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Original Contribution

FREQUENCES OF C3 ALLELES AND GENOTYPES IN SOME SHEEP BREEDS

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ABSTRACT

This research included sheep and rams of the following three breeds: *Trakia merino* – 80 sheep and 9 rams; *Ile-de-France* – 107 sheep; *Charollais* – 107 sheep; and milk crossbreeds: *Stara Zagora* × *East-Friesian*, and (*Stara Zagora* × *East-Friesian*) × *Blackhead Pleven breeds* – 118 sheep and 14 rams. The genetic structure of the studied sheep populations was determined by the calculation of the relative frequency of the observed alleles of the gene coding for the C3 complement component (S, F, F1, S1), and the genotypes that they formed (SS, FF, FS, F7S, F5S, F10S, FF1, SS1, S1S1). The S allele had the highest frequency in the milk type, *Ile-de-France* and *Trakia merino*. The *Charollais* breed demonstrated the reverse phenomenon as it had the F allele with a higher frequency. In this breed, another allele, F1, was observed. Its frequency was low, yet it was present in the genetic structure of the studied population of *Charollais* sheep. In the sheep of the *Trakia merino* breed, we discovered a fourth allele – S1. It also had a very low frequency – 0.04. The four alleles described formed the nine genotypes established by us - SS, FF, FS, F7S, F5S, F10S, FF1, SS1, and S1S1. Their frequencies differed in the different breeds of sheep, yet most common was the homozygous genotype SS (milk type, *Ile-de-France*, and *Trakia merino*). Second was the heterozygous type FS that had the highest frequency in the *Charollais* breed. The heterozygous type F7S should be noted as it had rather high frequencies in two of the breeds (*Ile-de-France* – 0.33 and *Trakia merino* – 0.32).

Key words: C3; polymorphism; frequency; sheep; inheritance

INTRODUCTION

The study of the genetic structure within animal populations is of significant importance for the improvement of their selection with regard to higher productivity and enhanced resistance against infectious and parasitic diseases For this purpose, various polymorphic systems are used (protein, erythrocyte, lymphocyte antigens, etc.). In humans, the polymorphism of C3 complement component has been fully studied. A relationship has been discovered between some C3 alleles and the susceptibility (or resistance) to some diseases (1, 2, 3, 4, 5, 6, 7). Very few reports about polymorphism of this factor in sheep currently exist. This situation consequently underlined our desire to carry out the present study with a view to determining the gene and genotype frequency of the polymorphic C3 system in the sheep.

MATERIALS AND METHODS

Animals

The sheep included in this study were from 3 productive types:

A. *Milk type crossings*: 118 sheep and 14 rams - *Stara Zagora* × *East-Friesian* and (*Stara Zagora* × *East-Friesian*) × *Blackhead Pleven breeds*;
B. *Merino type*: 80 sheep and 9 rams from the *Trakia merino* breed;
C. *Meat type*: 107 sheep from the *Ile-de-France* breed and 107 sheep from the *Charollais* breed;

At the beginning of the experiment, the sheep and rams were aged between 2 and 3 years. They were housed in separate premises. Blood for analysis was drawn from the *v. jugularis* and stored in 10 ml tubes. Subsequently, the

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blood was allowed to clot for one hour at room temperature (25°C) and the samples were finally centrifuged for 10 min. at 2000g.

Methods

The polymorphism of C3 complement component was determined according to the method of Teisberg (8) described briefly as stated below:

1. Buffers
   a. Stock solutions for gels - 5,5-diethylbarbituric acid sodium salt (*Diemal Na, Loba-Chemie, Austria*) 0.0230 M; 5,5-diethylbarbituric acid (*Reanal, Hungary*) 0.0037 M; Calcium-L(+)- lactate (*Fluka AG, Switzerland*) 0.0009 M; pH=8.6.
   b. Stock solution for tray buffer – 5,5-diethylbarbituric acid sodium salt (*Diemal Na, Loba-Chemie, Austria*) 0.061 M; 5,5-diethylbarbituric acid (*Reanal, Hungary*) 0.01 M; Calcium-L (+)- lactate (Fluka AG, Switzerland) 0.0018 M; pH=8.6.
1. The first buffer was used for preparation of agarose gel (1%) where serum samples were applied and electrophoresis carried out for about 2.5 h at 20 V/cm. Subsequently the gel was stained for 1 min with Amido Black 10 B and destained overnight with the destaining solution described below.
2. Destaining solution: ethanol, distilled water and glacial acetic acid (5:5:1) were mixed (for example, 1 l destaining solution contains 450 ml ethanol, 450 ml distilled water and 100 ml glacial acetic acid). Then 2 tablespoons of active charcoal powder were added in order to allow for the manifold use of the destaining solution.
3. Staining solution – 1% amido black 10B was added to 1 l destaining solution (without active charcoal ethanol), shaken well and left overnight. If necessary it could be filtered after a week.

