported a higher mean value of inbreeding (10.7%) for a population of 598 lambs of Hampshire breed which was predominantly closed for 30y. Álvarez *et al.* (2008) found the higher mean inbreeding (2.6%) and the lower mean average relatedness (2.7%) for the whole pedigree population of Xalda sheep. With various subpopulations formed, the values were either almost the same and higher (inbreeding from 6.1 to 14.1%) or smaller (inbreeding from 1.1 to 2.3% and average relatedness from 2.4 to 4.8%) when compared to the former Valachian sheep.

The individual increase in inbreeding and the realized effective size of the reference population are given in Table 4. The mean value of individual increase in inbreeding was 2.43% and the realized effective size for the reference population was 20.6 animals. When variant reference populations (the multiplier flock consisted of offspring of recent imports from the Czech Republic and/or commercial populations), apart from nucleus population, were investigated (unpublished results), the value for the realized effective population size remained almost stable (difference did not exceed 20%) and was similar to those reported by Gutiérrez *et al.* (2008) for Cartusian horse subpopulations. Maiwashe and Blackburn (2010) found a higher effective population size (92 animals) for Navajo Churro sheep (genealogical data of 2950 animals) kept in 4 regions of the USA. In accordance with Meuwissen (1999), who stated that due to mutation and drift the critical effective population size should be between 50 and 100 animals, the authors recommended development of ex-situ cryo-preserved germplasm bank that might be the best long-term strategy for maintaining the breed genetic diversity, also with respect to the transient nature of breeders (only small number of owners maintained their ownership for more than 7y). Ghafouri-Kesbi (2010) reported the realized effective population size at 71 animals for a close population of Zandi sheep (a reference population consisted of 2566 animals). Li *et al.* (2009) reported the mean increase in inbreeding at 0.148% and the realized effective population size of 122 animals

Table 1: Mean values of parameters to assess pedigree completeness

Parameter		Whole population	Reference population
Max number of generations traced	Mean	2.61	6.22
	Standard deviation	2.444	0.974
	Min	0	5
	Max	8	8
Number of full generations traced	Mean	0.87	2.19
	Standard deviation	0.938	0.623
	Min	0	1
	Max	3	3
Number of equivalent generations traced	Mean	1.16	3.71
	Standard deviation	1.472	0.746
	Min	0	2.25
	Max	4.84	4.84

Table 2: Generation intervals

		Whole population		
Age of parent at offspring birth	N	Mean age	Standard deviation	Standard error of mean
Father-Son	22	3.52	1.533	0.327
Father-Daughter	59	2.64	1.127	0.147
Mother-Son	31	4.70	1.804	0.324
Mother-Daughter	59	5.36	2.093	0.273
Total	171	4.07	2.047	0.157
		Reference population		
Age of parent at offspring birth	N	Mean age	Standard deviation	Standard error of mean
Father-Son	4	4.96	2.163	1.082
Father-Daughter	23	2.69	1.521	0.761
Mother-Son	4	6.94	0.802	0.404
Mother-Daughter	23	6.84	1.452	0.726
Total	54	4.94	2.489	0.339

N – number of observations

Table 3: Mean inbreeding and average relatedness

Parameter		Whole population	Reference population
Individual inbreeding, %	Mean	1.69	6.48
	Standard deviation	4.222	6.496
	Min	0	0
	Max	15.65	15.65
Individual average relatedness, %	Mean	5.36	10.55
	Standard deviation	4.054	1.860
	Min	1.10	5.82
	Max	16.47	13.65

Table 4: Individual increase in inbreeding and realized effective population size

Parameter	Reference population	
Individual increase in inbreeding, ΔF_i %	Mean	2.43
	Standard deviation	2.333
	Min	0
	Max	5.61
Realized effective population size, N_e	Mean	20.6
	Standard deviation	4.35

Table 5: Founders' and ancestors' analysis

Parameter	Reference population
No. of founders	62
Effective no. of founders	20
No. of ancestors contributing to the reference population	20
Effective no. of ancestors for the reference population	5
No. of ancestors explaining 50% diversity	2

for Finnsheep breed. Nomura *et al.* (2001) reported the low effective population size (17.2 animals) for Japanese Black cattle over the period 1993-1997 when an approach, which took into account the variance of family size, was used. According to the authors, this low value underlined the fact that only a few prominent sires were used for breeding, i.e. almost 43% of the total number of registered animals in 1998 was the progeny of five bulls. In our study, 75% animals of the reference population were offspring of 4 sires.

The reference population was derived from 62 founder animals (Table 5). A total of 20 effective founders were found. A big difference between the number of founders and the effective number of founders shows the excessive use of some animals as parents and disequilibrium between the founder contributions (Ghafouri-Kesbi, 2010). The number of founders/effective number of founders ratio was 3.1 for the reference population of the former Valachian sheep and indicated that the expected contributions of founders were more unbalanced in comparison to Romanov-INRA nucleus sheep (1.9) as reported by Danchin-Burge *et al.* (2010). On the contrary, this ratio was lower than those reported for Roussin de la Hague (11.7) and Charmoise (10.1) sheep. The effective number of ancestors contributing to the reference population of the former Valachian sheep was 5 animals. Only 2 animals were needed to explain 50% of its genetic diversity. Similar characteristics for concentration of gene origin were found by Pjontek *et al.* (2009b), who reported 7 animals as the effective number

of ancestors, and only 3 animals needed to explain 50 % of total genetic variability for the reference population of Slovak Sport pony. The effective number of founders/effective number of ancestors ratio for the reference population of the former Valachian sheep was 4. The ratio of 3 was found for Abundance and Normande dairy cattle (Boichard *et al.*, 1997). According to the authors, this ratio was unfavourable and was a result of bottleneck occurred due to fact that only few sires were preferred for reproduction. The bottleneck, which occurred in the investigated population of the former Valachian sheep, was probably due to fact that a limited number of sires was used to act as fathers of next generation.

CONCLUSION

This was the first analysis of pedigree of the former Valachian sheep. According to the realized effective population size, the mean individual increase in inbreeding and the other parameters calculated, the estimated genetic variability of this breed is low and inbreeding depression of production and fitness-related traits may occur. The analysis revealed the findings which may be useful for implementing into breeding strategies, providing that these are combined with the findings based on molecular analysis. The experience from many countries indicate that neither pedigree nor molecular information is sufficient to monitor small populations, including situations where pedigree is incomplete or of short history.

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