The gene and genotype frequencies were calculated by the method of Nicholas (9), since it has been earlier established that the genes coding for the C3 complement component in sheep are inherited co-dominantly (10).

RESULTS AND DISCUSSION

In our previous study (10), we had reported that the C3 complement component of the complement's system in sheep had a polymorphic structure; hence the frequency of the established alleles and genotypes could be calculated. For this purpose, we established the frequencies of the alleles S, F, F_1, and S_1, coding for the C3 complement component in the complement's system in sheep of three types – milk type, meat type (*Ile-de-France* and *Charollais*), and I type (*Trakia merino*). **Table 1** shows that the frequency of gene S was highest in the *milk type*, *Ile-de-France*, and *Trakia merino*. In the *Charollais* breed, the opposite phenomenon was observed as it had the F allele with a higher frequency. Typical of that breed was the appearance of another allele – F_1. Its frequency was low, yet it was present in the genetic structure of the studied population of the *Charollais* sheep. In the *Trakia merino* sheep, we observed a fourth allele – S_1. It also had a very low frequency – 0.04.

Table 1*. Relative frequencies of C3 alleles in different sheep breeds*

Alleles	***Milk type crossings***	***Il de France***	***Charolais***	***Trakia merino***
S	0,64	0,64	0,46	0,53
F	0,36	0,36	0,50	0,43
F_1	-	-	0,04	-
S_1	-	-	-	0,04

We determined that the four alleles described above formed nine genotypes – SS, FF, FS, F_7S, F_5S, $F_{10}S$, FF_1, SS_1, and S_1S_1 (**Table 2**). Their frequencies were different and depended on the breed; the most common was the homozygous genotype SS (*milk type, Ile-de-France* and *Trakia merino*). Next was the heterozygous genotype FS that had the highest frequency in the *Charollais* breed. It was interesting to observe that the F_7S genotype had a very high frequency in two of the breeds (*Ile-de-France* – 0.33 and *Trakia merino* – 0.32).

As already stated, the C3 polymorphism in humans has been thoroughly studied. Wieme and Demeulenaere (11) studied the polymorphism of human serum proteins and found that in some individuals, the β_2C globulin zone presented 2 bands in electrophoresis. The electrophoresis was

performed in 1% agar gel but when the traditional starch gel was used the results were identical. Family analysis on these bands showed that they were inherited as an autosomal sign; but it did not indicate how. An interesting observation was the fact that the person with two β_2C bands was a girl suffering from the Hodgkin's disease. This first observation motivated the work of many investigators thereafter.

Table 2. *Relative frequencies of C3 gentypes in different sheep breeds*

Genotypes	***Milk type crossings***	***Il de France***	***Charolais***	***Trakia merino***
SS	0,36	0,36	0,18	0,25
FF	0,08	0,09	0,17	0,17
FS	0,34	0,22	0,20	0,20
F_7S	0,15	0,33	0,13	0,32
F_5S	0,07	-	0,14	-
$F_{10}S$	-	-	0,10	-
FF_1	-	-	0,08	-
SS_1	-	-	-	0,03
S_1S_1	-	-	-	0,03

It was only one year later that Alper and Propp (12), using agarose gel with a better resolution, reported about 4 (probably 5) alleles, coding for C3 variants, and having different electrophoretic mobility. They determined 15 various phenotypes. According to the authors, the F_1, F and S alleles were inherited co-dominantly in an autosomal fashion, whereas the S1 and F0.8 alleles – via an unknown mechanism. Furthermore, the investigators studied the influence of various C3 genotypes on the blood serum C3 complement component concentration, the total complement activity and the adhesive properties of C3 molecules, but no significant differences had been observed.

The actual study on C3 polymorphism, however, began when Teisberg (8) published a truly perfect method for C3 complement component electrophoresis in humans. The author reported that the frequency of the C3S allele was 0.80, and that of the C3F allele – 0.19. The following year Teisberg (13) conducted a much broader research on Lapland people and discovered that the frequency of C3S was 0.9369, while C3F had a frequency of 0.0631. The determined frequencies of those alleles in Norwegians were much different from those in Lapland people. In that same year, the researcher performed a new broad research that covered the whole territory of Norway (without Lapland) and discovered that the C3S allele had a frequency of 0.7865, while the frequency of C3F was 0.2082. Comparing those two values, he noticed a difference of 0.0053 that was created by the existence of seven very rare alleles ($C3F_{0.5}$; $C3F_{0.8}$; $C3F_{1.1}$; $C3S_{0.6}$; $C3S_{0.8}$; $C3S_{0.4}$; $C3S_{0.9}$). The totality of those nine alleles formed 13 different genotypes. The author was right to talk about genotypes because the mentioned alleles are inherited co-dominantly, as he personally found out.

Alper and Rosen (14) summarised in a detailed overview that, besides the two common alleles (C3S and C3F), there were 14 other rare variants that have very low frequencies. They determined another very important fact – those versions were not different in terms of antigen and function.

The molecular mechanism that leads to the polymorphism of the C3 complement component in humans was clarified by Botto et al. (15). They determined that the difference between the genome organisation of C3S and C3F was a point mutation (cytosine to guanine) at nucleotide 364, exon 3. At a translation level, this resulted in replacement of arginine (at C3S) with glycine (at C3F). This replacement changes the polymorphous restriction area of the HhaI enzyme. Using a genome DNA, RFLP was studied. Afterwards, the number of fragments was increased via PCR (polymerase chain reaction) and an absolute conformability between genome polymorphism and the distribution of C3S and C3F in 50 normal individuals was observed. The molecular basis of the secondary structural polymorphism was determined via a HAV4-1 monoclonal antibody. The polymorphous determinate was established at codon 314 in exon 9 of the β-chain where leucine ($HAV4\text{-}1^+$) was replaced by proline ($HAV4\text{-}1^-$). The determination of amino acid sequences of those polymorphic variants would facilitate the characterisation of the probable functional differences among

the various C3 allotypes. The authors evidenced an increased incidence of the C3F allele in humans with partial lipodystrophy, IgA nephropathy, and Indian juvenile liver cirrhosis. Most probably, C3F could be used as a genetic marker for the determination of the susceptibility (or the resistance) to some diseases. In two studies, Dissing et al. (1) and Sörensen and Dissing(4) determined that the frequency of the C3F allele was higher in people suffering from arteriosclerosis. The relative risk of this disease was 1.87 times greater in C3F-positive individuals, in comparison with C3F-negative. Brönestam and Cedergren (2) reported a very interesting relationship between the high serum antibody titres and blood type antigens A and B in mothers that had recently given birth, and the carriers of the C3S allele. That phenomenon is observed very often in cases of AB0 incompatibility between the mother and the infant. That fact leads to the idea that the carriers of the C3S gene would react with a higher immune response after vaccinations against infectious diseases. During the same year, Brönestam (3) discovered a statistically feasible association between the C3F allele and the ratio of people affected by rheumatoid arthritis.

So far, the overview has shown that the polymorphism of the C3 complement component in humans has been studied thoroughly. Unfortunately, this problem has not been worked on sufficiently with animals. Gorman et al. (16) reported the existence of C3 polymorphism in dogs. It was determined that C3F and C3S alleles were inherited co-dominantly in an autosomal fashion, but no other alleles were found. The locus of C3 is not linked and is inherited in accordance with neither the main histocompatibility complex genes (DLA) nor with the loci of C6 and C7 complement components.

The studies of Kay et al. (17) in horses evidenced that in this animal species, the principal alleles were three: $C3_1$, $C3_2$ and $C3_3$, inherited co-dominantly as well. Those three alleles formed six various genotypes. Four years later, Bowling and Dileanis (18) reported the existence of a fourth allele that was with a higher frequency only in the wild Prjevalsky horse and with very low frequencies (0.01-0.04) in three other breeds.

Gahne and Amorena (19) discovered a C3-like protein in sheep blood plasma. Unlike the earlier studies, they used an ordinary agarose gel electrophoresis followed by immunoblotting. Thus, they found 4 principal alleles, three of them (F, I, M) presenting one principal band and the fourth (S) –3 to 5 bands. The family analysis showed that the alleles were inherited co-dominantly in an autosomal manner. Until now, we have not found any information concerning the relationship between C3 polymorphism and resistance or susceptibility to diseases in animals.

CONCLUSION

The relative frequencies of the alleles, coding for the C3 complement component, and the genotypes they form, were determined in the sheep.

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